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EDITORIAL NOTE

There is a need for strong Science and Technology base for Environmental Research and Development. Research in Environment related problems is an essential prerequisite for generating reliable data. It also provides knowledge for the development of sound evidence-based policies and strategies towards the conservation of natural resources. Globally it has provided important inputs for devising strategies to ensure ecological security and sustainable development. The National Conference on Impacts of Pollution on Health and Restoration of Quality Environment through Biotechnology Applications (EBAC-2018) held on 2-3 February 2018 at The American College, Madurai organised by the Postgraduate & Research Department of Zoology and Department of Immunology & Microbiology has enabled the researchers to share their ideas and knowledge.

This journal is the compendium of the papers presented in the conference published by Roots International Journal of Multidisciplinary Research. In the conference forty papers were presented orally, and fifty papers as posters in addition to five invited lectures. Among them, sixteen papers submitted as full papers were edited and included in this issue. We are indebted to the management of our college especially our Principal & Secretary, Dr.M.Davamani Christober, members of the organizing committee of the conference and the contributors of the papers.

We wish you a happy reading.

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Academic Excellence in research is continued promoting in research support for young Scholars. Multidisciplinary of research is motivating all aspects of encounters across disciplines and research fields in an multidisciplinary views, by assembling research groups and consequently projects, supporting publications with this inclination and organizing programmes. Internationalization of research work is the unit seeks to develop its scholarly profile in research through quality of publications. And visibility of research is creating sustainable platforms for research and publication, such as series of Books; motivating dissemination of research results for people and society

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Communication

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CONTENTS

EFFECT OF VERMICOMPOST, JEEVAMRUTHA AND G5-SOIL ENRICHER GRANULES ON THE GROWTH OF *AMARANTHUS ARITIS*

M.Bernath Rosi & A.Joseph Thatheyus
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Abstract

Organic agriculture aims at human welfare without any harm to the environment, which is the foundation of human life itself. It can be done by improving soil fertility, enhancing resource recycling, improving the production environment for production by farmers and enlarging the freedom of choice for consumers by supplying products of good quality at reasonable price. Effect of Vermicompost, Jeevamrutha and G5 soil enricher granules separately and in combination on the growth and yield of Amaranthus aritis was determined in the present study. Application of Vermicompost and Jeevamrutha enhanced growth parameters (germination percentage, shoot length, root length, wet weight, dry weight, chlorophyll content, and protein content) in A. aritis. The maximum levels were found in the combination of Vermicompost and Jeevamrutha while minimum values were noticed in the case of G5-Soil enricher granules.
Keywords: *Vermicompost, Jeevamrutha, G5 soil enricher granules, Amaranthus aritis, growth.*

Introduction

Efforts to improve the standard of living for humans through the control of nature and the development of new products have resulted in pollution, or contamination of the environment. Much of the world's air, water, and land are now partially poisoned by chemical wastes and some places have become uninhabitable. This exposes people all around the world to new risks from diseases. Many species of plants and animals have become endangered or are now extinct. As a result of these developments, governments are taking efforts to limit or reverse the threat to environment (Hall and Harhoff, 2012).

Nowadays organic farming practices are gaining importance as farmers have realized the benefits of organic farming in terms of soil fertility, soil health and sustainable productivity. They are aware of the use of organic liquid manures such as Panchagavya, Beejeevamrutha, Jeevamrutha and Biodigester in organic farming. These organic liquid manures play a key role in promoting growth and providing immunity to plants. The sprays of Panchagavya on chillies produced dark green coloured leaves within ten days. Its role as plant growth promoter has already been reported by Sreenivasa *et al*. (2010). The seed dipping in beejamrutha is known to protect the crop from harmful soil-borne and seed-borne pathogens.

The current global scenario firmly emphasizes the need to adopt ecofriendly agricultural practices for sustainable food production. The cost of inorganic fertilizers is increasing enormously to an extent that they are out of reach of small and marginal farmers. The Panchagavya, Jeevamrutha and Beejamrutha are ecofriendly organic preparations made from cow products. The use of organic liquid products such as Beejamrutha, Jeevamrutha and Panchagavya result in higher yield, growth and quality of crops. These liquid organic solutions are prepared from cow dung, urine, milk, curd, ghee, legume flour and jaggery. They contain macronutrients, essential micronutrients, many vitamins, essential amino acids, growth promoting factors like IAA, GA, and beneficial microorganisms (Sreenivasa *et al.,* 2010). Hence the present study has been designed to determine the effect of Vermicompost, Jeevamrutha and G5-soil enricher granules on the growth of *A.aritis.*

Materials and Methods

The present study was carried out for testing the effect of Vermicompost, Jeevamrutha, and G5- soil enricher granules and their combinations on the growth of the plant, *Amaranthus aritis.*

Study Area

A pot experiment was carried out during the growing season of 2016 at Mullai nagar in Madurai to study the effect of organic materials on the growth of *A.aritis* grown in red soil. The minimum and maximum temperature during the cropping period was 30 and 35^{0}C respectively. The experimental site had red soil with pH 5.57. Pots (8 pots) with 25.cm diameter and 35 cm depth were used in this study. Each pot was filled with 1.5 kg of the used fine

soil and planted with 35 of *Amaranthus* seeds. The soil was mixed well, labeled and brought to laboratory for analyses. The soil was mixed with the organic materials in each pot.

Chemical Parameters

Chemical analysis of the soil was carried out according to the methods described by Jackson (1973). Their chemical properties such as pH, Electrical conductivity, nitrogen, phosphorus, potassium, copper, zinc, iron and manganese were estimated and recorded. Soil organic matter was determined by using Walkley and Black method according to Jackson (1973). The samples were also analyzed for available nitrogen by Micro Kjeldhal method (Bremner and Mulvaney, 1982). Available phosphorus was determined by ascorbic acid method (Jackson, 1962) using atomic absorption spectrophotometer. Available potassium was determined according to Jackson (1962) using flame photometer. The DTPA-extractable micronutrients were measured (Lindsay and Norvell, 1978). Physical and chemical parameters were analysed in soil, Vermicompost, Jeevamrutha and G5-soil enricher granules (Dresboll and Thorupkristensen, 2005).

Vermicompost

Vermicompost was purchased from The Agriculture College and Research Institute at Madurai. Its characteristics were analyzed using the standard methods (Edwards, 2007). Experimental medium was maintained by mixing 2 kg of Garden soil and 1/2 kg of Vermicompost in the treatment pots of T1, T4, T5 and T7.

Jeevamrutha

Jeevamrutha was prepared by mixing 500g cow dung, 500 ml cow urine, 100g of green gram (soaked overnight and ground), 25 g undisturbed soil, 100 ml coconut water and ten liters of water and kept for three days by covering with muslin cloth. Stirring was done twice a day in clockwise direction. Jeevamrutha was stored in plastic vessels covered with muslin cloth in open condition and the quality analysis was done. Fresh preparation of Jeevamrutha was analyzed for the physical properties such as color (visual evaluation), odour (sensory evaluation), presence of mould growth and chemical properties such as pH, total macro and micronutrients (Kasbe *et al*., 2009). Jeevamrutha was applied in the treatment pots of T2, T4, T6, and T7.

G5 - Soil Enricher Granules

G5 soil enricher granules were purchased from the market. They are multi activity soil enricher granules, and organic certified granule fertilizer for all types of crops. G5 Granules are a blend of seaweed extracts, amino acid blends, herbal extracts and organic antiroot substance. 250 g of G-5 granules was applied per pot once in a week in the morning. G5 was applied in T3, T5, T6 and T7 treatments.

Amaranthus aritis

It has common names such as Red spinach, Chinese spinach, and Spleen amaranth. It belongs to the economically important family Amaranthaceae. Usually it grows to a size of 80–120 cm and it has both green and red varieties, as well as some with mixed colours. It flowers from summer to fall in the tropics, but can flower throughout the year in subtropical conditions. Leaves of young plants grown for grain are used not only for human consumption but also as animal feed, in South America, Africa, Asia and Eastern Europe (Muyonga *et al.*, 2008).

Treatment

The eight treatments were conducted for the seeds of *A.aritis* (Table 1). For each treatment, thirty five seeds were used in each pot. The seed germination in different treatments was observed after 96 hours of sowing. Observations were made only when the plumules had come through microphyll of the seed. The germination process was analyzed upto ten days. The shoot length, root length, wet weight, dry weight, protein and chlorophyll content were measured four times, once in ten days. The analysis of protein, and chlorophyll content was carried out using standard procedures (Atiyeh *et al.,* 2002).

Table 1 The Various Types of Treatments Used in the Present Study

Treatments	Products
T1	Vermicompost
T2	Jeevamrutha
T3	G5 soil enricher granules
T4	Vermicompost + Jeevamrutha
T5	Vermicompost + G5 soil enricher granules
T6	Jeevamrutha + G5 soil enricher granules
T7	Vermicompost + Jeevamrutha + G5 soil enricher granules
T8	Control

Seed Germination

Seed germination percentage was recorded up to tenth day after seeding.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds applied}} \times 100$$

Shoot Length and Root length

Shoot length was measured from above the root surface to the tip of the leaf and root length was measured from the ground level to the tip of the longest root hair (Danish and Patra, 2004).

Wet Weight and Dry Weight

The fresh plants from each pot were weighed on electronic balance to obtain the wet weight. Each of the plant was packed in separate envelope and dried at 80°C in an oven for two days and weighed using electronic balance to obtain dry weight (Devendra et *al.,* 2003).

Preparation of the Samples

Green leaves of *A.aritis* were cleaned, washed with distilled water and allowed to air dry. Two gram portion of the dry sample was weighed and made into paste using pestle and mortar. The juice was extracted and made up to 5ml with distilled water and poured into a centrifuge tube and after centrifugation the aqueous extract collected was packed in polythene pouches and stored in the refrigerator for the estimation of total protein and chlorophyll content.

Total Protein Estimation

Protein content of leaf extract was determined by Lowry's method using Folin-ciocalteau reagent. The absorbance of each sample was determined using spectrophotometer after 30 minutes and standard graph was prepared using BSA as standard (Singh *et al*., 2008).

Estimation of Chlorophyll

The estimation of chlorophyll was done in the laboratory. Chlorophyll was extracted from one gram of leaf sample using 20 ml of 80% acetone. The supernatant was transferred to a volumetric flask after centrifugation at 5000 rpm for five minutes. The extraction was repeated until the residue becomes colourless. The volume in the flask was made up to 100ml with 80% acetone. The absorbance of the extract was read in a spectrophotometer at 645 and 663nm against 80% acetone blank. The amount of total chlorophyll in the sample was calculated using the following formulae (Berova and Karanatsidis, 2009).

mg chlorophyll a/g tissue = 12.7 (A_{663}) - 2.69(A_{645})×V/1000×W

mg chlorophyll b/g tissue = 22.9 (A_{645}) - 4.68(A_{663})×V/1000×W

mg total Chlorophyll /g tissue = 20.2 (A_{645}) + 8.02 (A_{663}) ×V/1000×W

Where,

V = final volume of the extract

W =fresh weight of the leaves

The values are expressed as mg chlorophyll/g sample.

Results and Discussion

The growth parameters of *A. aritis* were determined in the present investigation with respect to various treatments like Vermicompost, Jeevamrutha, and G5-soil enricher granules and their combinations. The chemical parameters of soil, Vermicompost, Jeevamrutha, and G5- soil enricher are shown in Table 2, 3, 4 and 5 respectively.

In Table 2 the highest value was found for manganese while the lowest value was noticed for iron. Chemical parameters of vermicompost are shown in Table 3. Organic carbon, nitrogen, phosphorous, potassium and sodium, were observed in Vermicompost. The chemical parameters of Jeevamrutha are exhibited in Table 4. The highest value was found for nitrogen while the lowest was found in zinc. Chemical parameters of G5-soil enricher granules are shown in Table 5. The highest value was found for magnesium and the lowest for zinc.

Figure 1 shows the germination percentage of *A. aritis* in various treatments. It was found to be maximum in Vermicompost treatment (T1) while the minimum was recorded in G5-soil enricher granules treatment (T3). Figure 2 exhibits the effects of various treatments on the shoot length of *A. aritis* 10, 20, 30 and 40 days after seeding, maximum was recorded in Vermicompost combined with Jeevamrutha treatment (T4) while the minimum was recorded in G5-soil enricher granules treatment (T3). Figure 3 highlights the effects of various treatments on the root length of *A. aritis* 10, 20, 30 and 40 days after seeding. Highest value was recorded in Jeevamrutha treatment while the minimum was noticed in Vermicompost combined with G5-soil enricher granules treatment (T5).

Figure 4 indicates the effects of various treatments on the wet weight of *A. aritis* 10, 20, 30 and 40 days after seeding. Wet weight was found to be maximum in Jeevamrutha (T2) and Vermicompost treatments (T1). Treatment with G5-soil enricher granules (T3) showed the minimum wet weight. Figure 5 illustrates the effect of various treatments on the dry weight of *A.aritis* 10, 20, 30 and 40 days after seeding. Dry weight was found to be maximum in Vermicompost + Jeevamrutha + G5-soil

enricher granules treatment (T7). Treatment with G5-soil enricher granules (T3) showed the minimum dry weight.

Figure 6 divulges the effect of various treatments on the chlorophyll A content of *A.aritis* 10, 20, 30 and 40 days after seeding. Chlorophyll A was found to be maximum in Jeevamrutha treatment (T2) while G5-soil enricher granules treatment (T3) exhibited the minimum level of chlorophyll A. Figure 7 exhibits the effect of various treatments on the chlorophyll B content of *A.aritis* 10, 20, 30 and 40 days after seeding. Chlorophyll B was found to be maximum in Jeevamrutha combined with Vermicompost treatment (T2) while G5-soil enricher granules treatment (T3) showed the minimum chlorophyll B. Figure 8 highlights the effect of various treatments on the total chlorophyll content of *A.aritis* 10, 20, 30 and 40 days after seeding. Total chlorophyll was found to be maximum in Jeevamrutha combined with Vermicompost treatment (T4) while G5-soil enricher granules treatment (T3) exhibited the least total chlorophyll content.

Table 2 Chemical Characteristic of Soil Used in the Study

Parameter (unit)	Value	Interpretation
Moisture content (%)	1.35	-
pH	5.57	Acidic
Electrical conductivity (ds/m)	0.30	Harmless
Nitrogen (mg/kg)	476	High
Phosphorus (mg/kg)	50	High
Potassium (mg/kg)	518	High
Organic Carbon (g/kg)	5.14	Medium
Exchangeable Calcium (meq/100g)	3.50	-
Exchangeable Magnesium (meq/100g)	1.50	-
Exchangeable Sodium (meq/100g)	1.25	-
Exchangeable Potassium (meq/100g)	0.81	-

Table 3 Chemical Characteristics of Vermicompost

Parameter (Unit)	Value
pH	7- 8.2
Organic carbon (%)	17.98
Nitrogen (%)	1.50
Phosphorus (%)	0.30
Potassium (%)	0.56
Sodium (%)	0.30
Calcium and Magnesium (meq/100mg)	22.67
Copper (mg/kg)	9.50
Iron (mg/kg)	9.30
Zinc (mg/kg)	5.70
Sulphur (mg/kg)	128

Table 4 Chemical Characteristics of Jeevamrutha

S.No	Parameter (Unit)	Value
1	pH	7.07
2	Soluble salt (%)	3.40
3	Nitrogen (%)	7.70
4	Phosphorus (ppm)	166
5	Potassium (ppm)	126
6	Zinc (ppm)	4.29
7	Copper (ppm)	1.58
8	Iron (ppm)	282
9	Manganese (ppm)	10.7

Table 5 Chemical Characteristics of G5- Soil Enricher Granules

S.No	Parameter (unit)	Value
1	Iron (ppm)	20
2	Magnesium (ppm)	10
3	Sodium (%)	0.8
4	Zinc (ppm)	3
5	Sulphur (%)	0.45
6	Vitamin B (ppm)	13
7	Vitamin E (ppm)	1.71
8	Chromium (ppm)	13
9	Cobalt (ppm)	6

Figure 9 shows the effect of various treatments on the protein content of *A.aritis* 10, 20, 30 and 40 days after seeding. Protein content was found to be maximum in Jeevamrutha treatment (T2) while treatment with G5-soil enricher granules (T3) showed the minimum protein content. Table 6 highlights the variation due to treatment types and treatment period for the various factors. Variations due to treatment types are not statistically significant at 5% level for the factors, germination percentage, shoot length, wet weight and protein content of the leaves. Variations due to treatment period are statistically significant for all the factors.

Figure 1 Effect of Various Treatments (T1 to T8) on the Seed Germination (%) of *Amaranthus aritis*

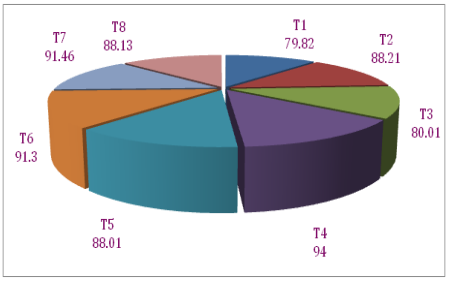

Figure 2 Effect of Various Treatments on the Shoot Length of *Amaranthus aritis*

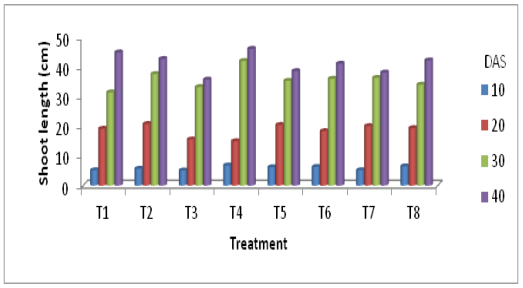

Figure 3 Effect of Various Treatments on the Root Length of *Amaranthus aritis*

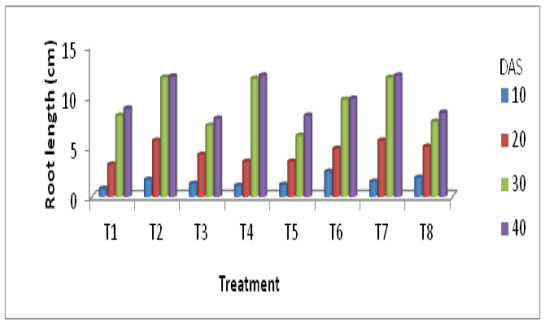

Figure 4 Effect of Various Treatments on the Wet Weight of *Amaranthus aritis*

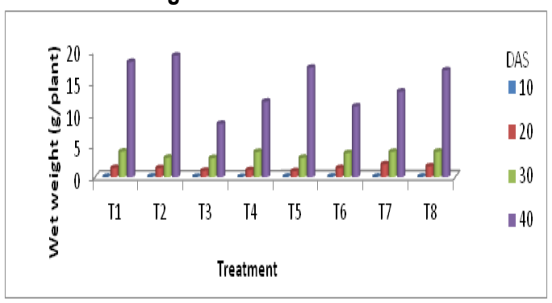

Figure 5 Effect of Various Treatments on the Dry Weight of *Amaranthus aritis*

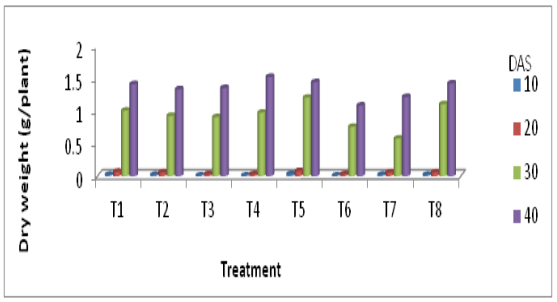

Figure 6 Effect of Various Treatments on the Chlorophyll A Content of the Leaves of *Amaranthus aritis*

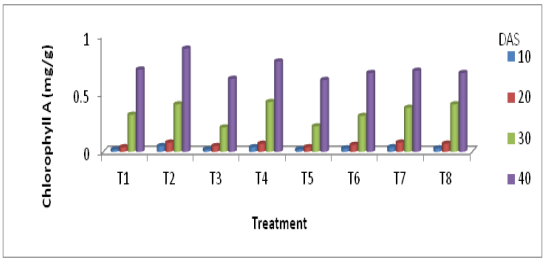

Figure 7 Effect of Various Treatments on the Chlorophyll B Content of the Leaves of *Amaranthus aritis*

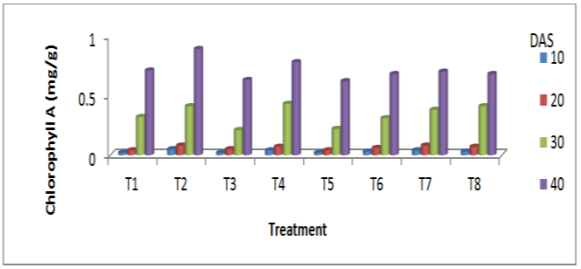

Figure 8 Effect of Various Treatments on the Total Chlorophyll Content of the Leaves of *Amaranthus aritis*

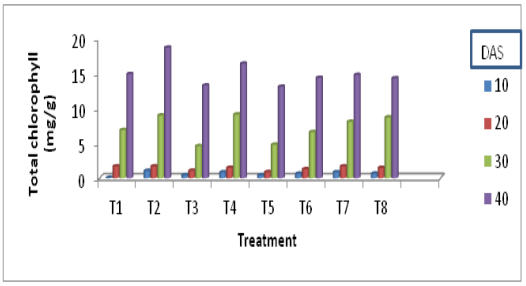

Figure 9 Effect of Various Treatments on the Protein Content of the Leaves of *Amaranthus aritis*

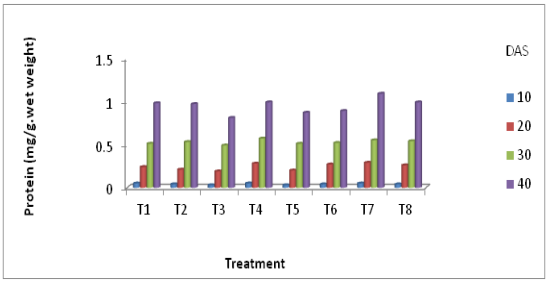

Table 6 Two Way Analysis of Variance (Anova): Variations Due to Treatment Type and Treatment Period for the Various Factors of *Amaranthus aritis*

S. No	Factor	Source of variation	ss	df	MSS	Calculated F value	Table F value at 5% level	Level of Significance
1	Germination percentage	Treatment type	40.85558	7	5.836511	0.284616	2.203232	NS
		Treatment period	2020.336	7	288.6194	14.07444	2.203236	S
2	Shoot length	Treatment type	61.33	7	8.761429	1.367447	2.487578	NS
		Treatment period	6302.58	3	2100.86	327.8939	3.072467	S
3	Root length	Treatment type	42.8094	7	6.115628	4.197625	2.487578	S
		Treatment period	385.6586	3	128.5529	88.23571	3.072467	S
4	Wet weight	Treatment type	28.68919	7	4.098455	1.080588	2.487578	NS
		Treatment period	1062.505	3	354.1684	93.37916	3.072467	S
`5	Dry weight	Treatment type	0.3921	7	0.056014	14.00357	2.487578	S
		Treatment period	0.5184	3	0.5184	129.6	3.072467	S
6	Chlorophyll A content	Treatment type	0.0598	7	0.008543	3.687564	2.487578	S
		Treatment period	2.39015	3	0.796717	343.9065	3.072467	S
7	Chlorophyll B content	Treatment type	1058	7	352.9882	207.5981	2.487578	S
		Treatment period	47.60964	3	1.700344	523.566	3.072467	S
8	Total chlorophyll	Treatment type	0.0381	7	0.005443	3.690073	2.487578	S
		Treatment period	3.715925	3	1.238642	839.75712	3.072467	S
9	Protein content	Treatment type	0.031747	7	0.004535	2.453667	2.487578	NS
		Treatment period	3.0151559	3	1.005053	543.7531	3.072467	S

S-Significant; Ns- Not Significant

Agricultural research is focused on evolving ecologically sound, biologically sustainable and socio economically viable technologies. Vermicompost is a good source of nutrients and growth promoting substances. In the present work, the effect of organic substances like vermicompost, Jeevamrutha and G5- soil enricher were studied using *A. aritis* as model plant. The higher uptake of nutrients namely nitrogen, phosphorous, potassium and sulphur in this particular treatment might be attributed to higher content of these nutrients, presence of beneficial microflora such as N-fixers and P- solubilizers, in the vermicompost. The findings are in line with those of Jagtap *et al.* (2007). The Jeevamrutha contains kinetin which has a role in enhancing chlorophyll content in plant leaves, thus in turn, enhances photosynthetic activity, growth and yield. The combination of Vermicompost and Jeevamrutha resulted in the highest growth rate in *A. aritis* compared to G5-soil enricher granules. The vermicompost contains humified organic matter characterised by high molecular weight and enzymatically active humic fraction which stimulates seed germination and plant growth. Thus, the organic manures like vermicompost, Jeevamrutha and G5-soil enricher granules when used as components in various strategies, show promising results in *A. aritis*. It has also been found that treatment in combination with Vermicompost and Jeevamrutha enhanced yield. Among the organic sources of nutrients, application of Jeevamrutha and vermicompost exhibited higher wet

weight and dry weight. The higher yield parameters might be due to higher dose of nitrogen, phosphorus and potassium through organic sources which might have helped in inducing growth parameters (Siddaram et al., 2010). Increase in chlorophyll content due to nitrogen application could be due to greater availability and uptake of nitrogen and phosphorus by plants. Phosphorus might have increased the uptake of nitrogen by the plants due to which the chlorophyll content increased. The presence of such beneficial microorganisms in liquid organic manures like Panchagavya and Jeevamrutha was earlier reported by many workers (Bhat *et al*., 2014; Xu et al., 2000). Lower growth was recorded with the application of G5-soil enricher granules due to fewer uptake of nutrients. Consequently the grain yield was lower besides the poor growth and yield components. These findings are in agreement with those of Jayaprakash *et al.* (2003) and Chandrashekara *et al.,* (2000). The results showed higher biomass yield in the plants grown on Vermicompost and Jeevamrutha than in the garden soil (control).

Acknowledgments

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POLYMER BASED SOLAR CELL WITH BEETROOT DYE AND CADMIUM TELLURIDE QUANTUM DOTS

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Abstract

In the present work, Polymer based solar cell was fabricated by the conjugation of natural dye, Cadmium telluride quantum dots and polymer solution. It is environmentally safe, lightweight, and efficient. The fabricated solar cell was characterised by XRD, UV-Vis spectroscopy. I-V characterization study was done to analyse the solar cell. Absorbance peak and Band gap of fabricated solar cells were studied from UV-Vis spectroscopy and it shows a strong absorbance in the blue wavelength region. Efficiencies of the solar cells were calculated for different temperatures and the maximum efficiency of 2.25% was obtained at 80˚C.

Keywords*: Solar cell, PMMA, CdTe quantum dots, Beetroot extract*

Introduction

Energy is the greatest challenge facing mankind in this century. Energy sources are grouped into non-renewable sources such as fossil fuel and renewable sources such as solar energy. Nonrenewable sources such as fossil fuels have been the energy sources for human society for a long time. Fossil fuel is being rapidly depleted by excessive consumption and its burning has caused and is still causing damage to the earth environment. Renewable energy sources are considered clean alternatives to the traditional sources [1].

Solar energy is one of the renewable sources, and is environmentally friendly. It is also universal and versatile with the ability to be made available even to the remotest parts of the world [2]. Solar energy causes no public harm and can be used by anybody, without limits. Therefore, many countries are actively endeavoring to develop solar energy [3].

Solar energy is clean, quiet, and visually unobtrusive [4]. A solar cell or photovoltaic cell is an electrical device that converts the energy of light directly into electricity by the photovoltaic effect, which is a physical and chemical phenomenon. It is a form of photoelectric cell, defined as a device whose electrical characteristics, such as current, voltage, or resistance, vary when exposed to light [5]. Polymer based solar cells (PSCs) is the third generation of the solar cells [6]. PSCs technology is a promising cost effective, alternative to silicon based solar cells. PSCs can be utilized to generate truly clean and renewable energy from sunlight [7]. There are many natural dyes used as a sensitizers such as Mangoostein, Rambutan, Mango, Tomato, Carrot, King coconut, Pumpkin, Red Banana, Beetroot, Turmeric, Venivel, Orange, Grape, Spinach, Wattakka, Ginger, Blueberry, Purple Cabbage, Lawsonia inermis, Pandan leaves, and Strawberry [8-12].

In this study, Polymer based solar cell was fabricated by the conjugation of natural beetroot dye, Cadmium telluride quantum dots and polymer solution and its I-V characterization study was done for different temperatures.

Materials and Methods

Extraction of the Natural Dyes

Fresh Beetroots were purchased from the local market. The collected beetroots were thoroughly washed with water to remove the adhering particles and dust from the surface. The beetroot extracts were prepared using 50g of finely cut beetroot crushed, filtered through filter paper. The filtered extract was added to 15ml of ethyl alcohol(C_2H_5OH) which was remove unwanted waste and the mixture was stirred at different temperature for 50˚C, 60˚C, 70˚C, 80˚C with 900 rpm for 30 minutes to obtain the liquid solution. After filtration, natural dye extracts were obtained. The extract was preserved inside a refrigerator for future use.

(a)

(b)

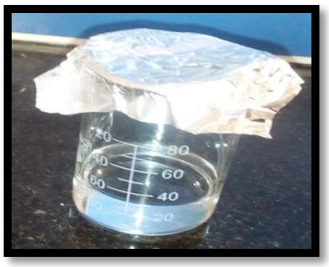
(c)

Figure 1 Schematic Illustration of Beetroot Dye Preparation: (a) Raw Beetroots (b) Beetroot Dye Extracts (c) Stirring the Dye with the Mixture of Ethanol

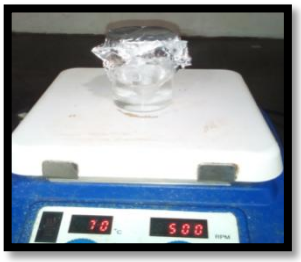
Synthesis of PMMA Solution

0.6g of Polymethylmethacrylate (PMMA) was carefully transferred into a 100 ml beaker and 30 ml of chloroform was added to dissolve the PMMA. The solution was stirred at 70˚C with 500 rpm for 30 minutes to obtain the clear solution. The prepared polymer solution is ready for coating on the glass substrate.

(a)



(b)



(c)

Figure 2 Schematic Illustration of PMMA Solution Preparation: (a) PMMA (0.6g) (b) PMMA Dissolved In 30ml of Chloroform (c) Stirring the Mixture of PMMA and Chloroform

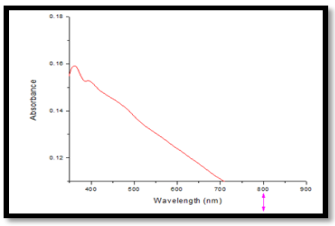
Preparation of the Glass Substrates

For making solar cell, the glass substrates were cut into pieces of dimensions (4.5×2.5) centimeter. The glasses were cleaned in a detergent solution followed by water, ethanol respectively and then dried. The prepared PMMA solution was coated on the cleaned glass substrates by dip coating process. These coated glass substrates were dried for few minutes. After drying, cadmium telluride quantum dots were coated on it. After coating, it was dried for one day. Then the prepared dyes for different temperatures were coated on the substrates. Various characterization studies were done and the efficiencies of the prepared solar cell were calculated.

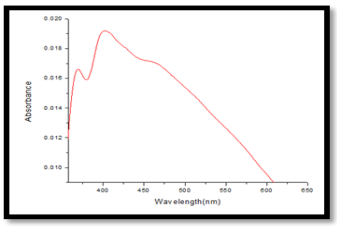
Results and Discussion

Optical Absorption Study

The absorption spectra of fabricated solar cells were carried out using JASCO UV-Vis NIR-V670 spectrometer. Figure 3(a) and (b) shows the UV of PMMA with Beetroot dye, PMMA with CdTe quantum dots and observed the absorption peaks at 380nm and 410nm respectively. And their maximum absorption wavelengths of the solar cells are listed in Table 1.



(a)



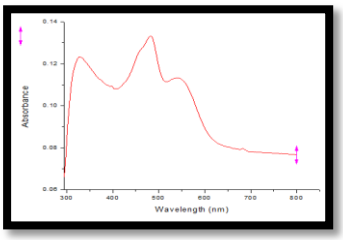
(b)

Figure 3 (a) and (b) Shows the UV of PMMA with Beetroot Dye, PMMA with CdTe Quantum Dots

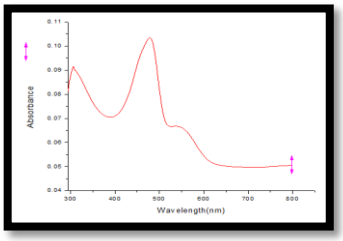
Table 1 Absorption Wavelength of the Fabricated Solar Cells

S.No	Cell type	Wavelength(nm)
1.	PMMA with CdTe quantum dots	380
2.	PMMA with Beetroot Dye	410

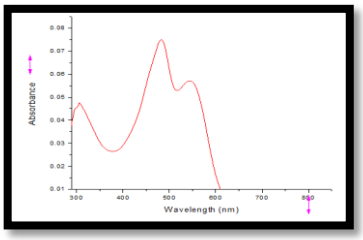
Figure 4 (a) to (c) shows UV spectra for various temperatures and observed the absorption peaks at 478nm, 479nm and 482nm respectively [13]. The maximum absorption wavelengths of the solar cells are listed in Table 2.



(a)



(b)



(c)

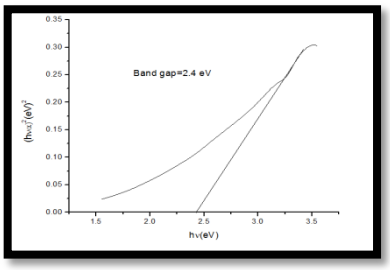
Figure 4 Absorption Spectra of Mixture of PMMA with Beetroot Dye and CdTe Quantum Dots at (a) 60˚C (b) 70˚C (c) 80˚C.

Table 2 Absorption Wavelength of the Fabricated Solar Cells

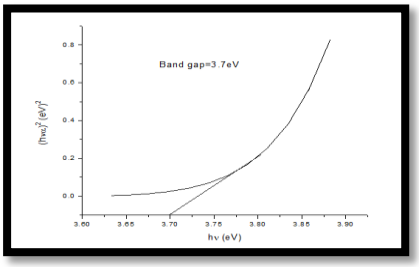
S. No.	Mixture of PMMAwith CdTe Quantum dots and Beetroot dye at	Wavelength (nm)
1	60˚C	478
2	70˚C	479
3	80˚C	482

Band Gap Analysis

Fig.5 (a) and (b) shows the band gap of PMMA with Beetroot dye, PMMA with CdTe quantum dots. Figure 6(a) to (c) shows mixture of PMMA with Beetroot dye and CdTe quantum dots at different temperatures. And their graph was plotted between photon energy (hv) and $(h\nu\alpha)^2$ and the band gap values determined are listed in Table 3 and 4 respectively. It was observed that when the temperature is increased, the bandgap value is decreased.



(a)

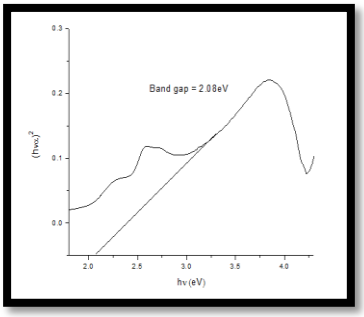


(b)

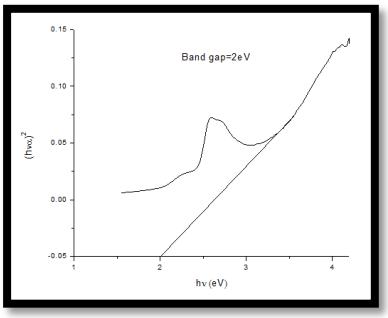
Figure 5 hv vs (hvα)² graph of (a) PMMA with Beetroot dye (b) PMMA with CdTe quantum dots

Table 3 Band Gap Values

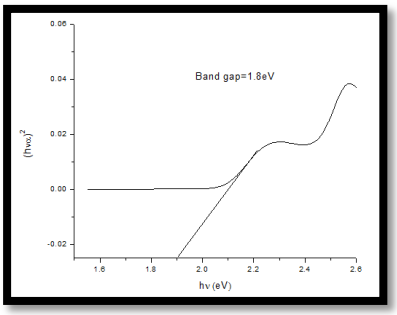
S.No	Cell type	Bandgap(eV)
1.	PMMA with CdTe quantum dots	2.4
2.	PMMA with Beetroot Dye	3.7



(a)



(b)



(c)

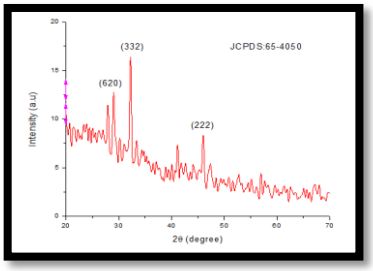
Figure 6 hv vs (hvα)² Graph of (a) Mixture of PMMA with Beetroot Dye and CdTe Quantum Dots at 60˚C (b) 70˚C (c) 80˚C

Table 4 Band Gap Values

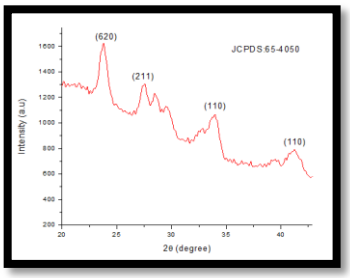
S. No.	Mixture of PMMA with Beetroot dye and CdTe Quantum dots at	Band gap in eV
1	60˚C	2.08
2	70˚C	2.00
3	80˚C	1.9

Particle Size Analysis

The XRD patterns of the dyes were carried out using Rigaku miniflex 600 instruments. Fig. 7(a) shows the diffraction pattern of dye at 50˚C and it reveals that as synthesized dye particles are crystalline in nature. The peaks at the 2θ values 27.3˚, 34˚, and 41.1˚ were fitted and their hkl planes were identified as (620), (332), and (222) respectively, which coincides with the JCPDS card no 65-4050. Figure 7(b) to (d) shows the diffraction pattern of dye at various temperatures. It reveals that as synthesized dye particle are crystalline in nature.



(a)



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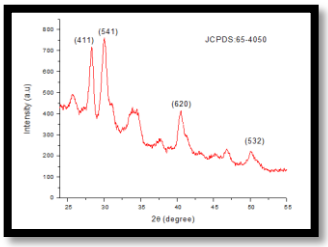
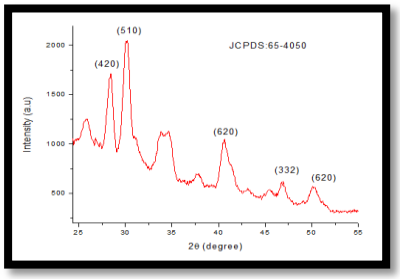


(c)



(d)

Figure 7 XRD Patterns of the Dye Particles at Different Temperature (a) 50˚C, (b) 60˚C, (c) 70˚C, and (d) 80˚C

The average crystallite size of the synthesized dye particle was calculated using Scherrer formula and displayed in Table 5.

$$D=\frac{k\lambda}{\beta\cos\theta} \quad \text{.....(1)}$$

Where

K is the Scherrer constant,

λ is the wavelength of light used for the diffraction,

β is the "full width at half maximum" of the sharp peaks,

θ is the angle measured.

Table 5 Particle Size of the Beetroot Dye at Different Temperature

S.No	Temperature	Particle size (nm)
1.	50˚C	5
2.	60˚C	8.6
3.	70˚C	9
4.	80˚C	9.5

Current Voltage Measurement

The current–voltage (I-V) measurement of fabricated solar cells was carried out using Keithley-2450. Figure 8(a) shows the current–voltage (I-V) curve for the fabricated solar cells based on beetroot dye and CdTe quantum dots with dye. Figure 8(b) shows the I-V characteristics of the as prepared solar cells for different temperatures. The characteristic curve shows the smooth variation when the temperature is increased, which indicates the smooth output voltage of the cells. The efficiencies were calculated using the formula. Table 4 shows the efficiencies of the fabricated solar cells.

$$\eta=\frac{P}{ExA}X100 \quad \text{.....(2)}$$

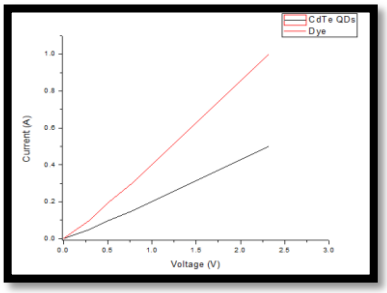
$$P=V \times I \quad \text{.....(3)}$$

Where

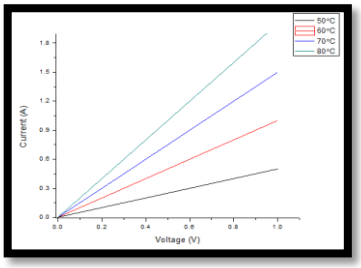
η =Efficiency of the cell (%)

V= Cell's voltage (V)

I= Cell's current (A)



(a)



(b)

Figure 8 I-V Curves of Polymer Based Solar Cells with (a) Different Sensitizers (b) Different Temperature

Table 6 Efficiency of the Fabricated Solar Cell

S.No	Cell Type	Efficiency (%)
1.	PMMA with CdTe quantum dots	0.29
2.	PMMA with Beetroot Dye	0.5.
3.	PMMA with Beetroot Dye and CdTe quantum dots at 50˚C	0.6
4.	PMMA with Beetroot Dye and CdTe quantum dots at 60˚C	0.75
5.	PMMA with Beetroot Dye and CdTe quantum dots at 70˚C	1.5
6.	PMMA with Beetroot Dye and CdTe quantum dots at 80˚C	2.25

From the results, it was found that the efficiency of the solar cell is increased when it is coated with Beetroot dye and CdTe quantum dots. It was also observed that the efficiency increased with the increase in temperature of the dye as the particle sizes were reduced with temperature. The maximum efficiency was observed for the dyes at 80˚C.

Conclusion

Polymer based solar cell was fabricated by the conjugation of natural dye, Cadmium Telluride quantum dots and polymer solution. The fabricated solar cells were studied by XRD, UV-Vis spectroscopy, and I-V characterization. From the U-V studies, it was observed that it shows a strong absorbance in the blue wavelength region and the band gap decreases with increase of temperatures. From XRD study, it was found that the particle sizes of the as prepared dye at 50˚C, 60˚C, 70˚C,

80˚C temperature was 5nm, 8.6nm, 9nm, and 9.5nm respectively. Efficiencies of the solar cells were calculated and the maximum efficiency of 2.25% was observed for 80˚C. The future work may be towards the fabrication of the solar cell.

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BIODEGRADATION OF THE SYNTHETIC PYRETHROID PESTICIDE, DELTAMETHRIN BY *BACILLUS SUBTILIS*

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Abstract

Synthetic pyrethroids are widely used as pesticides in agriculture and they are highly toxic to non-target organisms such as bees, fish and aquatic invertebrates. Therefore, it is important that we augment the rate of degradation of these pesticides in the environment by the addition of pesticide- degrading microbes. With that objective, Bacillus subtilis MTCC 2423 from IMTECH, Chandigarh, India was evaluated for its ability to degrade deltamethrin. B. subtilis was found to be able to grow in minimal media supplemented with 100 ppm deltamethrin. Biodegradation of different concentrations of deltamethrin was assessed by measuring the carbon di oxide production, change in pH and esterase activity. In the short term biodegradation study, deltamethrin degradation was highest on fourth day and in the long term treatment, it peaked on the eighth day. In both cases, maximum degradation was observed in 100 ppm concentration. The untreated deltamethrin (control) and deltamethrin treated with Bacillus subtilis were subjected to HPLC analysis. Deltamethrin degradation by B. subtilis was confirmed by the presence of intermediates showing peaks with different retention times compared to the peaks observed for the control deltamethrin sample. Therefore, the present investigation presents B. subtilis MTCC 2423 as a candidate for biological treatment of deltamethrin contaminated-environment and adds it as one more strain to the vast array of bacteria capable of removal of xenobiotics.

Keywords*: Pesticides, biodegradation, deltamethrin, Bacillus, Pyrethroids*

Introduction

Deltamethrin is a synthetic pyrethroid, a diverse group of broad spectrum pesticides analogous to the naturally occurring pyrethrins. It is a second generation synthetic pyrethroid and it is used widely around the world along with other synthetic pyrethroids. Deltamethrin is highly persistent in the environment and it is extremely toxic to non-target organisms such as aquatic organisms and fishes [1]. The biodegradation of pyrethroids is slower in the environment than the degradation of pyrethrins. This persistence in the environment highlights the importance of finding ways to increase the rate of degradation of synthetic pyrethroids. Several bacteria have been found to be capable of degrading synthetic pyrethroids [2]. Microbial degradation is the most important way in which xenobiotics like pesticides are degraded in the environment [3]. Augmentation of the natural degradation of pesticides is important to clear persistent residues of synthetic pyrethroids from the environment. Hence, it is important to screen for bacterial species capable of biodegradation of deltamethrin and to develop a consortium of bacteria for use in *in situ* biodegradation in contaminated soils and aquatic environments. Keeping this in mind, an attempt was made to isolate deltamethrin-degrading bacterium and to test its efficiency of degrading deltamethrin.

Materials and Methods

The bacterial strain *Bacillus subtilis* MTCC 2423 was obtained from the Microbial Type Culture Collection (MTCC) maintained by the Institute of Microbial Technology, Chandigarh, India.

Efficacy of Deltamethrin Degradation by *B. subtilis* MTCC 2423

The deltamethrin-resistant *Bacillus subtilis* MTCC 2423 was inoculated into minimal broth containing different concentrations of deltamethrin (0, 50, 100, 150, and 200 ppm) and incubated at room temperature. In the short term study, the degradation of deltamethrin was monitored by

analysing parameters such as pH, carbon dioxide, esterase activity, biomass, and degradation products every 24 h for 4 days. The long term study was conducted for 16 days and the same parameters mentioned above were tested every 4 days, till the 16th day of treatment.

Estimation of pH

The change in pH was monitored every 24h in the short term study and on 4th, 8th, 12th and 16th days in the long term treatment with the help of a pH meter.

Estimation of Free Carbon Dioxide

Carbon di oxide production during pesticide degradation was measured by titration method [4].

Estimation of Esterase Activity

Esterase activity during the biodegradation of various concentrations of deltamethrin was measured [5]. Briefly, 100 µL of the sample was mixed with 1.5 ml of 0.42 mM 1-naphthyl acetate, 0.5 ml Na_2HPO_4 buffer (0.2 M, pH 7.0) and 0.4 ml glass distilled water at 39°C for 10 minutes. 500 µl of 10% lauryl sulfate containing 2.5 mg of Fast Garnet GBC was added to the mixture and incubated at room temperature for 15 minutes for colour development. Esterase present in the sample acts on the susbstrate 1-naphthyl acetate and the released 1-naphthol combines with diazonium salt (Fast Garnet GBC) to form a coloured azo dye which is measured at 560 nm. The optical density value was compared with the absorbance of standard 1-naphthol curve (linear from 0- 0.08µM) to estimate the esterase concentration in the sample.

Estimation of Biomass

Biomass of the sample was analysed by turbidometric method. The absorbance of the test sample was measured using a colorimeter at 600 nm for 16 days at an interval of four days [4].

High Pressure Liquid Chromatography

The samples containing minimal medium, inoculum and 100 ppm concentration of the deltamethrin were taken on the 4th and 8th days of treatment and were subjected to HPLC analysis for confirming deltamethrin degradation.

Statistical Analysis

Two way analysis of variance (ANOVA) for the parameters pH, CO_2, esterase activity and biomass was done using MS excel package. Variations were considered significant only when the F value was greater than the tabulated value at 5% level.

Results and Discussion

Among the different genera of pesticide-degrading bacteria, genus *Bacillus* is special as they are capable of metabolizing a wide range of organic compounds. Hence, they are widely used in biodegradation studies [6, 7]. Along with deltamethrin, *Bacillus* sp. was found to be capable of degrading synthetic pyrethroids such as cypermethrin, bifenthrin and cyhalothrin [8]. *B. subtilis* was able to degrade pyrethroids such as Cypermethrin, Deltamethrin, Cyfluthrin and Cyhalothrin [9]. Several species of *Bacillus* are excellent biodegraders of various synthetic pyrethroids [10] which strengthens the case for developing a microbial consortium of various *Bacillus* sp. for cleaning up contaminated soils.

Figure 1 illustrates the changes in the pH of the medium during the degradation of deltamethrin by *B. subtilis* and it is found to be constantly decreasing for a time period of about eight days after which the pH remained constant, or a little increased. A steady decrease in pH was found in the short term degradation of deltamethrin by *B. subtilis* (Figure 2). 3- phenoxy benzoic acid is one of the major products of pyrethroid degradation (Maloney et al., 1992). This could have been the reason for the reduction in pH observed during the short term and long term degradation of deltamethrin by *B. subtilis.* It was observed that acidic conditions slowed down pyrethroid degradation while neutral or alkaline pH increased the rate of pyrethroid degradation [10]. The pH change was seen both in the short term treatment and long term treatment and in both the test conditions, it was significant (Table 1). Two way ANOVA also revealed that the change in pH in different concentrations of deltamethrin used was also statistically significant.

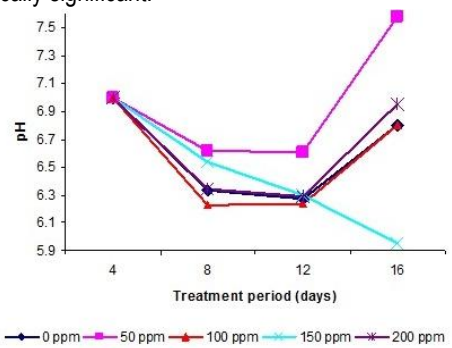


Figure 1 Changes in the pH of the Medium During the Degradation of Deltamethrin by *B. subtilis* in the Long Term Treatment

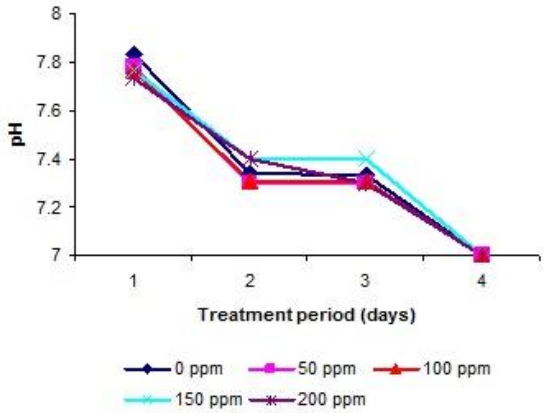


Figure 2 Changes in the pH of the Medium during the degradation of deltamethrin by *B. subtilis* in Short Term Treatment

One of the end products of the degradation of deltamethrin was CO_2. The free CO_2 present in the medium changed with as the treatment period progressed (Figures 3 and 4). During the degradation of pesticides by microbes, the mineralization product was found to be carbon di oxide [11, 12]. Carbon dioxide was found to vary with the treatment period and also with the concentration of deltamethrin used and this change was found to be statistically significant (Table 1). *Bacillus* sp. and other bacterial strains were able to completely mineralise synthetic pyrethroids such as cypermethrin and bifenthrin within days [6, 8, 13, 14].

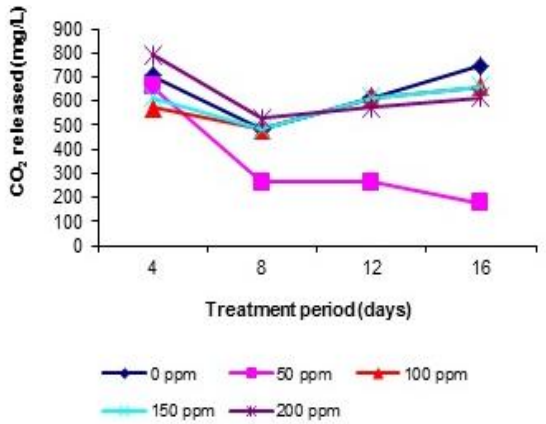


Figure 3 Carbon Dioxide Released during the Degradation of Deltamethrin by *B. subtilis* in the Long Term Treatment

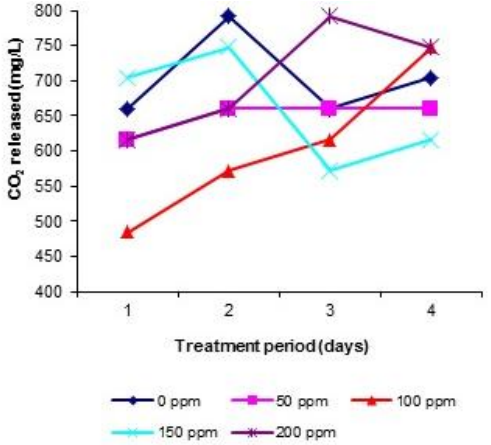


Figure 4 Carbon Dioxide Released during the Degradation of Deltamethrin by *B. subtilis* in the Short Term Treatment

Figure 5 and 6 exhibit esterase activity during the degradation of deltamethrin by *B. subtilis* during long and short terms respectively. The enzyme esterase is responsible for the breakdown of deltamethrin to 3-phenoxybenzoic acid and further to CO_2. Esterase activity varied significantly due to treatment period and the concentration of deltamethrin (Table 1). Enzymatic degradation of pesticides is rapid compared to chemical hydrolysis and carboxylesterases are found to be involved in the detoxification of pyrethroids besides other agrochemicals [15, 16]. Pyrethroid degrading thermostable esterases have been identified from the Turban basin [17].

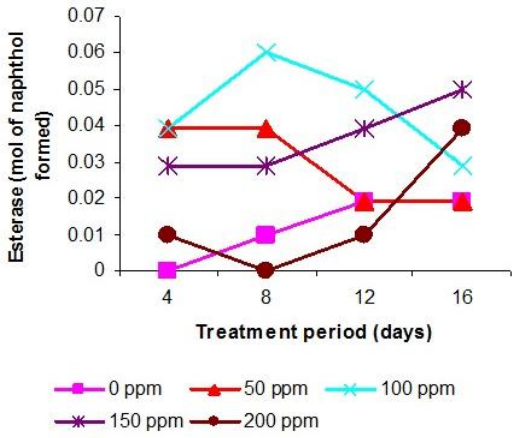


Figure 5 Esterase Activity During the Degradation of Deltamethrin by *B. subtilis* in the Long Term Treatment

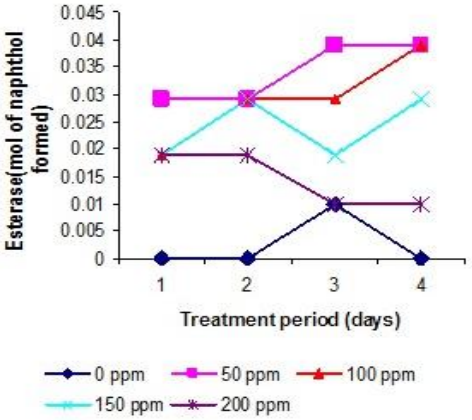


Figure 6 Esterase Activity during the degradation of Deltamethrin by *B. subtilis* in the Short Term Treatment

In the measurement of biomass, the isolate showed increasing turbidity when measured in a colorimeter. Figure 7 and 8 show the fluctuations in biomass during the long term and short term study respectively. In the long term treatment, biomass fluctuations did not vary significantly between treatment period whereas the fluctuation between different deltamethrin concentration was statistically significant (Table 1). Though the variations were statistically significant, difficulty was encountered when measuring the biomass by turbidity as the opaqueness of the minimal media supplemented with deltamethrin interfered with the colorimetric process. Therefore, in such cases, biomass estimation by plating method is recommended in order to understand the increase in biomass clearly. Biomass or inoculum size has been found to be an important factor in the efficient degradation of deltamethrin. Two strains of *Serratia marcescens* have been found to be excellent candidates for bioaugmentation of deltamethrin- contaminated soils. It was observed that soil type, presence of microbial flora, inoculum and interaction between these factors play an important role in deltamethrin degradation [18]. The exact impact of the inoculum size of *Bacillus subtilis* MTCC 2423 on deltamethrin degradation requires further study.

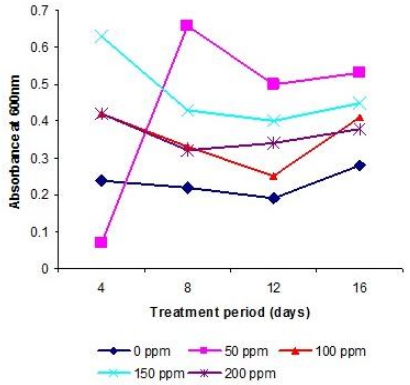


Figure 7 Turbidity during the degradation of Deltamethrin by *B. subtilis* in the Long Term Treatment

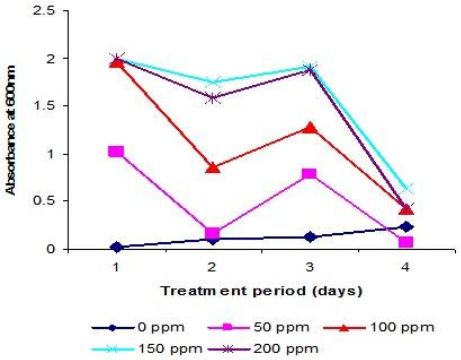
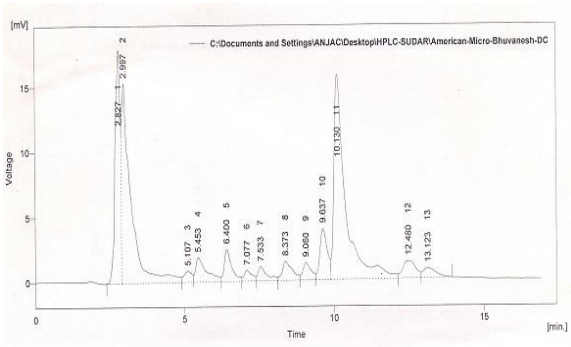


Figure 8 Turbidity during the degradation of Deltamethrin by *B. subtilis* in the Short Term Treatment

Table 1 Two Way Analysis of Variance for Various Factors with the Variables Treatment Period and Deltamethrin Concentration under Long Term and Short Term Treatment

Factors	*Treatment type*	*Source of Variation*	*SS*	*df*	*MS*	*F value*	*Table value at 5% level*	*Level of significance*
pH	Long	Concentration	0.56625	4	0.141563	1.729131	0.208136	Significant P < 0.05
		Treatment period	1.506695	3	0.502232	6.134564	0.009012	Significant P < 0.05
	Short	Concentration	0.00643	4	0.001607	1.288577	0.328429	Significant P < 0.05
		Treatment period	1.518655	3	0.506218	405.7862	2.5	Significant P < 0.05

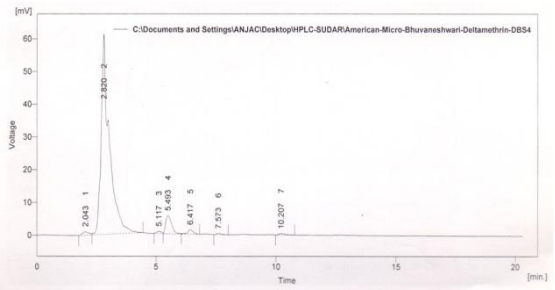
CO_2	Long	Concentration	240644.8	4	60161.2	5.475771	0.009588	Significant P < 0.05
		Treatment period	124194.4	3	41398.13	3.767988	0.0408	Significant P < 0.05
	Short	Concentration	27684.8	4	6921.2	1.201681	0.359848	Significant P < 0.05
		Treatment period	18972.8	3	6324.267	1.098039	0.387704	Significant P < 0.05
Esterase activity	Long	Concentration	0.003118	4	0.00078	4.279264	0.022184	Significant P < 0.05
		Treatment period	0.000152	3	5.08	0.278853	0.83962	Insignificant P > 0.05
	Short	Concentration	0.002505	4	0.000626	16.52507	8	Significant P < 0.05
		Treatment period	0.000101	3	3.37E-05	0.890062	0.474143	Significant P < 0.05
Turbidity	Long	Concentration	0.14253	4	0.035632	1.771366	0.199405	Significant P < 0.05
		Treatment period	0.016935	3	0.005645	0.280625	0.838379	Insignificant P > 0.05
	Short	Concentration	6.24985	4	1.562463	11.38634	0.000473	Significant P < 0.05
		Treatment period	3.08228	3	1.027427	7.487305	0.004377	Significant P < 0.05



Result Table (Uncal - C:\Documents and Settings\ANJAC\Desktop\HPLC-SUDAR\American-Micro-Bhuvanesh-DC)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.827	212.159	17.990	16.1	27.6	0.21
2	2.997	342.161	15.380	25.9	23.6	0.28
3	5.107	16.313	0.858	1.2	1.3	0.40
4	5.453	49.257	1.886	3.7	2.9	0.31
5	6.400	43.437	2.487	3.3	3.8	0.24
6	7.077	15.587	0.836	1.2	1.3	0.34
7	7.533	23.474	1.099	1.8	1.7	0.27
8	8.373	34.809	1.467	2.6	2.3	0.37
9	9.060	25.752	1.347	2.0	2.1	0.30
10	9.637	68.267	3.947	5.2	6.1	0.29
11	10.130	426.737	15.735	32.3	24.2	0.31
12	12.480	39.302	1.335	3.0	2.1	0.48
13	13.123	22.208	0.760	1.7	1.2	0.52
	Total	1319.463	65.126	100.0	100.0	

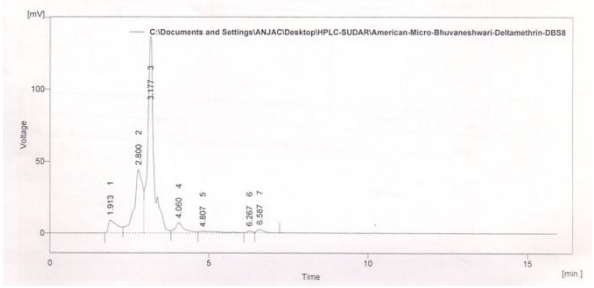
Figure 9 HPLC of 100 ppm Deltamethrin (Untreated Control)



Result Table (Uncal - C:\Documents and Settings\ANJAC\Desktop\HPLC-SUDAR\American-Micro-Bhuvaneshwari-Deltamethrin-DBS4)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.043	19.488	1.082	1.2	1.5	0.33
2	2.820	1446.216	61.155	90.2	86.7	0.33
3	5.117	9.344	0.661	0.6	0.9	0.25
4	5.493	94.449	5.473	5.9	7.8	0.27
5	6.417	21.835	1.381	1.4	2.0	0.21
6	7.573	5.017	0.370	0.3	0.5	0.20
7	10.207	7.038	0.389	0.4	0.6	0.28
	Total	1603.387	70.512	100.0	100.0	

Figure 10 HPLC of 100 ppm Deltamethrin Treated with *B. subtilis* after 4 Days



Result Table (Uncal - C:\Documents and Settings\ANJAC\Desktop\HPLC-SUDAR\American-Micro-Bhuvaneshwari-Deltamethrin-DBS8)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.913	181.196	8.970	6.0	4.5	0.37
2	2.800	801.432	44.153	26.3	21.9	0.27
3	3.177	1819.775	136.442	59.8	67.7	0.16
4	4.060	141.140	7.134	4.6	3.5	0.23
5	4.807	49.967	1.114	1.6	0.6	0.68
6	6.267	15.643	1.340	0.5	0.7	0.20
7	6.587	35.094	2.313	1.2	1.1	0.23
	Total	3044.247	201.466	100.0	100.0	

Figure 11 HPLC of 100 ppm Deltamethrin Treated with *B. subtilis* after 8 Days

In the HPLC analysis, the peak details exhibited the degradation of the pesticide and the presence of intermediate compounds which were detected by the difference in the retention time for the peaks observed. This shows that degradation has taken place more rapidly and effectively for the isolate and the reference strain (Figures 9 to 11). Microbial hydrolysis of most pyrethroids results in the production of intermediates such as 3-phenoxybenzaldehyde (3-PBA) or 3-phenozybenzoic acid [19]. Some bacterial strains are capable of degrading both the parental compound and the intermediate metabolites formed as well [20]. The metabolites formed by the degradation of pyrethroids vary depending on the pyrethroid degraded and the bacteria involved [10]. Further studies should be carried out to identify the intermediate compounds and the pathway of deltamethrin degradation by *B. subtilis.* The degradation efficiency exhibited by *B. subtilis* clearly shows that this strain can join the plethora of bacterial strains capable of transforming deltamethrin into less toxic compounds.

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BIODECOLOURISATION OF THE AZO DYE, CONGO RED USING *LACTOBACILLUS DELBRUECKII*

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Abstract

Waste water released from the use of synthetic dyes in different textile and other dyestuff industries require treatment before they are discharged into the environment to prevent groundwater contamination. Considerable interest has been on decolourisation and degradation of dyes by microorganisms due to their efficiency and duration of treatment. In the present study the natural isolate, Lactobacillus delbrueckii was isolated from dye contaminated soil and used in decolourisation of the dye, congo red. Decolourisation was achieved within fifteen days at lower concentration. The activities of α- amylase and protease produced by the bacterium were also determined for their activity. The results showed practical application potential of this bacterial strain in decolourisation of dye effluents that can help to solve pollution problems caused by textile and dyeing industries.

Keywords: *Azo dyes, Congo red, Lactobacillus delbrueckii, Biodecolourisation.*

Introduction

"Water" the elixir of life is being polluted by various means which should be prevented to avoid serious environmental hazards. Pollutants include chemical substances, organisms, products or physical properties that are released intentionally or inadvertently by man into the environment with actual or potential adverse, harmful, unpleasant and inconvenient effects. The most important and most precious natural resources and a regular and plentiful supply of clean water is essential for the survival and health of most living organisms. As a consequence of rapidly expanding industrialization and excessive population growth, most of our rivers, lakes, streams and other water bodies are being increasingly polluted. Therefore, treatment of waste water has to be done through state–of–art technique using microorganisms.

Tamil Nadu is dominated by textile industries that use varieties of dyes and chemicals. Their effluents are let out into the rivers without any pretreatment, causing environmental pollution. One of the very pressing environmental problems of textile industry is the removal of colour from effluents prior to discharge to local sewage treatment plant (Kousar *et al*., 2000). But, the dyes that are normally found in waste water treatment plants are highly resistant to microbial degradation. This is because dyestuffs are designed to resist chemical fading and light induced oxidative fading. Other factors involved in reducing biological degradation of dyes include properties such as high water solubility, high molecular weight and fused aromatic ring structures. Textile dyes are classified as azo, diazo, cationic and basic based on the nature of their chemical structure (Keharia and Madamwar, 2003). Among all, reactive azo dyes are most problematic due to their excess consumption and high water solubility. Furthermore, the sulfonic acid and the azo groups are rare amongst natural products and thus both confer xenobiotic character on this class of compounds. Therefore, bacterial isolates from dye effluent amended soils were used for decolourisation process. Some of the dyes and their degradation products have proved to be toxic, mutagenic and carcinogenic in nature. Thus, removal of dyes from effluents has been given utmost importance.

Numerous physical and chemical techniques based on coagulation, flocculation, precipitation, membrane filtration, ion-exchange, electrochemical destruction, photochemical degradation, ozonation and adsorption are available for removal of colour from textile waste water. The majority of colour removal techniques work either by

concentrating the colour into a sludge, or by partial to complete breakdown of the coloured molecule. Although some of the physico – chemical processes have been shown to be effective, their application is limited due to excess usage of chemicals, sludge generation with subsequent disposal problems, high installation as well as operating costs and sensitivity to a variable waste water input. Biodecolourization constitutes an attractive alternative to physico–chemical methods, mainly due to its reputation as low cost, ecofriendly and publicly acceptable treatment technology. Thus, a microbial capacity for the degradation of recalcitrant textile dye is developing that might be harnessed for dye removal by biotechnological processes. The objective of the present investigation is to isolate a bacterial strain capable of decolourising the reactive azo dye, congo red. The rate of decolourisation as well as protease and amylase activity of the selected bacterial strain was studied.

Materials and Methods

Isolation of the Bacterial Strain

For the isolation of bacteria, 1g of soil sample was serially diluted up 10^{-6}dilution. The isolation was carried out in nutrient agar medium by streaking a loopful of 10^{-6} dilution sample using streak plate technique.

Selection of Dye

Effluents which contain azo reactive dyes are very difficult to treat in environmental systems, due to the sulphonic acid groups, which make the dyes very water-soluble and polar. Therefore, such a reactive azo dye, congo red was selected. The molecular formula of congo red is $C_{32}H_{22}$ $NONa_2O_6S_2$ and the molecular weight is 696.68 (Finar, 2000). Its structure is shown in Fig.1.

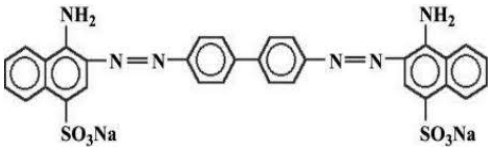

Figure 1 Chemical Structure of Congo Red

Preparation of Stock Solution

0.1g of congo red was dissolved in 100ml of distilled water. This was kept as stock solution and the concentration of this solution was 1000 ppm.

Minimal Broth for Decolourization Experiment

The composition of the minimal broth was as follows, K_2HPO^4 – 7.0g, KH_2PO_4 - 2.0g, $(NH_4)_2SO_4$ - 1.0g, Glucose - 1.0g, Sodium citrate - 0.5g, $MgSO_4.7H_2O$ - 0.1g, at PH 7.0 ± 0.2 at 25◦C.

Preparation of the Inoculum

The isolated colony was taken separately and inoculated on to Nutrient broth and kept in the shaker for overnight incubation.

Inoculation of the Isolated Strain

To the autoclaved minimal broth, the pure dye congo red was added in different concentrations like 10, 20, 30, 40, 50, 100, 200, 500, and 1000 ppm separately. To each of this, 0.1ml of culture was added and kept in the room temperature for incubation. O.D readings were taken for 1, 2, 3, 4, 5, 6 and the 7 hours using colorimeter. The readings were also taken for fifteen days using colorimeter.

Identification of the Isolated Strain

The schematic representation and tests used for the isolation and identification of the organism are given in Fig.2.The isolated bacterial strain was tentatively identified as, *Lactobacillus delbrueckii*

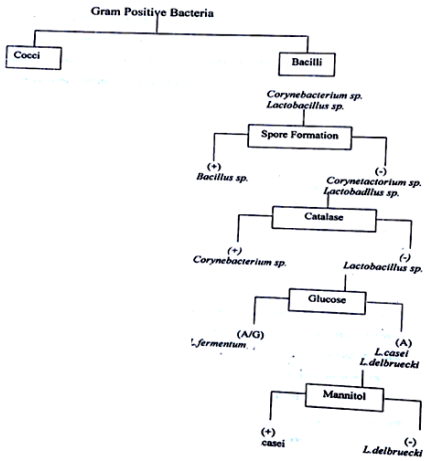

Figure 2 Identification Flow Chart of *Lactobacillus delbrueckii*

Decolourisation Assay

Decolourisation activity expressed in terms of percent decolourisation was determined by the following formula.

$$D\ (\%) = \frac{(A_{ini} - A_{fin})}{A_{ini}} \times 100$$

Where, D = Decolourisation

A_{in} = Initial absorbance

A_{fin} = Final absorbance after incubation time.

Two types of controls were used as uninoculated sterile control with dye and inoculated control without dye.

Enzyme Assay

Two ml of sample was taken from the dye concentrations 10, 20, 30, 40 and 50 ppm. The samples were centrifuged at 10,000 rpm for 15 minutes. The supernatant obtained were stored in a refrigerator by adding 1% sodium azide which inhibits the enzyme activity. This was done up to 5 days after inoculation of the bacterial strain. Enzyme activity of α- amylase and protease produced by bacteria were measured using different methods.

Results and Discussion

Soil samples that were analysed qualitatively had various bacterial colonies from which, one of the strains was identified and used for the decolourisation of the dye. Table 1 divulges the results of the various biochemical tests employed in the identification of the isolated strain. Most species of this non-spore forming bacterium ferment glucose into lactose, hence the name *Lactobacillus* (Diniz *et al*., 2002). In Gram staining, it was viewed as violet in colour which indicates the Gram positive bacilli. It did not form spores in spore formation test, and so it may be *Corynebacterium or Lactobacillus*. In catalase test, there was no bubble formation and so it was confirmed as *Lactobacillus sp.* By glucose test, it could be *Lactobacillus casei* or *Lactobacillus delbrueckii* because of the absence of colour change which indicates the absence of carbohydrate fermentation. In mannitol test, the organism did not ferment mannitol with the absence of yellow colour formation. So the organism was tentatively identified as *Lactobacillus delbrueckii. L.delbrueckii* are Gram positive, facultatively anaerobic non-motile and non- spore forming, rod shaped members of the industrially important lactic acid bacteria. Like other lactic acid bacteria, *L. delbrueckii* are acid tolerant, cannot synthesize porphyrins and possess a strictly fermentative metabolism with lactic acid as the major metabolic end product (Diniz *et al*., 2002).

Wastewater from textile dye units is one of the major environmentally undesirable pollutants (Murugesan and Kalaichelvan, 2003). The main cause of pollution in textile industry is due to desizing, bleaching, dyeing and printing (Patil *et al*., 2003). So, large amounts of dye stuff are being directly lost to the wastewater and find their way to the environment. Therefore, to solve this problem microbial degradation was carried out in the present study. The major environmental problem of colourants is the removal of dyes from the effluent. The untreated effluents of these industries may be highly coloured and thus particularly dangerous when discharged into open water bodies. The dye concentration may be much less than 1ppm but the dye is visible even at the less concentration (Murugesan and Kalaichelvan, 2003). Dyes are carcinogenic, mutagenic, allergenic and toxic in nature. So microbes are employed for dye decolourisation. In the present study, congo red was degraded by employing *L.delbrueckii.* The decolourisation has been observed and it is due to the enzyme production and utilization of dye by the organism.

Table 1 Biochemical Tests Used for the Identification of the Natural Isolate

S. No	Biochemical Test	Results
1.	Gram's staining test	Positive
2.	Spore formation test	Negative
3.	Catalase test	Negative
4.	Acid production from Glucose	Negative
5.	Acid production from Mannitol	Negative

L. delbrueckii was found to degrade and decolourise the dye when grown in minimal medium along with congo red in different concentrations like 10, 20, 30, 40, 50, 100, 200, 500 and 1000 ppm. After the inoculation of bacterial culture into the medium containing dye, O.D readings were measured after every one hour upto seven hours.

Figure3 illustrates the treatment of congo red using *L. delbrueckii* in hours and showed slight variation in all the concentrations after one hour of treatment. As the time duration increased, there was a decrease in optical density readings in 10, 20 and 30 ppm. But, when the time duration increased, no change was observed in 40, 50, 100, 200, 500 and 1000 ppm. For instance, from first hour to fourth hour the O.D reading was 0.54 and in fifth hour, it was 0.53. The decolourization activities of the strain are given in percentage in Fig. 4.

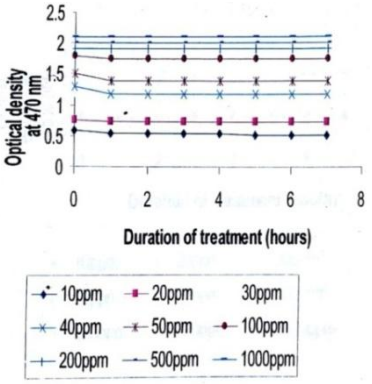

Figure 3 Decolourisation of Congo Red Using *L. delbrueckii* in Short Duration

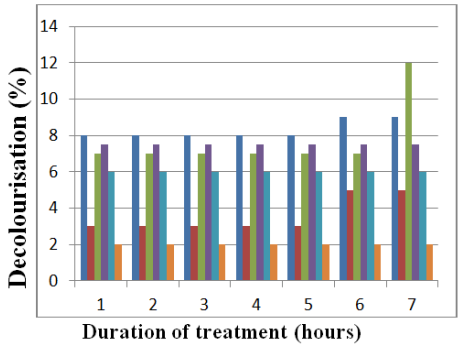

Figure 4 Decolourisation Activity of *L. delbrueckii* on Congo Red in Short Duration

Figure 5 shows *L. delbrueckii* exhibiting 100% decolourisation of congo red in 10 and 20 ppm with the maximum of 15 days of incubation. The percentages of decolourisation in higher concentrations were 61.4 to 78.3% which are shown in Fig.5.

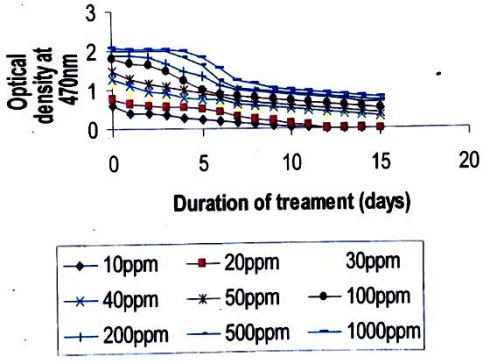

Figure 5 Decolourisation of Congo Red using *L. delbrueckii* in Long Duration

Dye decolourisation may take place through dye removal by simple adsorption of the dye at the cell surface or degradation by the cells. Cells would become deeply coloured if the dyes were removed by adsorption but cells remain colourless if they degrade the dyes (Puvaneswari *et al*., 2002). In the present study, the bacterial culture absorbed the dye and the culture present in the bottom of the flask had become deeply coloured which indicates the decolourization.

α-amylase and protease activities estimated during the decolourization process by the bacterial isolate in dye amended broth are given in Fig.6 and 7. The production of α-amylases in the decolourization of congo red varied from 0.7 to 1.6 mg/ml after five days of treatment (Fig. 6). As the days increased, the amylase production decreased. In congo red, five days of treatment showed maximum protease production ranging from 25 to 250 µg/ml.

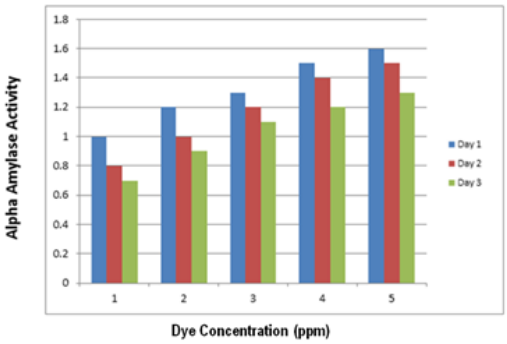

Figure 6 Alpha Amylase Activity during Decolourisation of Congo Red by *L. delbrueckii*

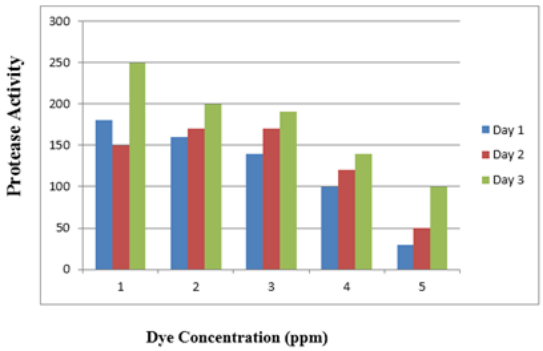

Figure 7 Protease Activity during Decolourisation of Congo Red by *L. delbrueckii*

L. delbrueckii are more efficient in degrading congo red. Generally, aromatic amines that are not mineralized accumulate in such environments and pose a major threat to human health and environment because of their well-documented toxic, mutagenic and carcinogenic activity (Keharia and Madamwar, 2003). Although numerous physical and chemical techniques based on coagulation, flocculation, precipitation, membrane filtration, ion-exchange, electrochemical destruction, photo-chemical degradation, ozonation and adsorption are available for removal of colour from textile wastewaters, they are not ideal (Keharia and Madamwar, 2003).

Table 2 divulges the two-way ANOVA results for the decolourisation efficiency of *L. delbrueckii* on congo red as a function of treatment period in hours and dye concentration. The variations due to dye concentration were statistically significant while they were not significant for treatment period in short duration. From Table 3, it is evident that the differences due to treatment period and dye concentration are not statistically significant in long duration.

Table 2 Two Way Analysis of Variance: Effect of *Lactobacillus delbrueckii* on the Decolourisation Activity of Congo Red In Short Duration

Source of Variation	SS	DF	MS	Calculated F - Value	Table F Value at 5% level	Level of Significance
Treatment Period	0.030104	6	0.005017	0.941683217	2.294598289	NS
Dye Concentration	17.52664	8	0.005017	411.1886172	2.138229149	S
Error	0.255746	48	0.005328			
Total	17.81249	62				

S-Significant, NS-Not significant

Table 3 Two Way Analysis of Variance: Effect of *Lactobacillus delbrueckii* on the Decolourisation Activity of Congo Red in Long Duration

Source of Variation	SS	DF	MS	Calculated F - Value	Table F Value at 5% level	Level of Significance
Treatment Period	648.9853	15	43.26569	1.072265548	1.75049637	NS
Dye Concentration	312.3766	8	39.04707	0.96771439	2.01642714	NS
Error	4841.975	120	40.34979			
Total	5803.337	143				

S-Significant**, NS**-Not significant

Therefore, biological methods involve the use of microorganisms such as a bacterium to turn pollutants into nontoxic and harmless substances to the environment. Biodegradation is the most environment friendly process as it does not require large amounts of energy and does not generate toxic substances (Wong, 2003). Decolourisation is a challenging process to the textile industry and a great potential for microbial decolourising system exists for achieving total colour removal within a few days.

Conclusion

Current investigation has confirmed the decolourisation of the azo dye, congo red by the isolate, *L.delbrueckii* under *in vitro* conditions. Bacteria from local dye environment are easily adapted to the environment or the bacteria originated from the dye contaminated soil of the local environment can develop enzymes for dye decolourisation. Therefore, treatment of wastewater contaminated with dye using *L.delbrueckii* is an effective method. Thus the study has confirmed the potential of *L.delbrueckii* in the decolourisation of the dye indicating its possible application in treatment of textile dye effluents.

Acknowledgments

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EFFECTS OF EFFLUENT FROM ELECTROPLATING INDUSTRY ON THE HISTOLOGY OF GILLS OF THE FRESHWATER FISH, *CYPRINUS CARPIO*

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Abstract

The present study evaluates the toxicity of electroplating industrial effluent (EIE) on the histopathological alterations of the gills of Cyprinus carpio.The 96 hour LC_{50} value of EIE was determined using Probit method and was found to be 0.128%. Fish were exposed to the chosen sublethal concentrations of 0.004, 0.007, 0.010 and 0.013% of EIE. After 7, 14, 21 and 28 days of exposure, the fish were taken out, sacrificed, the gills were excised out and the alterations in the structure of gills were observed after subjecting them to microtomy. The significant histological changes observed in the gills under light microscope includefusion of secondary lamellae, eroded respiratory epithelium, hyperplasia, hypertrophy, secondary lamellae with clubbed tip, eroded secondary lamellae, vacuolization, necrosis and complete erosion of secondary lamellae.The degree of damage in the gills was found to be EIE concentration and exposure period dependent.

Keywords*: Electroplating industrial effluent, histopathological alterations, gills, Cyprinus carpio.*

Introduction

Pollution of water resources is the most common complaint which has engrossed the greatest attention through the nations (Boyd and Tucker, 2000). Industrial wastes are the major sources of water pollution in addition to sewage, agricultural runoff and domestic wastes. Industries are mainly accountable for the release of heavy metals into the environment through their wastewater. Discharge of untreated effluents from industries has led to severe impact on ecological balance. Effluent of electroplating industry containing heavy metals put forth a huge impact on aquatic organisms (Kaur and Kaur, 2006). Heavy metals are hazardous to many organisms even at very low concentrations and cause several disorders in organisms. Water pollutants can stimulate biochemical, histological and morphological changes in fish tissues which can impair fish quality (Kaoud and El-Dahshan, 2010). The histopathological technique provides a real picture of the toxic effects of contaminants and their involvement in the vital functions of animals (Athikesavan *et al*., 2006). The exposure of fish to contaminants has induced a number of lesions in gill, liver, intestine and kidney (Pantung *et al*., 2008).

Gills are involved in respiration, osmoregulation, acid-base balance and nitrogenous waste excretion (Thopon *et al*., 2003), which also make them extremely sensitive to water contamination. Gills serve as a good indicator of water quality. They are sensitive to change in the water components and to any physical and chemical alterations of the aquatic medium as gill filaments provide a large surface area for direct and continuous contact with contaminants in water (Au, 2004). Acid and heavy metal pollution alter cell structure and persuade desquamation to lamellar epithelium and cause filament epithelium hyperplasia (Ayoola, 2008a). There are several reports of histopathological changes in fish exposed to organic compounds and heavy metals (Prashanth, 2011; Ganeshwade *et al*., 2013). Gills of *Labeo rohita* exposed to tannery effluent revealed fusion and clumping of primary lamellar epithelium (Fanta *et al*., 2003). Al-Mansoori (2004) reported copper and lead causing hyperplasia and fusion of lamellae in the gills of juveniles of *Carassius carassius*. Griffitt *et al*. (2007) noticed alterations in the morphology of

gills in *Danio rerio*treated with copper nanoparticles. Ogundiran *et al*. (2009) examined lesions in the gills of *Clarias gariepinus* subjected to soap and detergent effluents. Bhatkar (2010) investigated histological changes, fusion of secondary lamellae, moderate basal hyperplasia, epithelial lifting and secretion of mucus in gills of the Indian major carp, *Labeo rohita* when assessing the effects of chromium, nickel and zinc. Navaraj and Kumaraguru (2013) observed severe gill damage in Tilapia (*Oreochromis mossambicus*) exposed to electroplating industrial wastewater. Hassaninezhad*et al*. (2014) assessed the pathological responses of gill in yellowfin seabream under mercury exposure. In fish, metals absorbed into the gill tissues and localized in the membrane system of each cell induced the pathological changes (Mitsoura *et al*., 2013). Thus, gill histology is extensively used as an indicator of environmental pollution. Therefore, the present investigation is to highlight the effects of sublethal concentrations of electroplating industrial effluent on the histology of gills of the freshwater fish, *Cyprinus carpio.*

Materials and Methods

Healthy *Cyprinus carpio,* weighing 25±5g, were collected from a local fish farm and they were acclimated to laboratory conditions in aerated dechlorinated tap water for fifteen days in fibre tanks (150 L capacity). The effluent from electroplating industry was collected and transported immediately to the laboratory and stored in a refrigerator.The median lethal concentration (LC_{50}) values for 24 (0.203%), 48 (0.158%), 72 (0.132%) and 96 (0.128%) hours exposure to electroplating industrial effluent were calculated using probit analysis.For histopathological studies, fifty fish were selected and divided into five groups. First group served as control while other groups were exposed to the selected sublethal concentrations of 0.004, 0.007, 0.010 and 0.013% of electroplating industrial effluent for twenty eight days. Each group housed ten fish (n=10) and the effluent concentrations in the test groups were renewed every day.Both the control and the experimental fish were taken out and sacrificed after 7, 14, 21 and 28 days. The gills from fish of each sublethal concentration and control were excised out and rinsed in fish saline for removing the bloodstains. Gills were fixed in Bouin's solution for twelve hours and then dehydrated through an increasing gradient of alcohol series 30, 50, 70, 80, 90 and 100% for thirty minutes each. They were later dried in acetone, and cleared in xylene for thirty minutes. The processed tissues were infiltrated by embedding in paraffin wax. After this, thin sections were cut to a five micron thickness with a rotary microtome and the sections were mounted on a slide. The sections were stained with haematoxylin and counterstained with eosin. The sections were later observed under the lightmicroscope and photographed. The changes in the histology of gills were observed in the treated fish and compared with that of the fish from control set.

Results and Discussion

The histopathological effects of electroplating industrial effluent on the gills of *C. carpio* after 7, 14, 21 and 28 days are presented in Plate 1 to 17. The gill of control fish is with intact primary lamellae and secondary lamellae. The primary gill lamella is a flat leaf like structure with a rod like supporting axis in the centre and a row of secondary gill lamellae on either side of it which are free from each other and equally spaced (Plate 1). Minor alterations such as enlargement of primary lamella, erosion of respiratory and interlamellar epithelium from secondary lamellae and hypertrophic nuclei were noticed in gill of *C. carpio* treated with electroplating effluent after seven days (Plate 2 to 5). The changes like fusion of secondary lamellae, bending at the tip, shortening with clubbed tip, hyperplasia, hypertrophy, erosion of respiratory epithelium, and vacuolization in primary lamellae were noticed in gill after fourteen and twenty one days of treatment (Plate 6 to 13). More pronounced changes were noticed in gills of fish treated with high concentrations of effluent for twenty eight days. In twenty eight days treated fish, severe damages like necrosis, vacuolization leading to gap in primary lamella and complete erosion of secondary lamellae were noticed (Plate 13 to 17).The degree of damage was EIE concentration and exposure period dependent.

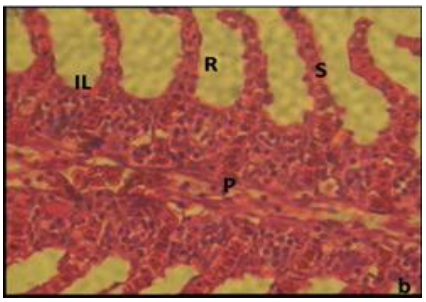

Plate 1 Sections Through the Gill of Untreated Cyprinus Carpio

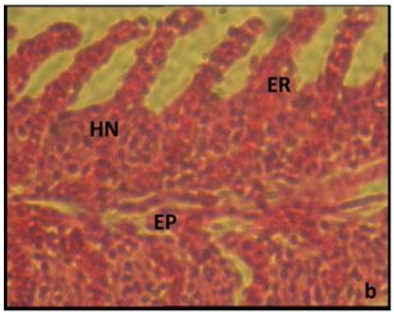

Plate 2 Sections through the gill of 0.004% of EIE treated fish after 7 days.

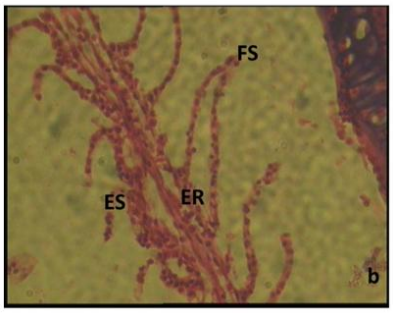

Plate 3 Sections through the gill of 0.007% of EIE treated fish after 7 days.

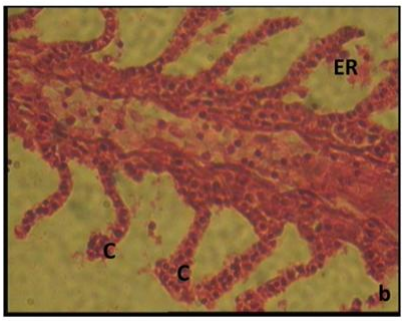

Plate 4 Sections through the gill of0.010% of EIE treated fish after 7 days.

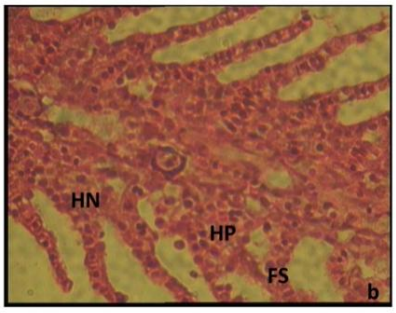

Plate 5 Sections through the gill of 0.013% of EIE treated fish after 7 days.

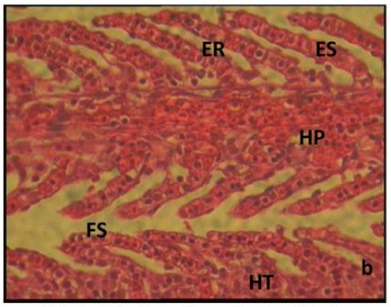

Plate 6 Sections through the gill of 0.004% of EIE treated fish after 14 days.

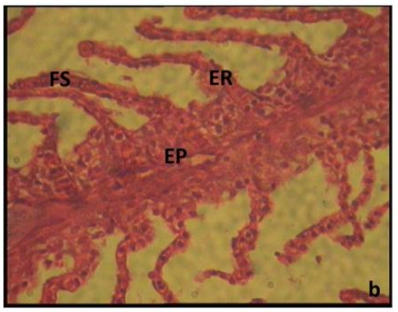

Plate 7 Sections through the gill of 0.007% of EIE treated fish after 14 days.

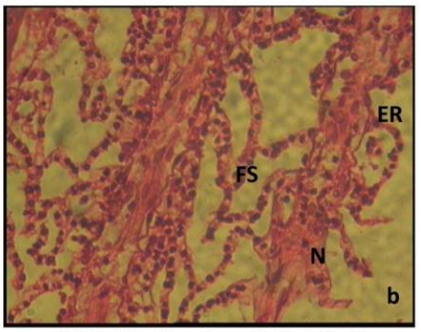

Plate 8 Sections through the gill of 0.010% of EIE treated fish after 14 days.

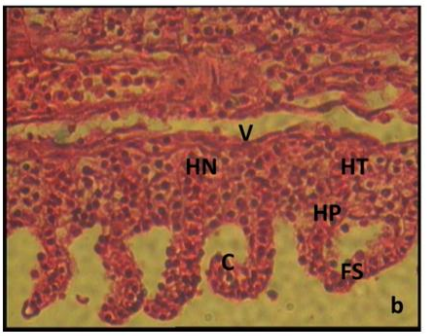

Plate 9 Sections through the gill of 0.013% of EIE treated fish after 14 days.

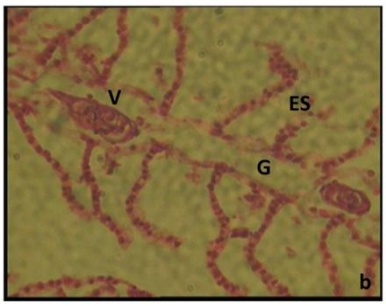

Plate 10 Sections through the gill of 0.004% of EIE treated fish after 21 days.

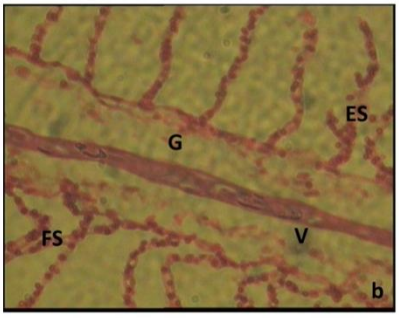

Plate 11 Sections through the gill of 0.007% of EIE treated fish after 21 days.

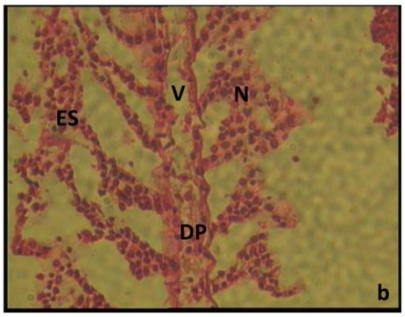

Plate 12 Sections through the gill of 0.010% of EIE treated fish after 21 days.

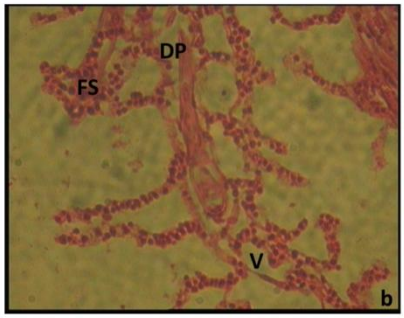

Plate 13 Sections through the gill of 0.013% of EIE treated fish after 21 days.

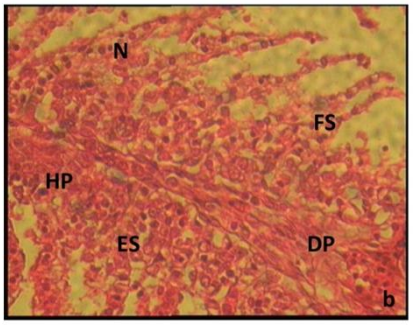

Plate 14 Sections through the gill of 0.004% of EIE treated fish after 28 days.

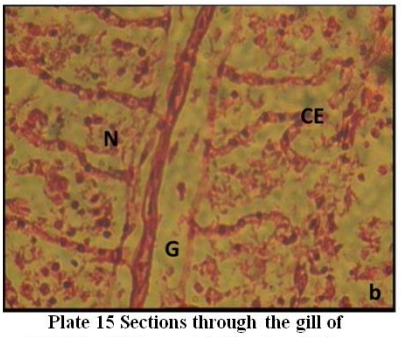

Plate 15 Sections through the gill of 0.007% of EIE treated fish after 28 days.

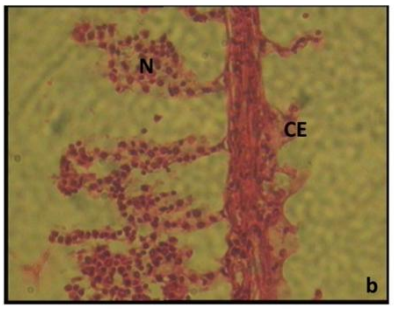

Plate 16 Sections through the gill of 0.010% of EIE treated fish after 28 days.

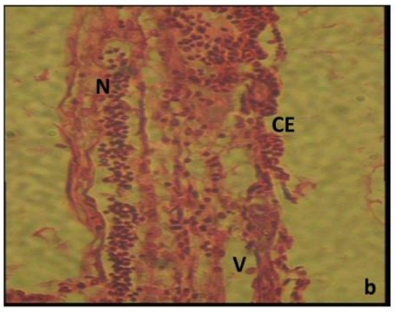

Plate 17 Sections through the gill of 0.013% of EIE treated fish after 28 days.

P – Primary lamella
S – Secondary lamella
IL – Interlamellar epithelium
R – Respiratory epithelium
ER – Eroded Respiratory epithelium
EP – Enlarged primary lamella
HN – Hypertrophic nucleus
FS – Fusion of secondary lamellae
ES – Eroded secondary lamella
HP – Hyperplasia
C – Clubbed tip
N – Necrosis
V – Vacuolisation
SS – Shortened secondary lamella
G – Gap
DP – Damaged primary lamella
HT – Hypertrophy
CE – Complete erosion of secondary lamella

Similar findings including hyperplasia and necrotic changes in secondary gill filaments leading to vacuoles, fusion and clubbed tips of gill lamellae were found in *O. mossambicus* exposed to electroplating effluent (Navaraj and Kumaraguru, 2013). Chezhian *et al*. (2010) reported mixed effluent of SIPCOT industrial estate showing lifting up of the epithelium, swelling, hyperplasia, hypertrophy, degenerative changes of epithelial cells and fused lamellar filaments, necrosis and disintegration of epithelial cells of lamellae in gills of *Lates calcarifer*. Edema, hyperplasia, fusion and desquamation in lamellae were found in *O. niloticus* exposed to heavy metals (Kaoud and El-Dahshan, 2010). Palanisamy*et al*. (2011) observed significant pathological lesions like hyperplasia, desquamation, and fusion of secondary lamellae and congestion of blood sinuses due to electroplating effluent in gills of *Mystus cavasius.* The current pathological findings are also in line with the report in *Channa punctatus* exposed to mercury (Gupta and Dua, 2002),*Oncorhynchus mykiss* treated with nickel (Pane *et al*., 2004), *Rasbora daniconius* treated with paper mill effluent (Pathan *et al*., 2010), *Labeo rohita* subjected to cadmium (Muthukumaravel *et al*., 2013) and red tilapia to lead (Aldoghachi *et al*., 2016).

The present investigation revealed the fusion of adjacent secondary lamellae due to hyperplasia which decreased the respiratory capacity, impaired the diffusion of respiratory gas in gills, and decreased free gas exchange. Hossam and Fagr (2007) also reported damage in gill due to hexavalent chromium inhibiting gaseous exchange of the gills. Navaraj and Kumaraguru (2014) have also observed the malfunctioning of gills owing to metals in electroplating industrial effluent which reduced the oxygen consumption rate of *O. mossambicus*. The fusion of gill lamellae reduces the respiratory area, and thus the fish fails to extract sufficient oxygen for metabolic activities (Olojo *et al*., 2005).

Epithelial lifting, hyperplasia and hypertrophy of the epithelium serve as a defense function. But these changes increase the distance between the environment and the blood and which serve as a barrier to the entry of pollutants (Fernandes and Mazon, 2003). As gills are the respiratory and osmoregulatory organs of fish, the pathological alterations in the gill might inhibit the respiratory function of the gills by reducing respiratory surface area resulting in hypoxia and respiratory failure (Yasser and Naser, 2011). Therefore, gill histology is widely used as one of the indicators of environmental pollution (Chavan and Muley, 2014).

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BIODEGRADATION OF LOW DENSITY POLYETHYLENE USING MICROORGANISMS

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Abstract

Polyethylene is linear chain with a thickness of 60 µm. It is an organic polymer and causes deleterious effect on earth. Microbial degradation is a natural process, when living organisms were able to depolymerize these synthetic polymers to monomers. Soil samples amended with polyethylene strips were enriched, serially diluted, plated on agar mediumand among the isolates one strain (PE-1) was characterized morphologically and biochemically found to be Bacillus cereus. The optimum pH, temperature was found to be pH 7 and 37^{0}C of temperature. BATH assay with Bacillus cereus showed higher hydrophobicilly. The percentage weigh loss of LDPE with Bacillus cereus due to degradation 14.29%. Whereas when LDPE was pretreated with UV exposure, revealed 35.71% weight loss. Simultaneously when LDPE was supplemented with nonionic surfactants revealed 50% degradation. On exposure to immobilized condition weight loss of polyethylene indicated 57.14%. FTIR study revealed prominent peaks of 1037.7cm-1 occurred due to symmetric CH vibration. Another wave length was of 1462.04 cm-1 exhibited a significant reduction in carbonyl peaks.

Keywords: *Polyethylene degradation, Bacillus cereus, Bacterial adhesion to hydrophobicity (BATH),LDPE, and FTIR.*

Introduction

Polyethylene is an organic polymer made of several monomer subunits. Polyethylene contains a lot of carbon and hydrogen atoms. It is of primary use in packaging materials such as plastic bags, plastic films, geomembranes, containers, including bottle, etc. The worldwide utility of polyethylene is expanding at a rate of 12% per annum and approximately 140 million tones of synthetic polymer are produced worldwide each year. They are of two types of polyethylene LDPE and HDPE and both compounds have very different physical properties. (Shimao, 2001)

It was estimated that it takes about 300 years to degrade LDPE films with thickness of 60µm (Ohtake*et al*.,1998). Being xenobiotic compounds, polyethylene are resistant to degradation and constitute about 5-8 percent of the dry weight of municipal solid waste. The polyethylene bags do not allow water and air to go into the earth. which cause infertility of soil. It causes depletion of underground water source. It causes danger to animal life. Several mega cities and coastal areas are affected by polyethylene blockage in drains causing flood. These problems cannot be solved by landfilling and incineration because these methods are expensive.

Incineration stimulates the emission of environmentally harmful gases such as nitrous oxide, sulphur dioxide, and CO_2. Hence biodegradation by microorganisms is an ecofriendly alternative.(Shoripritam Baral, 2014).

The breakdown of polyethylene caused by microorganisms can be of three different types:

1. A biophysical effect, in which cell growth can cause mechanical damage.
2. A biochemical effect, in which substance from the microorganisms can act on the polymer
3. Direct enzymatic action, in which enzymes from the microorganisms attack component of the plastic products, leads to splitting or oxidative breaking down.

Biodegradation is much simpler, economic and occurs in normal conditions of waste disposal without any extra prerequisites. (Crabbe,*et al*.,1994). Thus microbial degradation is the natural process where living organisms are able to depolymerize this synthetic polymers into monomers. The enzymes produced by the microbes are responsible for this biodegradation (Prabakaran, et al., 2013). Thus microbes are the best tool by which this ecological threat may be reduced. The heat resistance of a particular microorganism is needed to select process parameters such as temperature and time for inactivation of the target pathogen, (Chung *et al*., 2008).

Materials and Methods

Sample Collection

The municipal solid waste soil samples were collected from Madurai municipality. The soil samples were collected at a depth of 3-5cm and sealed in plastic bags immediately after sampling and then transported to the laboratory for the isolation of bacteria.

Enrichment of Municipal Solid Waste Soil with LDPE Strips

The collected municipal solid waste soil samples were enriched with LDPE strips in the laboratory for 20 days.

Serial Dilution Method

After enrichment, 1g of the enriched soil sample was weighed using electronic balance and then it was mixed with 9ml of distilled water and shaken well until it gets dissolved, then serially diluted upto 10^{-8} dilution. About 0.1ml diluted sample from 10^{-4}, 10^{-5} and 10^{-6} dilutions were spread plated on nutrient agar plates. The plate were incubated at 37°C/24 hours. After incubation, the colonies with different morphological characteristics were selected and re-streaked on nutrient agar to obtain pure colonies. The colonies thus obtained where used for further analysis.

Screening of Polyethylene Degrading Microorganisms by Clear Zone Method

The polyethylene degrading microorganisms were screened by using mineral salt medium amended with 0.1% of LDPE powder. After which the medium was sterilized at 121°C and pressure for 15lbs for 20 minutes. About 20ml of sterilized medium was poured before cooling into the plates. The isolated organisms were inoculated on polymer containing agar plates and then incubated at 25- 30°C for 2-4 weeks. The organisms producing zone of clearance around their colonies were selected for further analysis.

Morphological Characterization of Isolates

Morphological characteristics such as abundance of growth, pigmentation, optical characteristics, size and shape of the colony were studied on different agar media.

Biochemical Characterization

The efficient LDPE degrading microorganisms were characterized by Indole, Methyl red, Vogues Proskauer test, Citrate utilization test, Triple sugar Iron test, Starch Hydrolysis, Gelatin Hydrolysis, and Nitrate Reduction test.

Evaluation of Bacterial Hydrophobicity

In the BATH test, bacteria were cultured in NB medium until the mid-exponential phase, centrifuged and washed twice with phosphate-urea-magnesium (PUM) buffer containing (g l^{-1}): K_2HPO_4,0. 34; KH_2PO_4, 0.14; Urea, 0.036; $MgSO_4$ $7H_2O$, 0.04. The washed cells were resuspended in PUM buffer to an O.D.(540nm) value of 1.0-1.2. Aliquots (1.2ml each) of this suspension were transferred to a set of test tubes, to which increasing volumes (ranging: 0-0.2ml) of hexadecane were added. The test tubes were shaken for 10min and then allowed to stand for 2min to facilitate phase separation. The turbidity of the aqueous suspensions was measured at 540nm. Cell-free buffer served as the blank.The percentage of hydrophobicity was calculated using the formula:

Hydrophobicity % = OD of initial bacterial suspension – OD of aqueous phase ×100

Initial Bacterial Suspension

Consortium Preparation

About 1ml of the inoculum of isolated polyethylene degrading microorganisms or loopful of microbes was inoculated in nutrient broth and incubated at 37°C. This mixture of microbes was centrifuged at 3000 rpm and the pellet was used as consortium inoculum. Biodegradation of polyethylene bags by a mixed bacterial consortium in five different systems was tested.

Biodegradation in Synthetic Medium

Biodegradation tests were performed with polyethylene film LDPE cut into 1×1 cm and placed in an oven at 70°C for 20 days in order to achieve material disinfection. The strips were then added to flask containing sterilized mineral salt medium containing 5% bacterial inoculum separately in different flasks and incubated at 37°C for 60 days. The films were recovered after desired intervals for monitoring the changes brought about by the bacterial action. Simultaneously a set of control experimental flasks were performed without bacterial culture. Biodegradation was assessed by estimating the changes in weight and compared with control.

Biodegradation Studies of UV Treated LDPE

The polyethylene LDPE strips were subjected to UV exposure (UV light, 254 nm wavelength for about 60 hours). The polyethylene films were disinfected in 70% ethanol and air dried for 15 minutes in laminar flow hood after which films were transferred to mineralsalt medium containing 5% bacterial inoculum and incubated at 37°C for 60 days. Biodegradation was assessed by estimating the changes in weight and by using FTIR.

Biodegradation Studies of Polyethylene Using Non-Ionic Surfactant Supplementation

In the experiment, non-ionic surfactant (Tween-80) was added to the mineral salt medium at a concentration of 0.01% (v/v) to test the effect of these substances on the colonization and biodegradation of polyethylene. The flasks were incubated at 37°C for 60 days. The samples were withdrawn at desired intervals and the biodegradation was to be assessed and compared with ethanol.

Biodegradation of LDPE Using Immobilized
Preparation of Immobilized Beads

The bacteria solution was mixed with sodium alginate solution (2%) in 1:1 ratio. The bacteria-alginate mixture was added drop wise into calcium chloride (0.2m) solution with continuous shaking at 4°C. As soon as the drop of bacteria-alginate solution mixed with $Cacl_2$ solution, sodium ions of alginate were replaced by the Ca^{2+} ions of $Cacl_2$ solution, which finally formed Ca-alginate beads. The beads thus formed were washed 3-4 times with deionized water and finally with Tris-HCL buffer of pH 7.5. These beads were dried and weighed for further degradation studies.

Biodegradation of LDPE by Immobilized Beads or *Bacillus cereus*

Biodegradation of LDPE by immobilized beads of individual and consortium of were performed in 100ml of basal mineral salt medium with 1g of LDPE strips. The broth was inoculated with 1g of immobilized beads of microorganisms. After inoculation, the flasks were kept at 30°C at 150rpm for 60days and the process of degradation was monitored.

Determination of Dry Weight of Residual Polyethylene

To facilitate accurate measurement of residual polyethylene weight, bacterial biofilms were washed off from the polyethylene surface with 2% (v/v) sodium dodecyl sulfate (SDS) overnight, followed by rinsing with distilled water (10).

Weight loss % = Initial weight-Final weight ×100

Initial weight

Optimization of Polyethylene Biodegradation

Effect of pH

The effect of pH on the ability of *Bacillus cereus* to utilize polyethylene as a sole source of carbon and nitrogen was determined by supplemented Mineral salt medium (EM) with 0.1% of polyethylene at different pH values (6, 6.5, 7, 7.5), in an attempt to determine the suitable pH value, then cultures were to determine the shaker incubator (180rpm) at 30˚C for 7days. The optimum pH value was employed in the subsequent experiment.

Effect of Temperature

To determine the effect of temperature on the ability of *Bacillus cereus* to degrade polyethylene, Mineral salt medium (EM) (pH 6) supplemented with 0.1% of polyethylene films was inoculated and incubated in shaker incubator (180 rpm) at different temperatures 30, 37 and 45˚C for 7days. Optimal temperature was subsequently employed, depending on the growth density measurement.

FTIR

The changes in the polyethylene structure following UV irradiation and heating and subsequent incubation with bacterial was analyzed by FTIR spectroscopy.LDPE samples degraded by microorganisms were collected after 60 days of incubation. The LDPE residue was air dried and used for FTIR analysis LDPE samples were milled with pottassium's bromide (KBr) to form a very fine powder. This powder was then compressed into a thin pellet which can be analyzed KBr is also transparent in the IR.

Four types of polyethylene samples were analyzed

- Untreated control
- Non-irradiated polyethylene bags in the synthetic medium
- UV-irradiated polyethylene bags in the synthetic medium
- Non-ionic surfactant polyethylene bags in the synthetic medium
- Immobilization polyethylene bags In the synthetic medium

Protein Profile by SDS-PAGE

The LDPE degradation cultures were used to identified on protein separation by SDS-PAGE.

Result

The present study was focused with much attention to isolate and to identify the low density polyethylene degrading bacterium from municipal solid waste, Madurai. The efficient polyethylene degrading microorganism was used to check the in-vitro polyethylene degradation for a period of 60 days. In addition, the change in the polyethylene strips during degradation was also monitored.

Enrichment and Isolation of Polyethylene Degrading Microorganisms

The soil samples collected from the municipal solid waste Madurai was enriched in the laboratory with LDPE strips for a period of 25 days. After enrichment, the soil sample was serially diluted and plated on nutrient agar medium. Among different organisms isolated, only one isolate PE^{-1} was selected for further study.

Identification of the Efficient Bacteria

The PE^{-1} isolate was a gram positive rod shaped, motile endospore forming bacteria with colonies of large irregular and white colored on nutrient agar plate. Indole was not produced. It fermented glucose with high concentration of acid end products in methyl red test Acetoin was not produced in VP test. The organism utilized citrate as its sole carbon source. It fermented glucose but not lactose in TSI test. Nitrates were reduced to nitrites. Gelatin and starch were hydrolyzed. From the results, obtained it was found out that the organism PE^{-1} belonged to the genus *Bacillus cereus.* The result were compared with the Bergey's manual of determinative bacteriological. Carbohydrate fermentation test was performed for the isolate PE^{-1} with five different sugar such as dextrose, mannitol, sucrose, lactose and fructose. The result depicted in (Table.1) revealed that the organism ferment sugars like sucrose and fructose. Dextrose, mannitol and lactose were not fermented by *Bacillus cereus*.

Optimization of Polyethylene Degradation
Effect of pH

Mineral salt medium was prepared at different pH values (6, 6. 5, 7, and 7.5) to determine the optimum pH required for growth of *Bacillus cereus* on polyethylene. The obtained results as shown in the Figure.1 elucidated that an optimum growth was occurred at pH-7 with the optical density with 0.74 after 7 days.

Effect of Temperature

Bacillus cereus was grown and incubated at different temperature (25, 30˚, 37˚, and 45C). The result shown in (Figure.2) pointed out that optical density of bacterial growth at 37˚C with 0.67 after seven days of incubation which was suggested as the optimum temperature for bacteria growth. Relative result of bacterial growth was recorded at 30^{o}C where as, at 45^{o}C bacterial growth was lower than that of other incubation temperatures.

Hydrophobicity

Bacterial cell surface hydrophobicity was estimated by the bacterial adhesion to hydrocarbon (BATH) test, which is based on the affinity of bacterial cell for an organic hydrocarbon such as hexadecane. The more hydrophobic of the bacterial cells, the greater their affinity for the hydrocarbon, resulting in transfer of cells from the aqueous suspension to the organic phase and a consequent reduction in the turbidity of the culture. In the present study, the BATH assay result depicted in figure.3 showed the higher hydrophobicity of *Bacillus cereus*. The addition of bacterial cell to hexadecane was efficient even at the lowest concentration of the hydrocarbon resulting in a reduction of 83, 78, 72, and 67% for 0.08, 0.12, 0.16, and 0.20ml, of hexadecane respectively.

Determination of Mass Weight Loss of Polyethylene Film after 60 days of Incubation with *Bacillus Cereus*

Biodegradation of LDPE was monitored for a period of 60 days and the results are presented in (Figure.4 and Plate: 1 upto 5). The percentage of weight loss due to degradation was determined by subtracting the weight of the samples taken out on a particular day. It was observed that the percentage weight loss of LDPE strips when treated with *Bacillus cereus* revealed weight loss of 14.29%. Similarly, when the polymer LDPE was pretreated with UV exposure exhibited a percentage weight loss of 35.71%. On exposure to *Bacillus cereus* supplemented with Tween 80 showed remarkable increase in biodegradation of LDPE exhibiting 50%. When the LDPE strips were treated with immobilized *Bacillus cereus,* the weight loss of polyethylene was found to increase with 57.14%. The percentage of weight loss of LDPE polymer can be attributed to the breakdown of carbon backbone due to the enzymatic cleavage by the bacterium *Bacillus cereus.*

Analysis of Degradation of LDPE Treated with Immobilized Cells of *Bacillus cereus* by FTIR

In the present study, the changes in the structure of (LDPE) with subsequent bacterial inoculation were analyzed by FTIR in the frequency range 4000-800cm^{-1}. The FTIR spectra of the untreated LDPE film incubated with bacterial isolate, *Bacillus cereus* was carried out. In the FTIR peaks indicated a peak value of 1037.7cm^{-1} indicating CH symmetric vibration. Another peak with a spectral wavelength of 1462.04 denote a significant reduction in carbonyl peak thus found with respect to the internal bond absorbance. It is interesting to note the

spectra better for untreated polyethylene does not show the presence of carbonyl groups whereas the inoculation of *Bacillus cereus* amended with polyethylene after degradation due to a cleavage of polymer by the enzymatic action and abiotic oxidation let to the appearance of carbonyl groups in the polymer.

This behavior is the characteristic of degradation of polymer and another peak with appearance of 1377.17cm^{-1} indicated the presence of aromatic B-O-CH_2-CH_3 group formation.

Table 1 Morphological and Biochemical Characterization of the Efficient Polyethylene Degrading Microorganism

S.No	Morphological and Biochemical Test	Observation
	Colony Morphology on Nutrient Agar	On nutrient agar it produced, white color, irregular and large colonies
1	Gram's staining	Gram positive rod
2	Motility	Motile
3	Spore staining	Endospores present
4	Citrate utilization test	Positive
5	Triple sugar iron test	Acid butt and alkaline slant
6	Nitrate reduction test	Positive
7	Gelatin hydrolysis	Positive
8	Starch hydrolysis	Positive
9	Dextrose	Negative
10	Mannose	Negative
11	Sucrose	Positive
12	Lactose	Negative
13	Fructose	Positive

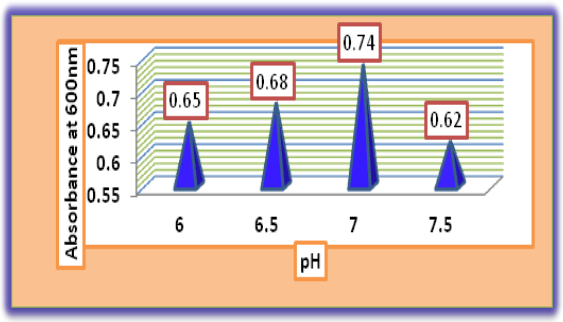

Figure 1 Effect of pH on Polyethylene Biodegradation by *Bacillus cereus* Growth in MSM Containing 1% Polyethylene in Orbital Shaker (180rmp, 30°c) for 7 Days

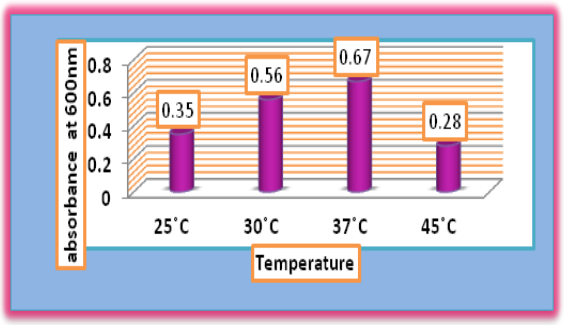

Figure 2 Effect of Temperature on Polyethylene Degradation by *Bacillus cereus* Growth in MSM Containing 1% Polyethylene in Orbital Shaker (180rpm, 30°c) for 7 Days

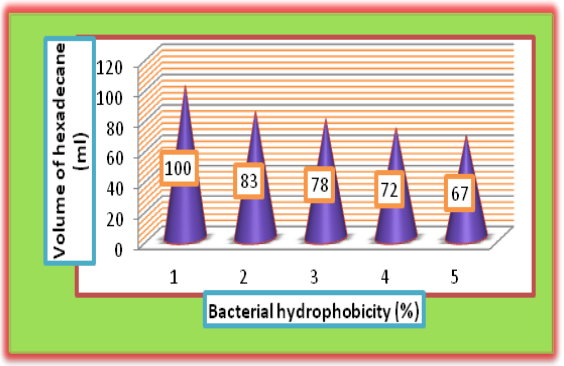

Figure 3 Hydrophobicity of Bacterial Isolates Determined by the Bacterial Adhesion to Hydrocarbon

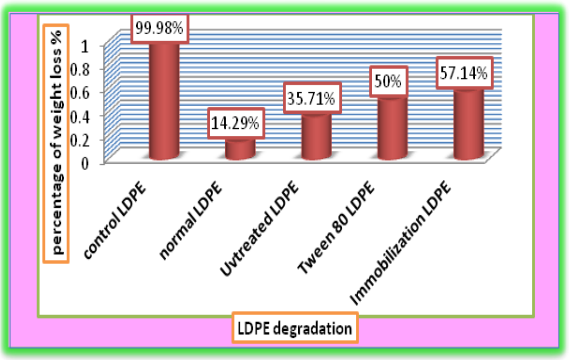

Figure 4 Determinationof Mass Weight Loss of Polyethylene Film After 60 Days of Incubation With *Bacillus cereus*

Plate 1 Biodegradation of LDPE Control Treated Flask without Inoculum

Plate 2 Biodegradation of LDPE by *Bacillus cereus* during 60 Days of Exposure

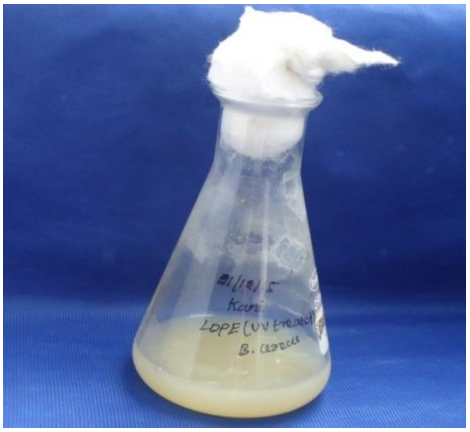

Plate 3 Biodegradation of UV Treated LDPE by *Bacillus cereus* during 60 Days of Exposure

Plate 4 Biodegradation of LDPE by Immobilized Beads of *Bacillus cereus* during 60 Days of Exposure

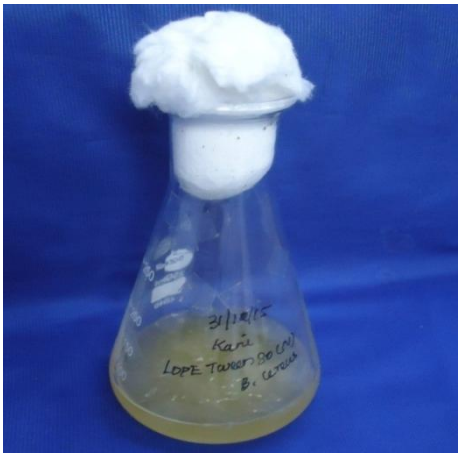

Plate 5 Enhancement of Biodegradation of LDPE by Supplementation of Nonionic Surfactant Tween 80 in Mineral Salt Medium during 60 Days of Exposure

Discussion

The polyethylene degrading microorganism was morphologically and biochemically characterized. PE-1 isolate was found to be gram positive rod shaped, fermented glucose and carbon sources. Gelatin were hydrolyzed, from the results obtained the PE-1 strain was found to be *Bacillus cereus*. The effect of pH and temperature optimized for the growth of microorganisms revealed optimum pH 7 and 37^{0}C of temperature. Bacterial cell surface hydrophobicity estimated by BATH assay revealed increased hydrophobicity even at low concentration. Our results were in total agreement with findings reported by Prabakaran et al., (2013). Biodegradation of LDPE was carried out for a period of 60 days.

Percentage weight loss due to degradation was checke out and LDPE strips treated with *Bacillus cereus*

showed 14.29%. Similarly LDPE pretreated with UV show weight loss of 35.71% on exposure of *Bacillus cereus* supplemented with tween-80 showed a remarkable increase of biodegradation exhibiting 50%. Whereas immobilized *Bacillus cereus* amended with polyethylene strips showed an enhanced degradation of 57.14% weight loss. Studied with FTIR. It is interesting to note that absence of carbonyl groups in untreated polyethylene(control). Degradation of polymer with a peak of 1377.17cm-1 indicated the aromatic CH_3 group formation, which occurred due to degradation.

Conclusion

Polyethylene is an organic polymer made up of several monomer supplements. Being xenobiotics they are resistant to degradation. Polyethylene strips amended with soil enriched serially diluted, plated and characterized morphologically and biochemically. It was later identified as *Bacillus cereus.* The optimum growth of microorganisms at an optimum pH was found to be 7 and temperature 37^0C. The percent weight loss of LDPE supplemented with non-ionic surfactant exhibited 54.1% compared to control. Whereas an exposure to immobilized condition weight loss of polyethylene indicated 57.14%. Similarly LDPE pretreated with UV exposure revealed 37.71% weight loss due to degradation. The degradation is further confirmed by appearance of prominent peaks which occurred due to symmetric CH vibration, indicating functional group modification.

Acknowledgement

I extend my sincere thanks to Co-ordinator T.S. Blessy Thayalin, Venture Institute of Biotechnology, Madurai.. I thank A. Soma Prabha, Research scholar, SBS, Madurai Kamaraj University for her kind help.

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BIODEGRADATION OF CYPERMETHRIN USING THE BACTERIUM, *ENTEROBACTER ASBURIAE*

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Abstract

The objective of this study was to isolate bacterial strains from agricultural fields that are subjected to the use of cypermethrin as pesticide, and to test its resistance to cypermethrin. Bacterial isolate that effectively resisted cypermethrin in varying concentrations from 100 to 2000 ppm was obtained as axenic cultures. The efficiency of the isolate to degrade cypermethrin was determined by measuring pH, CO_2, esterase activity, and biomass at appropriate intervals of the treatment period. The parameters of the free cells were compared against the immobilized cells of the isolate. The degradation was also observed by subjecting the treatment samples to UV-Visible spectrophotometry and HPLC analysis. The degradation efficiency was determined by statistical analysis. The bacterial isolate that exhibited potential for degradation was tentatively identified as ***Enterobacter asburiae,*** *by biochemical tests.*

Introduction

Cypermethrin is a non-systemic insecticide that is used to control a wide range of insects especially Lepidopterans, in fruits, vegetables, cereals, and various other crops (Roberts et al, 1998). It belongs to the second generation of synthetic pyrethroids which are more photostable and have greater stability in general. They are derivatives synthesized from naturally occurring pyrethrins, which are taken from pyrethrum, the oleoresin extract of dried *Chrysanthemum* flowers (Reigart et al, 1999). Their mode of action includes rapid penetration of insects by contact stomach action, interfering with nerve impulse transmission by acting on sodium channels, thus resulting in paralysis (Roberts et al, 1998). Deltamethrin and cypermethrin are commonly applied pesticides in Asian rice fields, especially targeting leaf-feeding insects such as rice leafholders. The damage caused by these pests are rather visible during early stages, provoking the farmers to spray the pesticide in large quantities often in formulations with other pesticides. These practices cause the emergence of either secondary pests such as brown planthoppers or pesticide resistant insects (Heong et al, 1997).

Synthetic pyrethroids are basically carboxylic esters whose acid and/or alcohol functional groups are optically active, and characterized by broad-spectrum high-level insecticidal activity. Their typical features include high hydrophobicity, resulting in low water solubility, high adsorptive ability to soil, rich stereochemistry, and high bioconcentration factor (BCF) to biota (Katagi, 2011; Gosselin et al, 1984). Lipophilicity and rapid biodegradation by vertebrates, including fish, mark the environmental fate of pyrethroids (Walker, 2008). Cypermethrin is classified by EPA as RUP (Restricted Use Pesticide) because of its toxicity to fish. While the compound itself is classified as moderately toxic, some of its formulations are categorized as Toxicity class III – slightly toxic. The oral LD_{50} value of cypermethrin in rats is 413 mg/kg dissolved in water. It is moderately toxic by dermal absorption or ingestion. It adversely affects the central nervous system, while high dermal absorption can cause numbness, tingling, itching, loss of bladder control, seizures and possible death. High dose of ingestion can cause nausea, prolonged vomiting, diarrhea, stomach pain that can progress to convulsions leading even to coma (Kamrin, 1997). The role of biological processes in the soil in degradation of the five pyrethroid insecticides permethrin, cypermethrin, decamethrin, fenpropanate, and fenvalerate was studied. The study showed that cypermethrin persisted in the autoclaved soil longer than

normal soil, indicating the role of microbial degradation (Chapman and Harris, 1981). In fact, bacterial degradation of pyrethroid pesticides measured by their half-lives was more rapid than the natural process in soil (Braganca et al, 2016). Misuse against wrong target organisms and frequent misconception among farmers that it can increase yield has caused the widespread use of the pyrethroids in agricultural fields more than necessary. This results in constant persistence of the pesticide in soils, sediments, and groundwater (Braganca et al, 2016; Heong et al, 1997). High sediment toxicity, and residual levels in dairy products are problems that arise due to abuse of pyrethroid pesticides.

Hence the present study has been designed to isolate a bacterial strain capable of degrading cypermethrin, and to test its efficiency of degradation.

Materials and Methods

Collection of Samples

The soil samples were collected from the agricultural field where cypermethrin was applied. The field was near Kombadi, about 18km away from Madurai city. The soil samples were collected in sterile containers and brought to the laboratory within six hours for bacteriological analysis. The selected pesticide cypermethrin was obtained from an agrochemical company near Nelpettai, Madurai.

Isolation of Pesticide-Resistant Bacteria

The soil samples were serially diluted up to 10^{-7}, plated on nutrient agar plates, and incubated at 37° C for 24 hours. The isolated strains were then tested for their resistance to the pesticides, by inoculating on minimal medium containing different concentrations of 100, 200, 500, 1000, 1500 and 2000 ppm of the pesticide, cypermethrin.

Identification

The taxonomic identification of bacteria was identified by Gram's staining and biochemical identification.

Degradation Efficiency

The isolate was inoculated on minimal broth containing different concentrations of cypermethrin like 500, 100, 1500, 2000, and 2500 ppm. The flasks were then incubated at room temperature for a period of 16 days and the samples were then subjected for the estimation of the pH, carbon dioxide, esterase activity, biomass, UV – Visible spectrophotometry and HPLC analysis.

pH

The pH of the sample was measured for the different test concentrations using pH meter on alternate days for a period of 16 days.

Carbon Dioxide Estimation

Free CO_2 was determined by titrating these samples using a strong alkali (NaOH) to pH 8. Sodium hydroxide was prepared in CO_2 free distilled water (boiled) from which 50 ml was diluted in 1000 ml of CO_2 free distilled water and titrated against 100ml of the sample. Phenolphthalein was used as the indicator and the endpoint is by the appearance of pink color. The free CO_2 was estimated by the formula

$$\text{Free } CO_2 \text{ (mg/l)} = \frac{(Volume\ X\ N) of\ NaOH\ X\ 1000\ X\ 44}{Volume\ of\ sample}$$

Esterase Activity

200 µl of the sample was added to 200 ml of naphthyl acetate solution. The enzyme reaction was allowed to run for 30 minutes. To this 500µl of the fast garnet disodium lauryl sulfate was added and kept for 5 minutes. After 5 minutes the absorbance was measured at 560nm. Blank used had 200µl of distilled water with 2ml of naphthyl acetate and 500µl of the dye. A standard graph was obtained from doing the same with different concentrations of naphthyl acetate.

Biomass

Biomass of the sample was analyzed by the turbidometric method. The absorbance of the test samples on alternate days was measured using a colorimeter at 600nm for 16 days (Kannan, 2002)

Immobilization

The 0.1 ml of the overnight culture of the isolate was mixed well. This was then placed as drops on calcium chloride to produce immobilized cells. The overnight culture was used as the free cells. Both the immobilized and free cells were then subjected to degradation experiments and were analyzed on alternate days for pH and CO_2.

UV-Visible Spectrophotometry

The samples containing the minimal medium 500 ppm of cypermethrin and the inoculum was taken on the 0^{th}, 10^{th} and 12^{th} day, and subjected to UV-Visible spectrophotometry. The absorbance was noted on different wavelengths.

High-Pressure Liquid Chromatography

The samples containing minimal medium, inoculum, and 500 ppm concentration of the cypermethrin were taken on the 0th and 10th day. They were dissolved in an equal amount of methanol and were subjected to HPLC analysis by UV detection.

Statistical Analysis

Two-way ANOVA for the parameters pH, carbon dioxide, esterase, activity, and biomass was done using MS Excel package. Variability was considered significant only when the statistic value was greater than the tabulated value at p<(or)=0.05.

Results

The bacterial strain isolated from soil sample was found to be gram negative coccus and on further testing on specialized media and subjecting it to biochemical analysis, the isolate was tentatively identified as *Enterobacter asburiae*.

Figure 1 implicates the changes in the pH of the medium during the degradation of cypermethrin by *Enterobacter asburiae.* pH decreases for a time of about 10 days after which it is constant. One of the end products of the cypermethrin degradation is CO_2, which was estimated by titration. The free CO_2 present in the minimal medium was found to increase constantly (Fig 2), and the maximum CO_2 was released by the minimal medium containing 500ppm cypermethrin.

As seen in Fig 3, the amount of naphthyl acetate utilized by the esterase enzyme increases until the 12th day and diminishes afterward. Again, maximum enzyme activity was seen in the minimal medium containing 500 ppm cypermethrin.

Biomass measurement by turbidity at 600nm also exhibited a similar case where maximum turbidity was observed in the case of minimal medium containing 500ppm cypermethrin (Fig 4). The degradation of cypermethrin is efficient at 500ppm of concentration, but an increase in the concentration of pesticide induces an increased lag phase in the growth curve of the bacterium. Similarly, degradation efficiency was maximal on the 12th day from inoculation.

From the figures 5 (a) & (b), it is visible that biodegradation of cypermethrin is faster and effective with immobilized cells rather than free cells. The decrease in pH and increase in the amount of carbon dioxide released by immobilized cells is higher than that of compared to free cells.

HPLC analysis showed the formation of peaks that were characteristic of the presence of intermediate compounds produced during the degradation of cypermethrin (Fig 6 a & b).

The results were subjected to two-way analysis of variance for the factors of pH, varying treatment period, and cypermethrin concentration. The factors were found statistically significant for cypermethrin concentration at 5% level.

Discussion

Recent studies have revealed that the microbial degradation process to detoxify pesticide contaminants can be effectively used to overcome the pollution problems. Soil bacteria with the ability to degrade several pesticides have been isolated from soil showing enhanced biodegradation. They include a metamitron degrading *Rhodococcus* sp (Parekh et al, 1994), a chlorpyrifos-degrading *Flavobacterium* sp (Ralebits et al, 2002), and an iprodione-degrading *Anthrobacter* sp (Mercadier et al., 1996). In further studies with the soil, two *Pseudomonas putida* strains were isolated, which were able to utilize diclofop-methyl as a source of carbon source (Karpouzas and Walker, 2000). It has been suggested that cultures of bacteria with the ability to degrade specific compounds can be used for bioremediation of pesticide-polluted sites.

At high concentrations of cypermethrin, the appropriate catabolic enzyme of *Pseudomonas* sp. gets repressed. The possible explanation that the microorganism may need an acclimation period to induce the necessary degradative enzymes and maybe because of this reason the prolonged lag phase observed at high concentrations of the pesticide. From these findings, it may be concluded that the isolated bacterial strain could be useful for the treatment of pesticide contaminants in industrial effluent and can detoxify agricultural waste (Jilani and Khan, 2004).

From the decrease in the pH it can be understood that the acidity of the medium increases i.e., during the process of degradation, an acid was formed as a product and thus the decrease in the pH was noted in the medium. The cypermethrin during degradation initially converts into 3-phenoxy benzoic acid and this may be the cause for the decrease in the pH of the medium. During the process of degradation of cypermethrin, after formation of 3-phenoxy benzoic acid in a particular amount it will then be converted into carbon dioxide. This is why the formation of carbon dioxide is observed only after six days of degradation.

The turbidity of the medium indicates the growth of the organism in the medium. From the increase in the turbidity of the medium, it can be inferred that the organism uses cypermethrin as a source of energy as it is grown on the minimal medium with cypermethrin and hence causing the degradation of cypermethrin.

Pseudomonas stutzeri strain S1 was found to degrade cyfluthrin, a synthetic pyrethroid under aerobic conditions (Saikia et al, 2005). The microbial enrichments with *Pseudomonas fluorescens*, *Achromobacter* sp and *Bacillus cereus,* were able to transform fenvalerate, fluvalinate, fastac and permethrin (Maloney et al, 1988).

The microorganisms exposed to the synthetic chemicals have evolved and acquired the ability to utilize some of them. Bacteria of several different genera have been shown to degrade xenobiotics. Most of the xenobiotic degrading microorganisms harbor plasmids which code for the catabolic genes. By understanding the biochemistry and the genetics of the plasmid-borne degradation and by using the recombinant DNA techniques, it is possible to characterize the appropriate genes and transfer them to constructed improved strains with enhanced ability for degradation of several toxic compounds (Chaudry and Chapalamadugu, 1991).

Cypermethrin is not soluble in water and has a strong tendency to adsorb to soil particles. It is therefore unlikely to cause groundwater contamination. In soils, cypermethrin photodegrades with a half-life of eight to ten days. Its major photodegradation products are DCVA and phenoxy benzaldehyde and phenoxy benzoic acid. It also undergoes microbial degradation under aerobic conditions. Under the laboratory conditions, cypermethrin degrades more rapidly on sandy clay and sandy loam soils than that of dry soils, and in soils low in organic material. In aerobic conditions, its soil half-life is two to eight weeks. Cypermethrin is more resistant under anaerobic conditions. In pond water and in laboratory degradation studies, pyrethroid concentrations decrease rapidly due to sorption of sediment and suspended particles (USEPA, 2000).

Immobilization is a process done to enhance the activity of the cell or an enzyme and in the present study when the free cells were compared with the immobilized cells, the degradation was effective and rapid in case of immobilized cells.

In the analysis of UV visible spectrophotometry, the peak formed by the 10^{th} and 12^{th} days' samples were steeper when compared to 0^{th} day's sample and this may indirectly indicate that there is a breakdown of cypermethrin and formation of different intermediate compounds. Pyrethroids and transformation products were identified and quantified by reverse phase HPLC. Quantification and identification of pyrethroids and transformation products by HPLC were based on retention times and peaks are of pure standards (Maloney et al, 1988). HPLC analysis also indicated the degradation of cypermethrin after ten days. The peak value shows the presence of an intermediate compound and the difference in retention time indicates the occurrence of intermediate compounds. The area and height of the peak illustrate the level of the intermediate compound formed during the degradation of cypermethrin.

Despite the early findings, the microbial population of soil is affected by cypermethrin. The ammonification and nitrification in treated soils are seen as a sign of the environmental impact of cypermethrin (Rangaswamy, 1999). Once applied, cypermethrin is bound strongly by soil components and is therefore not likely to enter groundwater. Cypermethrin is not persistent in soil and quickly degrades to less toxic products (Class, 1992). When organisms encounter a new organic chemical in their environment, they may obtain the new catabolic genes needed for degradation of that product from other microbes through conjugational and transformational events or they may modify existing genes through mutational processes (Chaudry and Chapalmadugu, 1991). During the process, the bacterium undergoes adaptation. It was observed that in the presence of high concentrations of insecticides, the bacteria were greatly stressed and their growth was slowed in consequence (Jilani and Khan, 2004).

The isolate *Enterobacter asburiae* was found to be effective in the degradation of the pesticide cypermethrin and hence it can be used in the effluent treatment for the pollution control especially to treat the effluents from the pesticide industry.

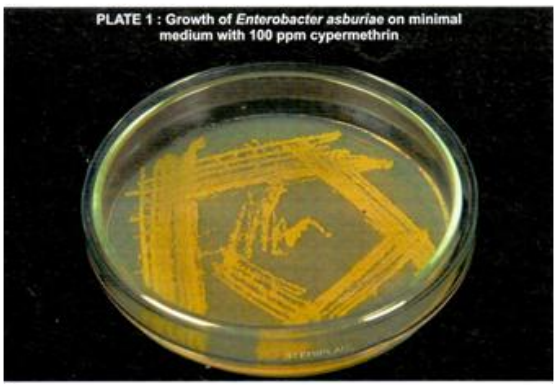
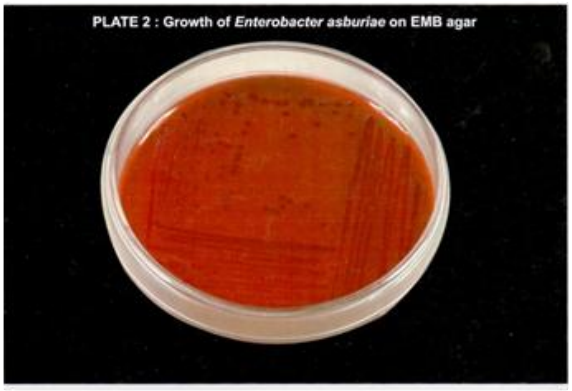


PLATE 1 : Growth of *Enterobacter asburiae* on minimal medium with 100 ppm cypermethrin



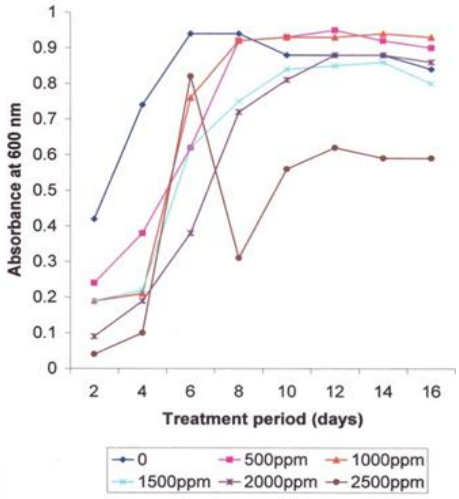


Figure 1 Changes in Turbidity of *Enterobacter asburiae* Containing 0-2500 ppm of Cypermethrin Concentrations

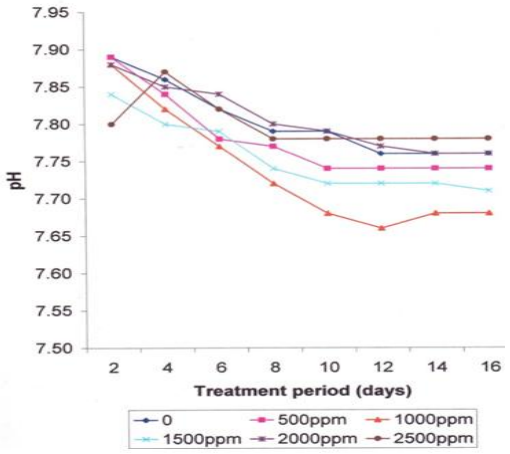


Figure 2 Change in pH in Culture Media with 0-2500 ppm Concentration of Cypermethrin for the Treatment Period

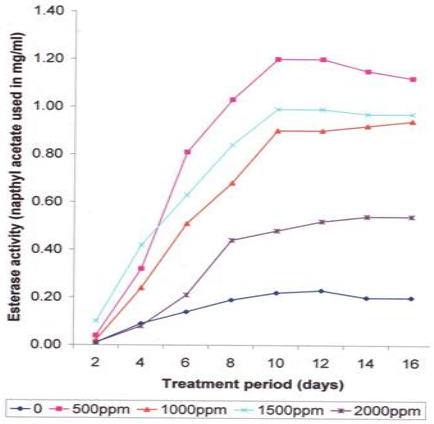


Figure 3 Esterase Activity of *Enterobacter asburiae* Grown in 0-2000 ppm Concentration of Cypermethrin

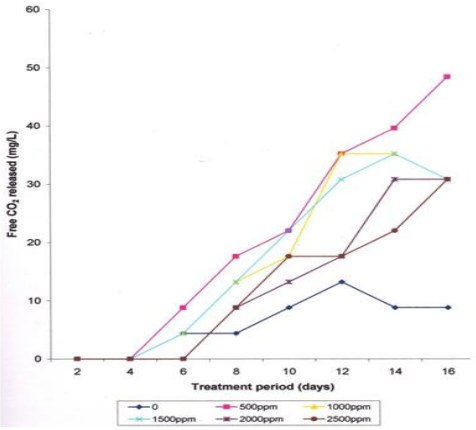


Figure 4 Changes in CO_2 Released by Culture Medium Containing 0-2500 ppm for the Respective Treatment Period

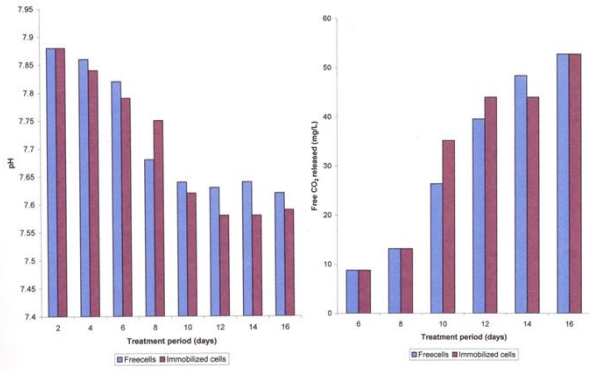


Figure 5 a) Changes in pH in Free Cells Versus Immobilized Cells b) Changes in CO_2 Released by Free Cells Versus Immobilized Cells

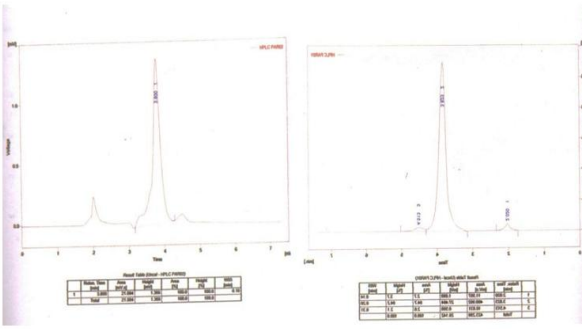


Figure 6 a & b HPLC results of after and before treatment of Cypermethrin with Enterobacter asburiae

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SEASONALITY AND ABUNDANCE OF BUTTERFLIES IN THE AMERICAN COLLEGE CAMPUS, MADURAI

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Abstract

The American College campus located in Madurai was selected as the study area to study the seasonality and abundance of butterflies. The campus had about thirty nine species of butterflies belonging to five families due to the undisturbed environment and diversity of trees. Nymphalidae family was represented with the highest number of species and the butterfly diversity was high during North West Monsoon season. Fourteen species of butterflies were seen in all the seasons and among them Blue pansy was recorded as the satellite species.

Keywords: *Lepidoptera, Blue Pansy, butterflies, abundance, diversity.*

Introduction

Seasonal changes are predominant in invertebrates especially in insects, where an active system for regulating body temperature is absent. Awareness regarding butterflies and their conservation is lacking nowadays. Urban areas have come under close investigation due to the recognition that conservation and management of urban habitat and species pose particular challenges (Angold et al, 2006). Butterflies are one of the best known and most charismatic groups of fauna (Asher *et al*, 2001). In Madurai there has been less scientific focus on any kind of major butterfly fauna survey until recently. Due to global destruction modern study of species is of vital importance for understanding biological communities and their conservation. Butterflies play a major role in pollination of flowers and caterpillars are vital food resources for animals and birds. Because of intensive machinery and heavy use of pesticides, arable land is now considered to support poor butterfly fauna (Sparks and Parish, 1995). Some large scale studies have focused wholly or partially on butterflies as indicators of urbanisation (Vanreusal and VanDyck, 2007). In India butterflies are treated as non-target species in the conservation and management of wildlife, as the prime focus has always been on vertebrate taxa. Research and documentation of butterflies with emphasis on their taxonomy need to be undertaken. Hence the present study has been planned to map the distribution of butterfly species in The American College campus, to create butterflies fact file, to aid subsequent research and to determine butterfly species richness, abundance, and distribution pattern between seasons.

Materials and Methods

The American College Campus is located in the heart of Madurai city with an area of forty five acres, with a canopy of trees and shrubs. There are different species of plants which support wide variety of butterfly species. The sampling of butterflies was done in six transects, T1 – Residential area, T2 – Binghamton Hall, T3-Ground Side, T4 – Stoffer hall T5 – Cycle Stand, T6 – Girls hostel (Fig.1). Transect counting method was employed for estimating the relative abundance of butterflies in The American college campus, Madurai. The study was carried out from July 2009 to June 2010. The survey and sampling were done in early morning hours and the records were based on visual sighting during transect walk. Prior to collection, a reference collection was maintained. The transect walk was carried out on days with favourable weather condition. The study period was divided into four seasons South west monsoon, North East monsoon, Winter and Summer. Butterflies that could be identified in the field after capture with netting were released. Butterflies were identified using standard monographs of (Wynterblyth, 1957, Kunte, 2000). The diversity pattern of butterflies was calculated by the method followed by

Rydzanicz and Lonc (2003) and the biodiversity richness by Shannon Weaver index.

Results and Discussion

A total number of thirty nine species of butterflies belonging to five families were found in the study area from July 2009 to June 2010 (Table 1). Among the families, Nymphalidae was the dominant family. The representation of the families Nymphalidae, Pieridae, Papilionidae, Lycaenidae and Hesperiidae was 38, 26, 18, 13, and 5 respectively (Table 2).

The butterflies common in all the seasons include Yellow pansy, Blue pansy, Lemon pansy, Peacock pansy, Angled castor, Tawny coster, Common Indian crow, Plain tiger, Glassy blue tiger, Tiny grass blue, Lime butterfly, Small salmon arab, Crimson tip and Small grass yellow. In the North West monsoon, an abundance of thirty five species was observed. Nymphalidae was the dominant family in the present work (Table 2) and the same was observed in the Silent valley National Park (Mathew and Rahamathulla,1992) and many members of this family are polyphagous. The American college campus (Fig 1) had higher diversity compared to Lady Doak college, Madurai (Priya, 2007) but lesser than Alagar hills for the study of seven months (Alex 2004).A few species were common in all sites of the campus (Table 3). The butterflies were abundant in the residential area of the campus. The butterflies seen in the campus were also recorded in the Alagar hills except for Peacock pansy and Small salmon arab. The rich diversity could be attributed to the serenity and density of trees. The high incidence of butterfly diversity in certain sites may be due to several biological factors as suitable ovipositor sites for gravid females, floral phenology, less number of predators and mimics. (Ramos, 2000). Certain species were found only in certain habitats and this can be due to reasons like suitable mud puddling sites, topography, temperature, humidity and light gaps (Leps and Spitzer, 1990). Some species are strictly seasonal and are found in certain seasons, which could be attributed to the phenology of food plants (Table 4 and 7).

Butterfly diversity was high in all seasons except for south west monsoon, where the Simpson index was 0.03 (Table 6). Blue pansy was recorded as the satellite species among the butterflies (Table 8). The residential area had prevalence of more species due to mud puddling on wet soil in open sunny patches near human habitation and foraging on plants like *Ixora coccinea, Hibiscus rosasinensis*, *Lantana camara* and V*itex negundo* that occurred in high abundance in residential area (Harcourt *et al.,* 2002). The high diversity may be due to high diversity of tress shrubs and climbers.

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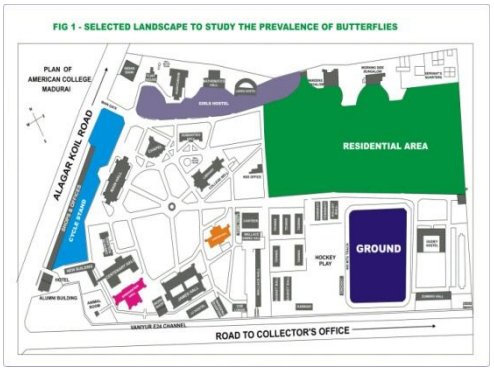

Figure 1 Selected Landscape to Study the Prevalence of butterfiles

Table 1 Butterflies of The American College Campus, Madurai from July 2009 to June 2010

Sl.No.	Common Name	Scientific Name	Family
1	Danaid Egg Fly	*Hypolimnas misippus*	Nymphalidae
2	Yellow Pansy	*Precis hierta*	
3	Blue Pansy	*Precis orithyia*	
4	Lemon Pansy	*Precis lemonias*	
5	Peacock Pansy	*Precis almana*	
6	Common Leopard	*Phalantha atella*	
7	Angled Castor	*Ariadne ariadne*	
8	Tawny Coster	*Telchinia violae*	
9	Common Evening Brown	*Melanitis leda*	
10	Common Indian Crow	*Euploea core*	
11	Plain Tiger	*Danaus chrysippus*	
12	Common Tiger	*Danaus plexippus*	
13	Striped Tiger	*Danus genutia*	
14	Chocolate Soldier	*Precis iphita*	
15	Glassy Blue Tiger	*Danaus limiace*	
16	Tiny Grass Blue	*Zizula hylax*	Lycaenidae
17	Common Cerulean	*Jamides celeno*	
18	Tailless Line Blue	*Nacaduba dubiosa*	
19	Round Six Line Blue	*Nacaduba berenice*	
20	Common Tit	*Aypolycaena erylus*	
21	Smallest Swift	*Parnara bada*	Hesperiidae
22	Black Flat	*Celaenorrhinus spilothyrus*	
23	Veined jay	*Zetides bathycles*	Papilionidae
24	Lime Butterfly	*Papilio demoleus*	
25	Common Mormon	*Papilio polytes*	
26	Blue Mormon	*Papilio polymnestor*	
27	Common Rose	*Tros aristolochiae*	
28	Crimson Rose	*Tros hector*	
29	Glassy Blue Bottle	*Zetides cloanthus*	
30	Psyche	*Leptosia nina*	Pieridae
31	Common Jezebel	*Delias eucharis*	
32	Lemon Emigrant	*Catopsilia pomona*	
33	Common Albatross	*Appias albina*	
34	Striped Albatross	*Appias libythea*	
35	Small Salmon Arab	*Colotis amata*	
36	Large Salmon Arab	*Colotis fausta*	
37	Crimson Tip	*Colotis danae*	
38	Mottled Emigrant	*Catopsilia pyranthe*	
39	Small Grass Yellow	*Eurema hecabe*	

Table 2 Relative Abundance of Butterfly Families in The American College Campus, Madurai from July 2009 to June 2010

Sl.No.	Habitat	Family				
		Nymphalidae	Lycaenidae	Hesperiidae	Papilionidae	Pieridae
1.	Residential Area	39.3	14.3	---	17..9	28..5
2.	Binghamton Hall	41.1	10	3.7	22.6	22.6
3.	PlayGround Side	42.4	7.6	---	26..9	23.7
4.	Cycle Stand	36.8	5.3	---	26.3	31.6
5.	Stoffer Hall	42.8	9.6	4.7	33..3	9.6
6.	Girls Hostel	42.8	4.8	---	33..3	19.1

Table 3 Common Species of Butterflies Present in all Transects in The American College Campus, Madurai from July 2009 to June 2010

Sl.No.	Common Name	Scientific Name
Family Nymphalidae		
1	Yellow Pansy	*Precis hierta*
2	Lemon Pansy	*Precis lemonias*
3	Peacock Pansy	*Precis almanac*
4	Common Indian Crow	*Euploea core*
5	Plain Tiger	*Danaus chrysippus*
Family Lycaenidae		
6	Tiny Grass Blue	*Zizula hylax*
Family Papilionidae		
7	Lime Butterfly	*Papilio demoleus*
8	Common Mormon	*Papilio polytes*
9	Lemon Emigrant	*Catopsilia pomona*
Family Pieridae		
10	Common Albatross	*Appias albino*
11	Psyche	*Leptosia nina*
12	Small GrassYellow	*Eurema brigitta*
13	Crimson Tip	*Colotis danae*

Table 4 Butterflies Species, Specific to Habitats in The American College Campus, Madurai from July 2009 to June 2010

Sl.No.	Common Name	Scientific Name	Habitat
Family Lycaenidae			
1	Common Cerulean	*Jamides celeno*	Residential Area
2	Round Six Line Blue	*Nacaduba berenice*	
Family Pieridae			
3	Large Salmon Arab	*Colotis fausta*	Binghamton Hall
4	Common Jezebel	*Delias eucharis*	
Family Nymphalidae			
5	Danaid Egg Fly	*Hypolimnas misippus*	
6	Striped Tiger	*Danaus genutia*	
7	Common Leopard	*Phalantha atella*	
Family Hesperiidae			
8	Smallest Swift	*Parnara bada*	
9	Black Flat	*Celaenorrhinus spilothyrus*	Stoffer Hall

Table 5 Simpson Diversity Index with Respect to Different Seasons on the Prevalence of Butterflies in The American College Campus

Seasons	Simpson diversity index
South West Monsoon	0.03
North East Monsoon	0.99
Winter Season	0.98
Summer	0.95

Table 6 Butterfly Sighting in Different Months at The American College Campus, Madurai from July 2009 to June 2010

Sl. No	Common Name	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
1	Danaid Egg Fly	4	7	5	--	--	--	--	--	--	2	--	--
2	Yellow Pansy	3	2	5	--	--	--	2	5	9	3	2	--
3	Blue Pansy	--	3	2	--	1	--	--	3	--	2	--	--
4	Lemon Pansy	15	13	21	17	7	3	10	12	15	19	17	2
5	Peacock Pansy	7	12	9	--	--	--	3	7	--	2	--	--
6	Common Leopard	4	3	7	--	--	--	2	3	--	--	--	--
7	Angled Castor	--	5	12	3	2	1	--	--	4	--	--	--
8	Tawny Coster	14	7	13	--	--	--	7	--	--	--	--	--
9	Common Evening Brown	--	--	--	--	--	--	2	5	4	3	--	--
10	Common Indian Crow	30	25	19	20	2	3	--	4	3	7	2	40
11	Plain Tiger	15	17	19	14	5	4	10	7	12	11	9	7
12	Common Tiger	--	--	--	2	--	--	3	--	2	--	1	--
13	Chocolate Soldier	--	--	3	7	--	1	4	--	15	19	7	--
14	Striped Tiger	--	--	--	4	--	2	--	--	--	--	--	--
15	Glassy Blue Tiger	17	19	24	--	4	2	15	10	30	--	7	20
16	Tiny Grass Blue	10	7	19	10	17	11	15	30	12	37	20	12
17	Common Cerulean	--	--	4	2	--	--	--	2	5	--	--	--
18	Tailess Line Blue	--	--	4	3	--	--	--	--	--	--	--	--
19	Rounded 6 Line Blue	--	--	--	3	2	--	--	--	--	--	--	--
20	Common Tit	--	--	2	--	--	--	1	--	--	--	2	--
21	Smallest Swift	--	--	--	3	--	--	--	--	2	--	--	--
22	Black Flat	--	--	--	2	--	--	4	3	--	--	--	--
23	Veined Jay	--	--	--	9	1	2	4	3	7	4	3	2
24	Lime Butterfly	9	4	7	12	2	3	--	--	7	3	2	2
25	Common Mormon	4	7	13	5	1	2	--	--	4	3	2	5
26	Blue Mormon	--	--	--	2	--	1	--	--	--	--	--	--
27	Common Rose	4	7	3	5	3	2	--	--	--	--	--	--
28	Crimson Rose	--	--	--	2	--	5	9	14	12	7	4	2
29	Glassy Blue Bottle	--	--	--	4	--	--	--	2	7	5	3	4
30	Psyche	--	--	--	--	--	--	2	9	12	14	8	12
31	Common Jezeebel	--	--	--	--	--	2	7	8	--	--	--	--
32	Lemon Emmigrant	5	7	9	12	2	2	9	7	12	8	15	9
33	Common Albatross	7	13	12	9	5	4	--	--	--	--	--	--
34	Striped Albatross	--	--	--	--	--	--	--	4	3	5	--	--
35	Small Salmon Arab	35	49	23	33	19	10	17	15	20	25	30	23
36	Large Salmon Arab	--	--	10	7	3	--	--	--	--	--	--	--
37	Crimson Tip	31	28	15	19	7	4	5	12	10	3	7	20
38	Small Grass Yellow	25	23	14	19	12	8	7	12	10	25	17	14
39	Mottled Emmigrant	--	--	--	8	12	--	5	--	9	--	--	3

Table 7 Prevalence of Butterflies in Different Seasons in The American College Campus from July 2009 to June 2010

Family Papilionidae	SW monsoon	NE monsoon	Winter	Summer
Danaid egg fly	√	√	---	√
Yellow pansy	√	√	√	√
Blue pansy	√	√		√
Lemon pansy	√	√	√	√
Peacock Pansy	√	√	√	√
Common leopard	√	√	√	---
Angled castor	√	√	√	√
Tawny coster	√	√	√	√
Common evening brown	---	---	√	---
Common Indian crow	√	√	√	√
Plain tiger	√	√	√	√
Common tiger	---	√	√	√
Striped tiger	---	√	---	---
Chocolate soldier	---	√	√	√
Glassy blue tiger	√	√	√	√
Family Lycaenidae				
Tiny grass blue	√	√	√	√
Common cerulean	---	√	√	√
Tailesss line blue	√	√	-	-
Round six line blue	---		---	---
Common tit	---	√	√	√
Family Hesperiidae				
Smallest swift	---	√	---	---
Black flat	---	√	√	√
Veined Jay	---	√	√	---
Lime butterfly	√	√	√	√
Common mormon	√	√	---	√
Blue mormon	---	√	√	√
Common rose	√	√	---	---
Crimson rose	---	√	√	√
Glassy blue bottle	---	√	√	---
Family Pieridae				
Psyche	---	---	√	√
Common jezeebel	---	---	√	---
Lemon emigrant	√	√	√	---
Common albatross	√	√	√	---
Striped albatross	--	--	√	√
Small Salmon arab	√	√	√	√
Large Salmon arab	---	√	---	---
Crimson tip	√	√	√	√
Mottled emigrant	√	√	√	√
Small grass yellow.	√	√	√	√

Table 8 The Density Pattern of Butterflies in The American College Campus from July 2009 to June 2010

Species Status	Name of the species	% Distribution of density pattern
Dominant	Lemon Pansy	9.9
	Common Indian Crow	9.0
	Plain Tiger	7.7
	Glassy Blue Tiger	7.7
	Salmon Arab	20
	Crimson Tip	9.3
	Small Grass Yellow	10.8
	Tiny Grass blue	11.5
Subdominant	Peacock Pansy	2.3
	Angled Castor	1.5
	Tawny Castor	2.8
	Lime butterfly	2.9
Satellite	Blue Pansy	0.6

INFLUENCE OF VERMICOMPOST, AMIRTHAKARAISAL AND AbdA GOLD ON THE GROWTH OF *CASSIA AURICULATA*

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Abstract

Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food long term sustainability, and concerns on environmental pollution associated with indiscriminate use of agrochemicals. The effect of vermicompost, amirthakaraisal, and AbdAgold separately and in combination on the growth of C. auriculata was determined studying the parameters germination percentage, root length, shoot length, wet weight, dry weight, chlorophyll A,B and total chlorophyll content, and protein content. Application of vermicompost, and amirthakaraisal, enhanced the growth parameters in C. auriculata.
Keywords: *Organic manure, vermicompost, amirthakaraisal, AbdA gold, Cassia auriculata, growth.*

Introduction

Agriculture remains the key sector for the economic development in most developing countries. It is critically important for ensuring food security, alleviating poverty and conserving the vital natural resources that the world's present and future generations will be entirely dependent upon them for their survival and well-being. The world population will inevitably double by the middle of the twenty-first century, which is in the space of just two generations. Over 90% of the developing nations, especially in Asia will be in the urban areas which follow up the green revolution strategy. Organic agriculture is one among the broad spectrum of production methods that are supportive of the environment (Vermitech, 2004). Vermicomposting is a biotechnological process in which earthworms are employed to convert the organic wastes into humus like material known as vermicompost. Certain earthworm species are capable of consuming a wide range of organic wastes from sewage sludge, animal wastes, agricultural residues, domestic wastes, and industrial wastes (Frederickson *et al.,* 2002). Under favourable conditions of temperature and moisture, earthworms maintain the aerobic condition in the vermicomposting process, ingest organic waste materials and egest a humus-like substance which is more homogeneous than the organic wastes or raw materials used. Amirthakaraisal is a foliar spray, which is a blend of two products from cow. It contains jaggery, water, cow urine and cow dung. When suitably mixed and used, it acts as an effective growth promoter and pest repellent. Some macronutrients (N, P, and K) and micronutrients (Zn, Fe, Mn, and Cu) along with reducing sugars are present in amirthakaraisal. Chemolithoautrotrophic nitrifiers, which colonize in the leaves, increase the ammonia uptake and enhance total nitrogen supply. Effective microorganisms present in amirthkaraial improved soil quality, growth and yield of crops (Bhat and Limaye, 2012). Hence the present work has been designed to study the effect of vermicompost, amirthakaraisal and AbdA gold on the growth of *C. auriculata.*

Materials and Methods

The present study was carried out for testing the effect of Vermicompost, Amirthakaraisal, and Abda gold plant vitalizer and their combinations on the growth of the plant, *C. auriculata*.

Chemical Parameters

Soil sampling for chemical analysis was done according to standard methods. Samples were processed and their chemical properties such as pH, Electrical conductivity, nitrogen, phosphorus, potassium, copper, zinc, iron and manganese were estimated and recorded. Vermicompost, Amirthakaraisal, and AbdA gold were applied once in a week. Chemical parameters were analysed in soil, vermicompost, amirthakaraisal and AbdA gold (Dresboll and Thorupukristensen, 2005).

Vermicompost

Vermicompost was obtained from the Agriculture College and Research Institute, Madurai. The nutrient composition of vermicompost was analysed following standard methods (Edward, 2007).

Amirthakaraisal

Amirthakaraisal is an organic formulation derived from the four ingredients, fresh cow dung, cow urine, jaggery and water. Amirthakaraisal is an effective stimulator, growth promoter and immunity booster. Amirthakaraisal proved its value by providing strength and resistance to the crop. All the ingredients were mixed well and stirred three times a day. Within 24 hours the manure was ready. Manure was kept under the shade covered with a white mesh or plastic mosquito net to prevent house flies from laying eggs and formation of maggots in the solution. This manure can be used as foliar spray or as a solution that can be mixed with water. One liter of solution is diluted with ten liters of water.

AbdA Gold

AbdA gold is an organic product that ensures balanced root: shoot ratio and early establishment of crops. It stimulates plant for enhanced nutrient uptake which leads to improved yield. It boosts the thickness of the stem and increases the height of the crops. AbdA gold increases leaf area, greenness and chlorophyll content leading to enhanced photosynthesis.

Dosage

The pots had red soil with pH 5.57. Eight pots with 15 cm diameter and 30 cm depth were used in this study. Each pot was filled with 1.5kg of the soil and planted with seeds. Fifteen days after seeding 100 g of vermicompost, amirthakaraisal and AbdA gold individually and in combinations were used per pot once in a week in the morning.

Cassia auriculata

C. auriculata commonly known as boran bean (*chedi avarai*) belongs to the family Fabaceae and the subfamily Faboideae. It is a herbaceous annual or biennial shrub found throughout India. It is growing up to 1-1.5m. Bright white flowers appear in racemes at the end of the branches. The flowers are 4-5 cm across and upper three stamens are reduced to staminoides.

Treatments

The eight treatments as given in Table 1 were conducted for the crop, *C. auriculata* and for each treatment the seeds were applied in the soil. The germination of seeds was observed after four days of sowing. The germination process was noticed for the first eight days. The root and shoot length, wet and dry weight of the plant were calculated fifteen days once. The analysis of protein and chlorophyll content was carried out using standard procedures.

Table 1 Treatment Types and Details

Treatment	Details
T1	Vermicompost
T2	Amirthakaraisal
T3	AbdA gold
T4	Vermicompost + Amirthakaraisal
T5	Vermicompost + AbdA gold
T6	Amirthakaraisal + AbdA gold
T7	Vermicompost +Amirthakaraisal+ AbdA gold
T8	Control

The following parameters were recorded 15, 25, 35, and 45 days after seeding (DAS).

1. **Shoot Length**: It was measured from the ground surface to the tip of the plant.
2. **Root Length:** It was measured from the ground level to the tip of the longest root hair.
3. **Wet Weight:** The plants were removed from the soil and washed out off any loose soil. The plants were gently blotted with soft paper towel to remove any free surface moisture and weighed immediately.
4. **Dry Weight:** The shoot and root were cut and packed separately and dried in a hot air oven at 50° C until the moisture content was totally lost and then weighed.
5. **Chlorophyll Content:** One gram of fresh leaves were taken and minced well with scissors. To this about 5ml distilled water was added and homogenized in a blender. The final volume was made up to 10 ml. An aliquot (10.5 ml) was taken and mixed with 4.5 ml of 80% acetone. The acetone extracts the pigment. The supernatant after centrifugation was collected and measured for optical density at two wavelengths 645nm and 663nm for chlorophyll A and B respectively with the solvent blanks (Berova and Karanatsidis, 2009). The amount of chlorophyll content (mg chlorophyll/g tissue) was calculated using the following equations.

mg chlorophyll a /g tissue= 12.7 (A_{663}) - 2.69 (A_{645})× V/ 1000×W

mg chlorophyll b/ g tissue = 22.9 (A_{645}) - 4.68 (A_{663})×V/1000×W

mg total chlorophyll /g tissue=20.2(A_{665})+ 8.02(A_{663}) ×V/ 1000×W

Where,

V= final volume of the extract

W=fresh weight of tissue extracted

Protein Content

Protein content of the seeds was analyzed by Lowry's method using Folin-ciocalteau reagent. *C. auriculata* beans were cleaned and washed with distilled water and allowed to air dry. Two grams of samples were weighed and made into paste using pestle and mortar. The juice was extracted and made up to 5ml with distilled water and poured into a centrifuge tube and after the process of centrifuging the aqueous extract collected was packed in polythene pouch and stored in the refrigerator.

The amount of 0.1ml to 1ml working standard solutions in five test tubes was taken and one ml of distilled water was added to each. After that 5.0ml of Folin-ciocalteau reagent was added and then 0.3ml of copper sulphate reagent was added in each test tube. Then all the test tubes were covered with cotton and they were kept undisturbed for thirty minutes. The optical density (OD) of each test tube was measured using colorimeter (Singh *et al., 2008*).

Statistical Analysis

The data collected were subjected to two way analysis of variance (ANOVA) for the different factors. Statistical analysis was carried out by taking the average of five plants from each pot. The level of significance used in 'F' test was P = 0.05.

Results and Discussion

The growth of *C. auriculata* was estimated in the present investigation in various treatments like vermicompost, amirthakaraisal, and AbdA gold and their combinations. The chemical composition of soil, vermicompost, amirthkaraisal and AbdA gold were tested and tabulated in Table 2, 3, 4, 5 respectively. The chemical parameters of soil are shown in Table 2. The highest value was found for potassium (50 mg/kg) and the lowest for Electrical conductivity (0.30 Ds/m). Chemical parameters of vermicompost are shown in Table 3. Organic carbon, nitrogen, phosphorous, potassium, and sodium, were found in vermicompost. The chemical parameters of Amirthakaraisal are shown in Table 4. The highest value was found for potassium (317 mg/kg) and the lowest value was found for zinc (0.22). Chemical parameters of AbdA gold are shown in Table 5. Vitamins, proteins, Fat, Carbohydrates, Phosphorous, Sodium, Zinc, and Magnesium were found in AbdA gold organic manure.

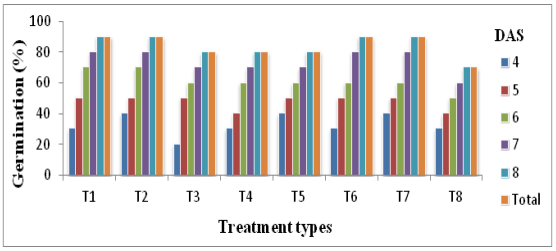

Figure 1 Effect of Various Treatments on the Seed Germination (%) of *C. auriculata*

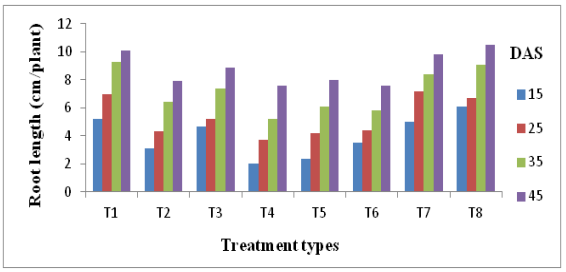

Figure 2 Effect of Various Treatments on the Root Length of *C. auriculata*

Table 2 Chemical Characteristic of Soil Used in the Study

Parameter (Unit)	value	Interpretation
Moisture content (%)	1.35	-
pH	5.57	Acidic
Electrical conductivity (ds/m)	0.30	Harmless
Nitrogen (mg/kg)	476	High
Phosphorus (mg/kg)	50	High
Potassium (mg/kg)	518	High
Organic Carbon (g/kg)	5.14	Medium
Exchangeable Calcium (meq/100g)	3.50	-
Exchangeable Magnesium (meq/100g)	1.50	-
Exchangeable Sodium (meq/100g)	1.25	-
Exchangeable Potassium (meq/100g)	0.81	-

Table 3 Chemical Characteristics of Vermicompost

Parameter (Unit)	Value
pH	7- 8.2
Organic carbon (%)	17.98
Nitrogen (%)	1.50
Phosphorus (%)	0.30
Potassium (%)	0.56
Sodium (%)	0.30
Calcium and magnesium (meq/100mg)	22.67
Copper (mg/kg)	9.50
Iron (mg/kg)	9.30
Zinc (mg/kg)	5.70
Sulphur (mg/kg)	128

Table 4 Chemical Characteristics of Amirthakaraisal

Parameter (Unit)	Value
Total Nitrogen (mg /kg)	273
Total Phosphorus (mg /kg)	281
Total Potassium (mg /kg)	317
Total sugars (mg /kg)	195
Sodium (mg /kg)	75
Calcium (mg /kg)	27
Total organic carbon (%)	0.68
Indole Acetic Acid (mg/ kg)	9.56
GA (mg/ kg)	4.0
Phenols (µg/ml)	0.71
pH	5.6
Electrical Conductivity (ds/m)	10.30

Table 5 Chemical Characteristic of AbdA Gold

Parameters (Unit)	Value
Protein (%)	30
Fat (%)	2
Carbohydrate (%)	75
Calcium (ppm)	7896
Iron (ppm)	332
Iodine (ppm)	532
Magnesium (%)	0.5
Manganese (ppm)	155
Sodium (%)	4
Zinc (ppm)	2
Phosphorus (%)	0.9
Sulphur (%)	0.43

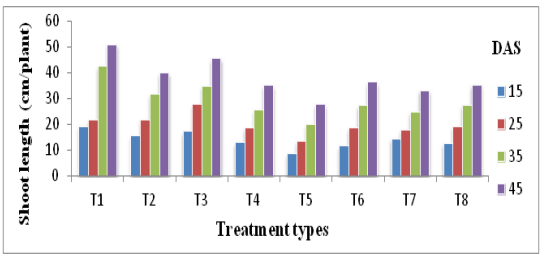

Figure 3 Effect of Various Treatments on the Shoot Length of *C. auriculata*

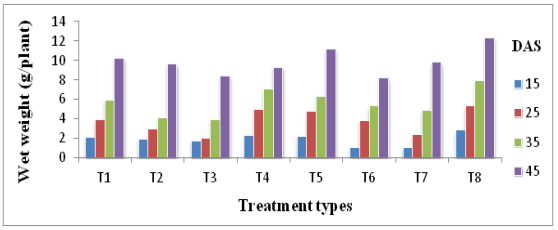

Figure 4 Effect of Various Treatments on the Wet Weight of *C. auriculata*

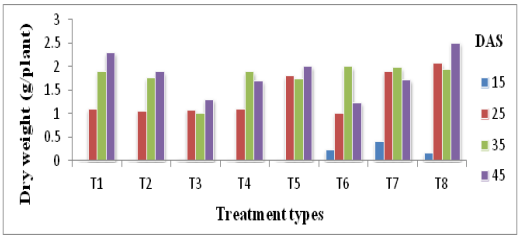

Figure 5 Effect of Various Treatments on the Dry Weight of *C. auriculata*

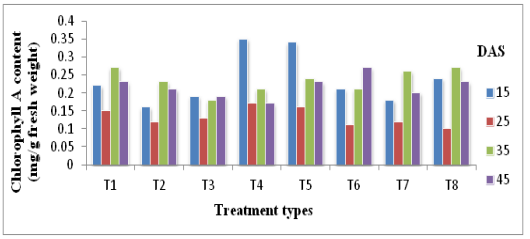

Figure 6 Effect of Various Treatments on the Chlorophyll a Content of Leaves of *C. auriculata*

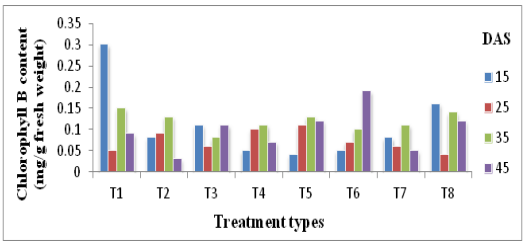

Figure 7 Effect of Various Treatments on the Chlorophyll B Content of Leaves of *C. auriculata*

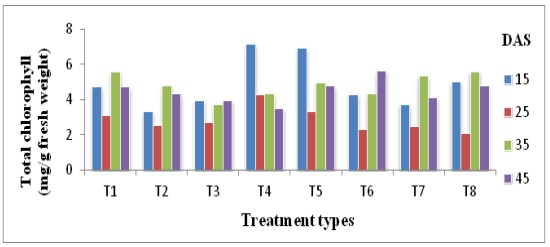

Figure 8 Effect of Various Treatments on the Total Chlorophyll Content of Leaves of *C. auriculata*

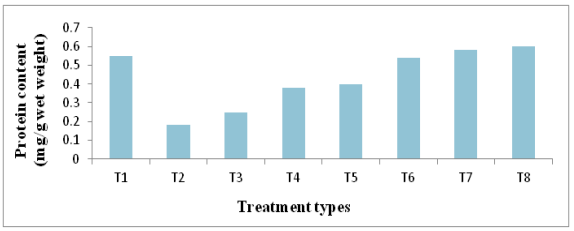

Figure 9 Effect of Various Treatments on the Protein Content of the Beans of *C. auriculata*

Table 6. Two way Analysis of Variance (Anova): Variation Due to Treatment Types and Treatment Period for the Various Factors of *C. auriculata*

S. no	Factor	Source of variation	SS	df	MSS	Calculated F value	Table F value at 5% level	Level of significance
1.	Germination percentage	Treatment type	77.5	7	11.07143	0.306931	2.35926	NS
		Treatment period	2590	4	647.5	17.9505	2.714076	S
2.	Root length	Treatment type	53.315	7	7.616429	54.91674	2.487578	S
		Treatment period	106.3475	3	35.44917	255.5991	3.072467	S
3.	Shoot length	Treatment type	756.6322	7	108.0903	14.78649	2.487578	S
		Treatment period	2678.111	3	892.7036	122.1196	3.072467	S
4.	Wet weight	Treatment type	30.14254	7	4.306077	8.925668	2.487578	S
		Treatment period	280.8043	3	93.60142	194.0177	3.072467	S
5.	Dry weight	Treatment type	1.7647	7	0.2521	2.773196	2.487578	S
		Treatment period	15.47103	3	5.157008	56.72905	3.072467	S
6.	Chlorophyll A	Treatment type	0.015672	7	0.002239	1.223071	2.487578	NS
		Treatment period	0.057484	3	0.019161	10.46785	3.072467	S
7.	Chlorophyll B	Treatment type	0.015288	7	0.002184	0.732774	2.487578	NS
		Treatment period	0.009512	3	0.003171	1.063911	3.072467	NS
8.	Total chlorophyll	Treatment type	7.245314	7	1.035045	1.309946	2.487578	NS
		Treatment period	22.11719	3	7.372398	9.330457	3.072467	S

S-significant, NS- Not significant

Cassia auriculata

Seed Germination

Figure 1 shows the germination percentage of *C. auriculata* in various treatments. It is found to be higher in pots treated with vermicompost, amirthakaraisal, and vermicompost mixed with amirthakaraisal pots. Seedlings emerged only four days after seeding and the germination percentage was slightly higher in T1 (vermicompost) when compared to T4 (vermicompost+amirthakaraisal). The lowest value of germination percentage was noticed in control when compared to other treatments.

Root Length

The effect of various treatments on the root length of *C. auriculata* 15, 25, 35, and 45 days after seeding is shown in Fig.2. The highest root length was recorded in control and vermicompost treated pots.

Shoot Length

The effect of various treatments on the shoot length of *C. auriculata* 15, 25, 35, and 45 days after seeding is exhibited in Fig.3. The highest length of 50.8cm was recorded in Vermicompost treatment (T1) and the lowest length 27.6cm was recorded in vermicompost + AbdAgold treated pots (T5).

Wet Weight

Figure 4 shows the effect of various treatments on the wet weight of *C. auriculata* 15, 25, 35, and 45 days after seeding. The highest value of wet weight was recorded in control pot. The lowest value was seen in amirthakaraisal + AbdA gold treated pot (T6).

Dry Weight

Figure 5 shows the effect of various treatments on the dry weight of *C. auriculata* 15, 25, 35, and 45 days after seeding. The highest value of dry weight was recorded in control pot (T8) while the lowest value was recorded in amirthakaraisal + AbdA gold treated pot (T6).

Chlorophyll Content

Figure 6 and 7 show the effect of various treatments on the chlorophyll content of *C. auriculata* 15, 25, 35, and 45 days after seeding. The highest value of chlorophyll A was recorded in vermicompost + amirthakaraisal treatment (T4), while T6 exhibited the lowest value of chlorophyll A. Fig 7 exhibited the effect of various treatments on the chlorophyll B content of *C. auriculata* 15, 25, 35, and 45 days after seeding. The highest value of chlorophyll was observed in vermicompost treatment (T1), while amirthakaraisal (T2) treatment exhibited the lowest value of chlorophyll B. Figure 8 highlights the effects of various treatments on the total chlorophyll content of *C. auriculata* 15, 25, 35, and 45 days after seeding. The highest total chlorophyll content was recorded in vermicompost + amirthakaraisal treated pot (T4) while control pot (T8) exhibited the lowest chlorophyll content.

Protein Content

Figure 9 shows the effect of various treatments on the protein content of seeds of *C. auriculata* 45 days after seeding. This was found to be maximum in control treatment (T8) with 0.60mg/g bean. The minimum protein content was recorded in amirthakaraisal treatment (T2) with 0.18mg/g bean.

Statistical Analysis

Table 6 exhibits the two way analysis of variance (ANOVA). Variations due to treatment types are statistically significant at 5% level for the factors root length, shoot length, wet weight, and dry weight and variations due to treatment period are statistically not significant at 5% level for the factor chlorophyll B alone in *C. auriculata.*

In the present work, the effect of organic manures, vermicompost, amirthakaraisal and AbdA gold was studied using *C. auriculata* as model plant. The combination of vermicompost and amirthakaraisal produced the highest germination percentage compared to AbdA gold plant vitaliser. The vermicompost contains micronutrients that stimulate plant growth. The shoots were longer and thicker. These plants recorded the maximum shoot length 25 and 35 days after seeding. The vermicompost contained nutrients like S, and Zn which might have enhanced the shoot growth. Eventually with the application of amirthakaraisal, 25 and 35 days after seeding, there was a significant increase in the height of the plant. The highest dry weight was found in T5, which had the combined effect of vermicompost and AbdAgold (Khatik, 2001). Vermicompost, amirthakaraisal and AbdAgold mixed treatment recorded low chlorophyll content in *C. auriculata*. Atiyeh *et al*. (2000) found that vermicompost tended to be higher in nitrates, which is the more plant-available form of nitrogen. The later study also showed that the supply rate of several nutrients, including P, K, S and Mg, was increased by vermicomposting as compared with conventional composting. These results are typical of what other researchers have found (Sudha and Kapoor, 2000). It appears that the process of vermicomposting tends to result in higher levels of plant-availability of most nutrients than does the conventional composting process. Vermicompost has consistently improved seed germination, enhanced seedling growth and development, and increased plant productivity much more than would be possible from the mere conversion of mineral nutrients into more plant-available forms (Arancon, 2004). That maximum benefit from vermicompost is obtained when it constitutes between 10 and 40% of the growing medium. It appears that levels of vermicompost higher than 40% do not increase benefit and may even result in decreased growth or yield. The combination of all manures similarly increased the protein content 45 days after seeding. Thus, the organic manures like vermicompost, amirthakaraisal and AbdAgold when used as components in various strategies, show promising results in *C. auriculata.* It has also been found that vermicompost treatment gives enhanced growth in combination with amirthakaraisal and AbdAgold.

Acknowledgement

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BIODEGRADATION OF CYPERMETHRIN USING *PSEUDOMONAS STUTZERI* (MTCC 2643)

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Abstract

Cypermethrin is an insecticide of synthetic pyrethroid family and over ninety percent of the cypermethrin manufactured worldwide is used to kill pests of cotton. It is generally non-persistent in soil with half-life period in sandy soils of 2-4 weeks but it degrades more slowly under anaerobic and water logged conditions. Hence in the present study Pseudomonas stutzeri was tested for the degradation of cypermethrin. The degradation was studied with the analysis of pH, CO_2, Esterase activity and Turbidity in different concentrations of cypermethrin. Maximum degradation efficiency was observed in 500ppm of cypermethrin on the tenth day. The UV-visible spectrophotometry and HPLC analysis were done to predict the change in chemical nature from initial stage and presence of intermediates by means of peaks with different retention time. Thus the biodegradation of cypermethrin can be done using this strain and further research is needed to emerge it as an effective biodegradation agent against pyrethroid insecticides.

Keywords: *Cypermethrin, Pyrethroid, Biodegradation, Pseudomonas stutzeri*

Introduction

Pesticides are applied to kill or control pests and considering the toxic effects of pesticides, it is essential to remove these chemopollutants from the environment (Kanekar et al., 2004). Pesticides vary greatly in their affinity for soil particles and their solubility in water while they can contaminate water supplies by moving their eroded soil particles that they are attached (Rao et al., 1979). Leaching occurs when pesticides move through soil profile and reach ground water sources of well water and it can allow contaminants to surface in seeps or streams. Sorption is the partitioning of chemicals between soil and water phases. Runoff is the transport of contaminants or suspension while volatilization is the evaporation of pesticides into the air and they may cause some pesticides to move from the intended field to other location (Kerle et al., 1997). Pesticides affect the soil microflora, soil chemistry and persist in the environment. Many approaches to pesticide waste treatment have been considered by the researchers (Yu, 2002; Huston and Pignatello, 1999; Arnol et al., 1995; Somich et al., 1990), but few are sufficiently broad-based and convenient to the user.

Cypermethrin is an insecticide in the synthetic pyrethroid family used to kill pests of Cotton. It is also used on pests of Lettuce and Pecans to kill Cockroaches, Fleas and Termites (Cox, 1996). Because of the potential risk by these pesticides, there is a serious need to develop remediation process to eliminate or minimize contamination in the environment. Cypermethrin is less persistent in soils with the typical half-life of 2-4 weeks (Chapman and Harris, 1981). Increased cypermethrin persistence was observed in soil with high organic matter, high clay content, reduced microbial activity and anaerobic condition (Chapman et al., 1981). Microbes play a significant role in the degradation of cypermethrin. Cypermethrin degrades more slowly under anaerobic and water-logged condition (Walker and Keith, 1992). Microbial degradation is more important than purely physical and chemical methods of degradation (Chapman and Harris, 1981). In the present study, the efficiency of degradation of cypermethrin was determined using *Pseudomonas stutzeri,* MTCC 2643 obtained from MTCC, IMTECH, Chandigarh, India, by means of determining esterase activity and the analysis of degradation products by employing UV- Visible spectrophotometry and HPLC.

Materials and Methods

Collection of Samples

The soil samples were collected from the agricultural field where cypermethrin was applied. The field was near Kombadi 18km away from Madurai. The soil samples that were collected in sterile containers were brought to the laboratory within six hours for bacteriological analysis. The selected pesticide cypermethrin, was bought from an agrochemical company near Nelpettai in Madurai.

Strain

Pseudomonas stutzeri MTCC 2643 was obtained from MTCC, IMTECH, Chandigarh, India.

Degradation Efficiency

The bacterial strain was inoculated on minimal broth containing different concentrations of cypermethrin. The flasks were then incubated at room temperature for a period of sixteen days and the samples were then subjected for the estimation of pH, biomass, UV-Visible spectrophotometry and HPLC analysis.

pH

The pH of the sample was measured for the different test concentrations using pH meter on alternate days for a period of sixteen days.

Biomass

Biomass of the sample was analyzed by turbidometric method. The absorbance of the test sample on alternate days was measured using a colorimeter at 600nm for sixteen days (Kannan, 2002)

Immobilization

The 0.1ml of the overnight culture of the bacterium was mixed with sodium alginate and mixed well. This was then placed as drops on calcium chloride to produce immobilized cells. The overnight culture was used as the free cells. Both the immobilized and the free cells were then subjected to degradation experiments and were analyzed on alternate days for pH.

UV-Visible Spectrophotometry

The samples containing the minimal medium 500ppm of cypermethrin and the inoculum were taken on the 0th, 10th and 12th days. They were subjected to UV-Visible spectrophotometry and the absorbance was noted on different wavelengths.

HPLC

The samples containing minimal medium, inoculum and 500ppm concentration of cypermethrin were taken on the 0th and 10th days. They were dissolved in equal amount of methanol and were subjected to HPLC analysis by UV detection.

Results and Discussion

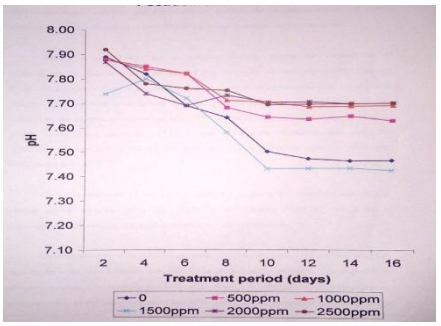

Figure 1 Changes in the pH of the Medium during the Degradation of Cypermethrin by *Pseudomonas stutzeri*

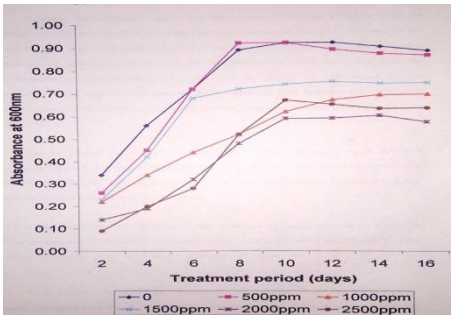

Figure 2 Turbidity during the Degradation of Cypermethrin by *Pseudomonas stutzeri*

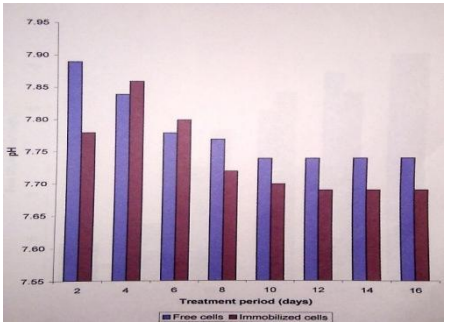

Figure 3 Changes in the pH of the Medium during the Degradation of Cypermethrin by the Free Cells and Immobilized Cells of *Pseudomonas stutzeri*

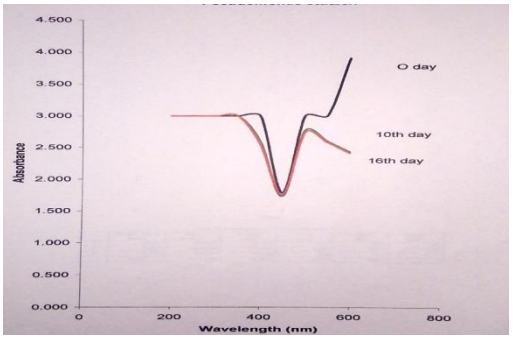

Figure 4 UV Visible Absorption Spectra for the Medium with 500ppm Cypermethrin after 0, 10 and 16 Days after Treatment with *Pseudomonas stutzeri*

The changes in the pH of the medium during the degradation of cypermethrin by *Pseudomonas stutzeri* is exhibited in Fig.1. The pH decreased for a period of about 12days and later remained somewhat constant. The change in pH indicates the process of degradation in which an acid is formed as a product. The cypermethrin during degradation initially converts into 3-Phenoxy benzoic acid and this may be the cause for the decrease in the pH of the medium.

In the measurement of the biomass, the strain showed a gradual growth with increasing turbidity of the medium. The strain had a maximal turbidity value at 500ppm concentration of cypermethrin (Fig.2). The increase in turbidity of the medium elicits that the organism is growing in the medium resisting cypermethrin and also using it as a source of energy as it is grown on the minimal medium and hence causing the degradation of cypermethrin.

The biodegradation for cypermethrin was faster and effective with the immobilized cells than that of free cells. The decrease in the pH was faster in the case of the immobilized cells than that of free cells. (Fig.3) Thus the effectiveness of the degradation by the organism is enhanced in the case of immobilized cells.

Figure 4 shows the UV-Visible absorption spectrum at different wavelengths for the 0^{th}, 10^{th} and 12^{th} day samples. The peak formed by the 10^{th} and 16^{th} day samples were steeper when compared to 0^{th} day sample. This figure directly predicts that there is break down of cypermethrin and formation of different compounds.

In HPLC analysis, the peak details exhibited the degradation of the pesticide and presence of intermediate compounds which were detected by the difference in the retention of the peaks observed. The area and height of the peak indicate the level of intermediate compounds formed during the degradation of cypermethrin.

Discussion

Soil bacteria with the ability to degrade several pesticides have been isolated from soil showing enhanced biodegradation. At high concentrations of cypermethrin the appropriate catabolic enzymes of *Pseudomonas sp.* get repressed. The possible explanation that the microorganism may need an acclimatization period to induce the necessary degradative enzymes and may be because of this reason the prolonged lag phase was observed at high concentrations of the pesticide. The cypermethrin during degradation initially converts into 3-phenoxy benzoic acid and this may be the cause for the decrease in the pH of the medium. In the medium the pH gets decreased more rapidly and from this it may be known that cypermethrin gets degraded rapidly by *P. stutzeri.*

During the process of degradation of cypermethrin, after formation of 3- phenoxy benzoic acid in a particular amount it will be then converted into carbon dioxide. This is why the formation of carbon dioxide is observed only after six days of degradation. The turbidity of the medium indicates the growth of the organism in the medium. The increase in the turbidity of the medium elicits that the organism is growing in the medium resisting cypermethrin and also using it as a source of energy as it is grown on the minimal medium and hence causing the degradation of cypermethrin. Immobilization is a process done to enhance the activity of the cells, that the degradation was effective and rapid in the case of immobilized cells.

In the UV visible spectrophotometry, the peaks found in the 10^{th} and 12^{th} day samples were steeper when compared to the 0-day sample and this may indirectly predict there is a breakdown of cypermethrin and formation of different compounds. HPLC analysis also indicated the degradation of cypermethrin after ten days. The area and height of the peak illustrate the level of intermediate compounds formed during the degradation of cypermethrin.

Conclusion

From these findings it may be concluded that *Pseudomonos stutzeri* can be used in the treatment of pesticide contaminants in industrial effluent and can detoxify agricultural wastes. Such findings may be useful in designing a scale-up *in situ* or on-site hazardous waste by bioremediation process.

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DRINKING WATER CONTAMINATION WITH FLUORIDE AND ITS EFFECTS ON HUMAN

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Abstract

In this study, groundwater quality with reference to fluoride and other chemical parameters such as pH, total alkalinity, hardness, calcium and salinity were tested in samples collected from Bodinayakkanur, a dental fluorosis prone and affected area. Nineteen water samples from various sources viz., well, bore well and hand pump collected in different sites of the study area were examined. The study reveals that all the parameters except salinity are within standard limits in municipal water. Six ground water samples were found to have high fluoride levels above the Indian standard for potable water (1.0 mg/l). The study shows that elevated fluoride level is associated with lowering of hardness and calcium levels in water. The present investigation suggests that people of Bodinayakkanur should be advised to consume only municipal water. Ground water can be used for potable purpose only after its treatment for fluoride. Awareness on the consequences of excessive fluoride intake and its preventive strategies must be given.

Keywords: *groundwater, fluoride, water.*

Introduction

Water is an essential natural resource for sustaining life and environment that we have always thought to be available in abundance and free gift of nature. However, chemical composition of surface or subsurface is one of the prime factors on which the suitability of water for domestic, industrial or agricultural purpose depends. Freshwater occurs as surface water and ground water. Though ground water contributes only 0.6% of the total water resources on earth, it is the major and preferred source of drinking water in rural as well as urban areas. In a country like India, where the majority of population lives in the villages with bare infrastructural facilities, lack of awareness, poor hygiene and sanitation, the concept of safe potable water assumes greater significance. It is estimated that only 77% of urban population and 31% of rural population in India are able to get potable water supply (Gupta *et a*l., 2002).

Ground water forms a major source of drinking water supply for urban and rural population of India due to nonavailability of other water resources and the consideration that surface soil strata acts as a natural filter providing safe and pure drinking water. However, various studies carried out in the previous years have clearly shown that ground water is also becoming highly susceptible to pollution from diffuse sources like deep percolation from intensively cultivated fields. The major sources of high fluoride in water are anthropogenic activities and leaching of soil (Sharma *et al*., 2002). The problem of high concentration of fluoride in underground water sources and the resultant disease 'Fluorosis' has been emerging as one of the most important toxicological and geo-environmental issues throughout the world. But this problem is very serious in India as the majority of the population living in rural areas has to depend on groundwater sources for their water requirements.

Materials and Methods

Study Area

Bodinayakkanur (also spelled as Bodinaickkanur; or shortened to just Bodi) is a Town and a municipality in Theni district in the state of Tamil Nadu, India.

Identification of Fluoride Endemic Areas

A door to door survey was conducted among people in the areas of study to determine the prevalence of dental fluorosis. Drinking water samples from all available sources of the study area were collected.

Sampling Strategy

Nineteen drinking water samples from different sites in Bodinayakanur were collected in precleaned polythene bottles of 500 ml capacity. The water samples collected

included hand pump water, bore well water, well water and municipal drinking water. Alkalinity of the samples was tested at the site itself. Other chemical parameters were analysed after transporting the samples to the laboratory.

Chemical Analysis of Water

The sample collected from different locations in Bodi were analysed for selected chemical parameters adopting standard methods (APHA, 1998).

Results

Table 1 depicts the nineteen sampling sites in Bodi and their codes.The estimated pH, Phenolphthalein alkalinity, Total alkalinity, Hardness, Calcium content, Salinity and fluoride concentration of the nineteen water samples analyzed are given in Table 2 and the mean values of each parameter are shown in Table 3. pH values of all the water samples examined varied from 6.77 to 8.15. A mean pH of 7.49 was obtained for all the water samples tested. Total alkalinity of the water samples was found to vary from 200 to 670 mg/l. Sample no. 5 showed high alkalinity (670mg/l). Six water samples (sample no. 1,3,5,6,10 and 18) showed phenolphthalein alkalinity in the range of 10-30 mg/ l. In the remaining water samples, no phenolphthalein alkalinity was observed. Hardness of the water samples ranged from 224 to 1184 mg/l. Twelve water samples (samples no.1,3,4,5,7,8,9,10,11,12,14 and 17) showed hardness above 600 mg/l and two (sample no.18 and 19) showed a value less than 300mg/l. In the remaining five water samples (sample no. 2, 6, 13, 15 and 16) hardness was found to be within the standard limits. A mean total alkalinity of 445.26 mg/l was obtained for the nineteen water samples examined.

Calcium content of the water samples varied from 29.66 to 150.70 mg/l. Lower levels of calcium (less than 75 mg/l) was found in twelve samples (sample no.2,3,4,5,6,7,9,10,13,15,18 and 19). In the other water samples calcium was found to be within standard limits. The mean calcium content of water samples was 70.33 mg/l. Salinity ranging from 0.73 to 10.34 ppt (mean = 5.31 ppt) was noticed in the water samples examined.

Figure 1 shows the OD values of standard fluoride solutions used for the estimation of fluoride from water samples. Fluoride concentration in the water samples tested ranged from 0.2 to 1.8 mg/l. Six water samples had fluoride content above 1.0 mg/l. They include sample no. 5 (1.6 mg/l), sample no. 6 (1.7mg/l), sample no. 10 (1.4mg/l), sample no.13 (1.8 mg/l), sample no 15 (1.5 mg/l) and sample no.19 (1.7 mg/l). The mean fluoride level was 0.94 mg/l.

Results of correlation analysis between fluoride concentration and other chemical parameters examined are given in Table 4. Correlation coefficients (r) between fluoride concentration and pH, alkalinity, hardness, calcium and salinity were found to be - 0.02, 0.7, - 0.4, - 0.5 and – 0.03 respectively.

Table 1 Sampling Places, Sources, Depths and Codes of Water Samples Collected from Bodinayakkanur

Sample Code	Water Source	Depth (feet)	Place
1	Borewell	150	Ahamalai
2	Municipal drinking water	-	Ammapatti
3	Borewell	250	B. Meenakshipuram
4	Panchayat union handpump	150	Bodi hill north
5	Samuthaya well	75	Body Hill West
6	Tank (water from samuthaya well)	33	Boothipuram
7	Panchayat union handpump	150	Dombuchery
8	Panchayat union hand pump	150	Kamarajapuram
9	Borewell	250	Kodangipatti
10	Well	32	Kooyonoolai
11	Private handpump	155	Kottaiyanur
12	Panchayat union borewell	260	B- Maniyam Patti
13	Panchayat union handpump	150	Melaichockanath apuram
14	Panchayat union hand pump	160	Nagalapuram
15	Panchayat union handpump	150	Rasingapuram
16	Panchayat union handpump	150	Silamalai
17	Panchayat union handpump	150	Sillamarathu patty
18	Borewell	150	Uppukottai
19	Handpump	150	Rasingapuram West

Table 2 Chemical Characters of Water Samples Collected from Bodinayakkanur

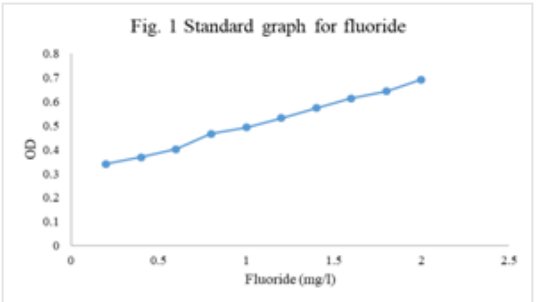
Sample no.	pH	PA$CaCO_3$/l	TA as $CaCO_3$ mg/l	Hardness (mg/l)	Ca (mg/l)	Salinity (ppt)	Fluoride (mg/l)
1.	7.90	30	420	800	84.97	7.03	0.5
2.	7.75	-	380	350	61.72	0.73	0.3
3.	7.92	20	470	940	37.67	4.92	0.6
4.	7.58	-	540	720	49.70	4.92	1.0
5.	7.77	10	670	720	29.66	7.53	1.6
6.	7.94	10	390	520	36.87	3.53	1.7
7.	6.77	-	560	650	72.14	3.03	0.7
8.	7.17	-	490	756	88.18	6.92	0.7
9.	7.35	-	410	930	63.33	7.23	1.0
10.	8.15	20	510	1000	69.74	10.34	1.4
11.	7.28	-	420	664	88.18	6.02	0.2
12.	7.83	-	250	630	117.83	4.63	0.2
13.	7.42	-	410	300	32.06	1.64	1.8
14.	7.11	-	390	890	141.88	6.35	0.8
15.	7.08	-	520	590	33.67	6.92	1.5
16.	7.15	-	460	530	82.56	5.43	0.8
17.	6.85	-	600	1184	150.70	10.03	0.8
18.	8.11	20	370	224	34.47	1.02	0.5
19.	7.18	-	200	284	60.92	1.02	1.7

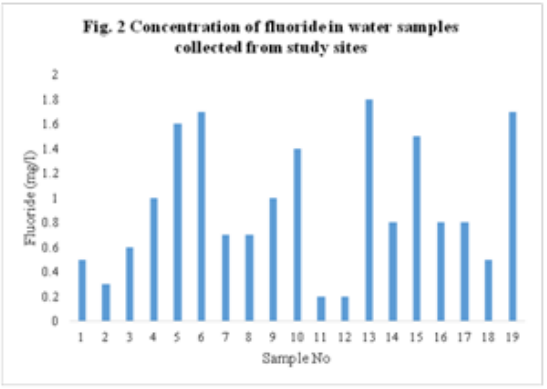
Table 3 Mean Values of Chemical Parameters of Water Samples Collected from Bodinayakkanur

S.No.	Chemical parameter	Mean Value
1.	pH	7.49
2.	Total alkalinity as CaCO3 (mg/l)	445.26
3.	Total Hardness as CaCO3 (mg/1)	667.47
4.	Calcium (as Ca) (mg/1)	70.33
5.	Salinity (ppt)	5.31
6.	Fluoride (mg/1)	0.94

Table 4 Results of Correlation Analysis between Fluoride and Other Chemical Parameters

S.No	Chemical Parameter	Mean Value
1.	pH	-0.02
2.	Total alkalinity as $CaCO_3$ (mg/l)	+0.07
3.	Hardness as $CaCO_3$ (mg/1)	-0.04
4.	Calcium (as Ca) (mg/1)	-0.5
5.	Salinity (ppt)	-0.03





Discussion

We often think of water as a matter of taste, clarity and odour and in terms of other properties which determine whether water is fit for drinking. For other uses different properties may be important. Most of these properties depend on the kinds of substances that are dissolved or suspended in the water. Water quality should always be taken in relation to the purpose for which the water is used because water suitable for one purpose may not be suitable for the other. For example, the water that we drink can be used for irrigation, but water used for irrigation may not be suitable for drinking. Pure water is tasteless and odourless. However, water is never found in a pure state in nature. Both groundwater and surface water

may contain many constituents, including micro organisms, gases, inorganic and organic materials. Water quality is assessed by measuring the amounts of various constituents contained in water. These amounts are often expressed as milligrams per litre (mg/l), which is equivalent to the number of grams of a substance per million grams of water. The suitability of water for a given use depends on many factors such as hardness, salinity and pH. Acceptable values for each of these parameters for any given use depend on use and not on the source of the water. Drinking water is regulated by guidelines stringent enough to protect human health. In the present study. quality of ground water and municipal water in Bodinayakkanur was tested and reported by comparing the drinking water quality guideline (Bureau of Indian Standards,1991).

pH

High pH and alkalinity of drinking water are not harmful to human beings. However, a low value of pH below 4.0 produces a sour taste and higher values of pH and alkalinity hasten the scale formation in pipes and water heating apparatus and also reduce the germicidal potential of chlorine. pH below 6.5 starts in corrosion in pipes. pH and alkalinity are also important in fixing alum dose in drinking water treatment (Trivedy and Goel, 1984).

While Sharma *et al*. (2002) reported a pH of 7.0 – 8.5 in the ground water of Unnao in Uttar Pradesh, Mariappan and Vasudevan(2002) found a pH ranging from 7.91 -9.10 in the ground water of dental fluorosis – affected Panamarathupatty block of Salem district in Tamil nadu. Santhi *et al* (2002) reported a pH of 7.34- 8.88 in the ground water of fluorotic areas and 7.70 to 8.49 in control areas of Vallioor union of Thirunelveli district in Tamil Nadu. Saxena and Shravastava (2002) studied ground water quality in Bhopal and found a pH of 6.2-8.5.In our study, the pH values obtained (6.77-8.15) are in general agreement with the above discussed literature for pH. Also, the pH values obtained are well within acceptable limit (BIS, 1991).

Total Alkalinity

Alkalinity in itself is not harmful to human beings (Pande and Sharma, 1999). Alkalinity is basically the dissolved minerals in water that help to neutralize the water. Sometimes it is favourable to have high values of alkalinity because it enhances the buffering capacity of water. Alkalinity ranges of 115 -500 mg/l,150 -200 mg/l and 390 – 660 mg/l were reported in the groundwater of Armori town in Maharashtra (Patil *et al*., 2001),drinking water of Thittagudi in Tamilnadu (Rani *et al*., 2002) and Kadathur canal water of Amaravathi river in Tamil Nadu (Karthikeyani *et al*., 2002) respectively. These values when compared with the total alkalinity values obtained in the present investigation are almost similar.

In our study, thirteen water samples (sample no 2,4,7,8,9,11,12,13,14,15,16,17 and 19) showed zero phenolphthalein alkalinity indicating the absence of carbonates in them. Thus, their alkalinity was mainly due to bicarbonates. In the rest of the six water samples, both carbonates and bicarbonates contributed to their alkalinity. The alkalinity of the water sample was within permissible limit except one (sample no 5), which showed an alkalinity value above 600mg/l. This was accompanied with increased fluoride concentration (1.6 mg/l in sample no 5). Values of alkalinity were lesser than hardness in sixteen samples (sample no 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, and 19). This suggests that in these samples, neutral salts of calcium and magnesium may be present as sulphate (Rani *et al*., 2002). In the remaining three samples (sample no 2, 13 and 18) alkalinity was found to be greater than hardness and may be due the presence of basic salts of sodium and potassium in addition to calcium and magnesium (Manivasakam, 1984).

Hardness

Hardness has no adverse effects on health. But it is undesirable due to the formation of heat retarding insulating scales in boilers and other exchange equipment, Hard water is not suitable for domestic use in washing, cleaning and laundering as it prevents the lather formation with detergents. Hardness is sometimes advantageous also as it prevents corrosion in pipes by forming a thin layer of scales (Trivedy and Goel, 1984).

Literature pertaining to the hardness of the water reveals an array of values such as 106-494 mg/l in the groundwater of Unnao in Uttar Pradesh (Sharma et al., 2002), 120-447 mg/l in the Kadathur canal water of Amaravathi river in Tamil Nadu (Karthikeyani et al., 2002).In the present study, a much higher range of hardness was obtained (224-1184 mg/l). It is noteworthy that two water samples (sample no. 18 and 19) showed hardness less than 300 mg/l and among these two, one sample (sample no 19) showed an elevated fluoride concentration of 1.7 mg/l.

Calcium

Calcium is one of the important nutrients required by organisms. It plays a major role in imparting hardness to water as calcium carbonate. High levels of calcium in natural waters are rare (Trivedy and Goel, 1984). In our study the calcium range in water samples (29.66-150.70 mg/l) was different when compared to 40.72-126.99 mg/l in the groundwater of Chitrakoot in Satna (Tripathi *et al*., 1996), 84.24-88.00 mg/l in Gadchiroli lake of Maharashtra (Patil and Tijarae, 2001) and 24.05-119.44 mg/l in the drinking water of Thittakudi in Tamil Nadu (Rani *et a*l., 2002).

Calcium content lower than 75 mg/l was noticed in twelve water samples (sample no. 2, 3, 4, 5, 6, 7, 9, 10. 13, 15, 18 and 19) in our study and out of these, six water samples (sample no. 5, 6, 10, 13, 15 and 19) showed increased fluoride concentration (above 1.0 mg/l). This shows that calcium content decreased with increase in fluoride concentration in water. The remaining seven water samples (sample no. 1, 8, 11, 12, 14, 16 and 17) showed calcium level less than the maximum permissible limit of 200 mg/l (BIS, 1991) and were associated with fluoride concentration less than 1.0 mg/l.

Salinity

Salinity is the amount of dissolved salts found in 1 kg of water. Salinity or salt content is expressed in parts per thousand (ppt) because there are 1000 gms in 1 kg. The average salinity of sea water is 35 ppt. Fresh water salinity is usually less than 0.5 ppt. Drinking water has to have salinity less than 0.2 ppt. Santhi *et al*. (2002) reported a salinity range of 0.49-1.49 ppt in the groundwater of Vallioor union of Thirunelveli district in Tamil Nadu. Rani *et al*. (2002) found 0.12- 0.58 ppt salinity in the drinking water of Thittakudi in Tamil Nadu. In our study, the salinity range was contrasting and very high also (0.73-10.34 ppt). From the salinity values it is clear that all the water samples including municipal water contained high amounts of salts. High salinity may be responsible for scale formation in the water stored vessels.

Fluoride

Fluoride is one of the important factors in water quality management due to its adverse health effects (Nemade, 1996). The effects of extensive intake of fluoride range from bone stiffness and rheumatism to permanent crippling and kidney damage (Odonnel, 1973; Waldbott, 1973). Dental fluorosis is most common occuring due to the presence of excess fluoride in public water supply (Elevove, 1940). Fluoride concentration in groundwater in India varies considerably. In some parts the fluoride levels were below 0.5 mg/l while at certain other places values as high as 20.0 mg/l were reported (Sarma, 1999). Santhi *et al*. (2002) found 2.39-5.66 mg/l and 0.74-1.20 mg/l fluoride in groundwater of fluorotic and control areas respectively in the Vallioor union of Thirunelveli district in Tamil Nadu. Latha *et al*. (1999) reported groundwater fluoride concentration of 1.0-7.4 mg/l in Karnataka. In the present investigation, fluoride range obtained (0.2-1.8 mg/l) is not very high when compared with the above ranges. Only 31% (6 out of 19) of the water samples analysed, showed elevated levels of fluoride (sample no. 5, 6, 10, 13, 15 and 19). Most of the people residing in the localities where the samples were collected showed signs of dental fluorosis. Groundwater from the sources considered in the present study was being used for drinking purposes a few years ago.

Most of the people in the study area are drinking municipal water nowadays which has fluoride content (0.3 mg/l) and other chemical parameters within normal limits. Though not for drinking purpose, the bore well and hand pump waters are being used for cooking and washing vegetables by many residents of Bodinayakkanur and it is to be pointed out here that fluorides in water are not destroyed by heating or boiling.

Correlation Analysis

The correlation of fluoride concentration with total alkalinity, hardness and calcium content in water samples is significant. Results of correlation analysis were in accordance with the report given by Teotaia and Mandsingh (1981) that when fluoride level in water is high, the water will be highly alkaline and become softer.

Swain et al. (2002) found a positive relationship between pH and fluoride concentration in water. But in the present study a negligible relationship was obtained between pH and fluoride content (r = -0.02).

Gupta (1991) found that fluoride content increased with decrease in salinity. However, in our study very less negative correlation (r = -0.03) was obtained between salinity and fluoride concentration indicating negligible relationship between salinity and fluoride concentration.

The chemical analysis of the water samples collected in Bodinayakkanur reveals that the distribution of fluoride, pH, total alkalinity, hardness, calcium and salinity are not even in the groundwater in the study area. The people of this area have been exposed to high fluoride from some source since many years and hence, there is prevalence of

dental fluorosis in the area. Municipal water is safe for drinking with reference to fluoride and other chemical parameters studied. The six water samples which showed high fluoride contents (above 1.0 mg/l) suggested that groundwater may be the source for fluoride exposure to the residents of Bodinayakkanur and such water is not advisable to be used for drinking and cooking purposes. Awareness about consequences of high fluoride in water and preventive strategies of dental fluorosis should be given to the people of Bodinayakkanur. They should be advised to use municipal water for potable purpose. During water scarcity, groundwater can be used for drinking and cooking only after suitable physical, chemical or biological treatment for fluoride. The urgent attention of the government is required to improve groundwater quality.

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PHYSICAL AND CHEMICAL ANALYSIS OF VAIGAI RIVER WATER IN MADURAI DISTRICT, TAMIL NADU, INDIA

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Abstract

One of the most important challenges that human beings confront today is providing clean water to a vast population of the universe. The demand for fresh water is a serious problem in today's world. Because of industrialization the wastewater continuously discharges into rivers, canals, estuaries, and other water bodies. The main aim of the present study is to monitor the physical and chemical characteristics of Vaigai river water in Madurai district, Tamil Nadu, India. The parameters like color, odor, total dissolved solids (TDS), pH, dissolved oxygen, BOD, COD, total alkalinity, calcium, magnesium, iron, manganese, free ammonia, nitrates, chlorides, fluorides and sulfates were analyzed. All the water samples analyzed had very high turbidity values. It was found to be between 149 to 605 mg/l was observed in the stations 3, 5, 6 and 7. The Hardness of the water samples collected from stations 5, 6 and 7 were higher than the acceptable limits in the range of 223 to 265 mg/l. Total alkalinity was high in the station 7 (263 mg $CaCO_3$/l). water sample of station 4 had 2.2 mg/l ammonia, 4 mg/l nitrates and 0.85 mg/l phosphates which are higher than standard. Water samples of the stations 6 and 7 had higher fluoride levels than the standard level. The order of potability of drinking water as analyzed from the results is as follows station one> station6> station7> station5> station3> station2.

Keywords: *Vaigai River, physicochemical characteristics, TDS, BOD, COD*

Introduction

Water is one of the most important and plentiful compounds of the environment. It is also a vital resource for agriculture, manufacturing sites, and other human activities. It is the most significant constituent of all living beings. Most of the biochemical reactions that take place during the metabolism and growth of living organisms require water. The river is the most significant and susceptible freshwater system that plays a serious role in the nourishment of all life. In urban areas, the careless discharge of industrial effluents and other wastes into rivers affect the quality of water (Agarwal and Saxena, 2011).

Begam and Selvakumar (2014) reported that the water quality of Indian rivers has been deteriorating due to persistent release of domestic sewage, agricultural and industrial wastes. The main problems related to river water are firm suspensions, turbidity, lack of oxygen, an excess of carbon dioxide and organic biomass. By the analysis of physicochemical factors, the presence of xenobiotic compounds in the river water has reduced its useful microflora (Venkateshraju *et al*., 2010). The deteriorating physicochemical characteristics of the river water support a heavy microbial load. The discharge of sewage and effluent into river changes the physicochemical properties of water which is not fit for human utilization (Islam *et al*., 2012). Rajini *et al*., (2010) documented that the excellent quality of river water is a declaration in human health, the defense of the ecosystems and sustainable development.

The quality of river water is influenced by a variety of natural factors like rain, temperature, and braving of rocks and human behavior which change the hydrochemistry of river water (Gupta *et al*., 2011). The chemical waste products like cyanide, zinc, lead, copper, cadmium, and mercury from the industrial process are often discharged into the river. The fish and other animals are killed by a high concentration of these heavy metals. Unexpected urbanization and rapid increase of industrialization add to river contamination crisis (Bhattacharya *et al*., 2012).

World Health Organization (WHO) recommended that *Escherichia coli* and total coliforms, chlorine residual, turbidity, pH, dissolved oxygen content and temperature are the essential parameters for determining the quality of drinking water (WHO, 2003).

Water quality of rivers in different places was reported by many researchers. For good and long life, a good quality water is extremely vital. Present scenario of Vaigai is that it is highly polluted by various pollutants and anthropogenic activities. Therefore, the present work has been planned to analyze the physical and chemical characteristics of Vaigai River water.

Materials and Methods

The water samples were taken from Vaigai River. Seven samples were collected and analyzed for physico-chemical and bacteriological parameters.

Sampling

The water samples were collected from Vaigai Dam, Thuvariman, Theekkathir, Arapalayam, Sellur, Goripalayam, and Anna Nagar. The samples were taken from the depth of 5-10 cm below the surface of the water. Sampling methods recommended by APHA (1998), were adopted for the collection and analysis of water samples. Before the acquiring the samples, the bottles were washed with acid for sterilization. Sampling was done between the months of December 2015 and March 2016. After sampling, the samples were kept in an ice box and transferred immediately to the laboratory for analysing physicochemical properties such as Total Suspended Solids (TSS), Temperature, Turbidity, pH, Electrical Conductivity (EC), Total Alkalinity, Total Hardness, Calcium, Magnesium, Ammonia, Nitrites, Nitrates, Chlorides Fluorides, Sulphates and Phosphates. Table 1 shows the Name and the Station code of water samples collected in Vaigai River. The sampling sites are depicted in Fig. 1.

Table 1 Sampling Stations

S.No.	Station Code	Name of the Station
1	S1	Vaigai Dam
2	S2	Thuvariman
3	S3	Theekkathir
4	S4	Arapalayam
5	S5	Sellur
6	S6	Goripalayam
7	S7	Anna Nagar

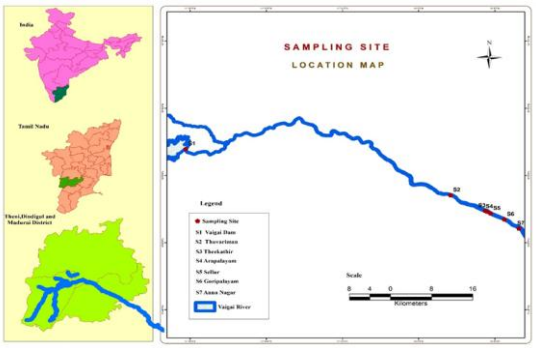

Figure 1 Map showing Vaigai River sampling stations

Determination of Total Coliforms by Multiple Tube Tests

For the preparation of a single strength lactose broth, and a series containing nine tubes, three tubes of LB 2X-10 ml; 3 tubes of LB 1X – 1 ml; 3 tubes of 1X - 0.1 ml was made. All aliquots were serially diluted according to protocol and incubated for 48 hours at 37˚C.

Identification of Bacteria using Spread Plate Technique

Media used in the experiment were UTI, EMB, MacConkey, SS and TCBS agar. An appropriate amount of media was prepared and sterilized by autoclaving. The water samples were diluted up to 10^{-6}. 1ml of the diluted sample was inoculated in the plate containing the appropriate medium. The inoculated sample was uniformly spread over the plate with the help of glass spreader. The plates were incubated at 37˚ C for 24 hours.

Results and Discussion

During the different seasons of December (post-monsoon), January and February (winter) and March (summer), the physicochemical and microbiological parameters were examined. Table 2 and 3 show the turbidity obtained ranging from 2 to 12.5 NTU. The turbidity value exceeded the acceptable limits in all the stations. All the stations had the pH value within the acceptable limits. Most of the samples had fewer amounts of total dissolved solids, but the high quantity of total dissolved solids was observed in stations 3, 5, 6 and 7 in various seasons. The total alkalinity ranged from 35 to165 mg/l in all the stations except station 7. The total hardness of water samples from the stations 5, 6 and 7 crossed the acceptable limits of 218 to 265mg/l. The level of calcium and magnesium were within the acceptable limits prescribed by Bureau of Indian Standards (BIS) presented in Table 4.

Table 2. Seasonal variations among Water Quality Protection Standard (WQPS) for water samples

WQP	S1			S2			S3			S4		
	POST MON	WIN	SUM	POST MON	WIN	SUM	POST MON	WIN	SUM	POST MON	WIN	SUM
Appearance	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid
Odour	Foul	Foul	Foul	Foul	Foul	Foul	Foul	Foul	Foul	Foul	Foul	Foul
Turbidity (NTU)	**2**	3	**5**	3	3.75	4.5	3	4	5	**9**	**11**	**12.5**
Temperature (◦C)	25	26.5	30	26	25	30	24	26	29	25	26	29
TDS (mg/l)	145	148	149	446	449	450	**502**	**505**	**507**	265	267	268
EC (micro mho/cm)	208	211	213	638	641	644	718	721	725	378	381	383
pH	6.20	7.7	8.5	6.15	7.4	8	6.94	7.65	8.15	7	7.6	7.94
Total Alkalinity (mg/l)	35	41	45	96	101	103	115	121	124	38	41.5	45
Total Hardness (mg/l)	48	51	53	158	161	165	179	180.5	183	98	1.1	103
Calcium (mg/l)	11	14	17	37	40.5	43	41	45.5	47	23	26	29
Magnesium (mg/l)	4	5.5	7	12	16	19	15	18	20	8	11	14
Ammonia (mg/l)	0.1	0.25	0.4	0.1	0.3	0.5	0.1	0.24	0.4	0.5	**2.2**	**3.3**
Nitrate (mg/l)	1	2.5	4	3	5	7	6	8.5	10	2	4	7
Nitrite(mg/l)	0.2	0.35	0.5	0.1	0.25	0.4	0.1	0.3	0.5	**0.6**	**0.95**	**0.9**
Chloride (mg/l)	38	42.5	47	117	121	125	128	131	133	58	61	63
Fluoride (mg/l)	0.7	0.85	0.8	0.7	0.85	0.9	0.7	0.85	0.9	0.6	1.25	**3**
Sulphate (mg/l)	4	6	8	12	15	17	15	19	21	9	12	15
Phosphate (mg/l)	0.1	0.15	0.3	0.1	0.25	0.4	0.2	0.4	0.5	**0.6**	**0.85**	**0.9**
MPN	6	6	6	9	17	14	9	14	12	**9**	**7**	**17**

Note: POST MON = post monsoon; WIN = winter; SUM= summer

Table 3. Seasonal variations among Water Quality Protection Standard (WQPS) for water samples

WQP	S5			S6			S7		
	POST MON	WIN	SUM	POST MON	WIN	SUM	POST MON	WIN	SUM
Appearance	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid	Slightly turbid
Odour	Foul	Foul	Foul	Foul	Foul	Foul	Foul	Foul	Foul
Turbidity(NTU)	9	11	12	3	5	5	2	4.25	6
Temperature(-C)	25	25	29	25	26	30	26	25	27
TDS (mg/l)	552	554	556	530	533	535	600	603	605
EC (micro mho/cm)	788	792	795	759	761	763	858	861	865
pH	6.91	7.6	8	6.8	7.29	7.9	6.80	4.85	8
Total Alkalinity(mg/l)	168	170	123	157	161	165	258	260	263
Total Hardness(mg/l)	228	231	233	218	220	223	258	261	265
Calcium(mg/l)	56	59	62	53	56	58	63	66	67
Magnesium(mg/l)	20	22.5	25	19	21.5	23	22	26	29
Ammonia(mg/l)	0.3	0.5	0.7	0.2	0.45	0.6	0.3	0.25	0.5
Nitrate(mg/l)	5	7	9	7	9	12	7	10	13
Nitrite(mg/l)	0.3	0.5	0.5	0.2	0.45	0.4	0.1	0.25	0.3
Chloride(mg/l)	118	121	125	113	117	119	118	121	125
Fluoride(mg/l)	1	0.95	0.9	0.6	1.4	2	1	1.5	2
Sulphate(mg/l)	13	15.5	19	11	13	15	14	17	20
Phosphate(mg/l)	0.1	0.24	0.5	0.2	0.4	0.6	0.1	0.3	0.5
MPN	9	9	12	6	7	9	7	9	6

Note: POST MON = post monsoon; WIN = winter; SUM= summer

Table 4. Standard parameters for water characterization

S.No	Parameters	Standard values	
		BIS	WHO
1.	Appearance	Slightly turbid	Slightly turbid
2.	Odour	Unobjectionable	Unobjectionable
3.	Turbidity (NTU)	5	2.5
4.	Total dissolved Solids (mg/l)	500	500
5.	Electrical conductivity (µ mho/cm)	750-2250	500
6.	pH	6.5-8.5	6.5-8.5
7.	Total Alkalinity(mg/l)	200	250
8.	Total Hardness(mg/l)	300	500
9.	Calcium(mg/l)	75	75
10.	Magnesium(mg/l)	30	30
11.	Ammonia(mg/l)	0.5	0.5
12.	Nitrate(mg/l)	45	45
13.	Nitrite(mg/l)	0.5	0.5
14.	Chloride(mg/l)	250	250
15.	Fluoride(mg/l)	1.5	1.5
16.	Sulphate(mg/l)	200	250
17.	Phosphate	0.5	0.5
18.	Most Probable Number (ml)	10/100	10/100

The sample collected from station 4 had a high level of ammonia during the winter and summer seasons, but in station five the ammonia level was higher in the summer season. The increased level of nitrates was observed in station 4. In station 4 and 6, the highest phosphates level was noted. In the stations 6 and 7, the fluorides level was found to be high. Table 2 and 3 represent that all the stations had the value of nitrates, chlorides, and sulfates within the acceptable limit. The turbidity of the water is frequently used to measure water quality. The desirable level is less or equal to 1 NTU as recommended by WHO. Turbidity value up to 5 NTU will indicate inadequate of the treatment plant and possibly correlate with increased total coliform bacteria (McCoy *et al.,* 1986). In this study, the turbidity value for all the stations was up to 5 NTU. The minimum turbidity value was recorded in station 1 (Vaigai dam), and the station 4 indicates maximum level (Arapalayam).

Kataria *et al.,* (1996) reported that the increased level of TDS indicates pollution by external sources. The high amount of dissolved solids of samples adversely affects the running water making it unfit for irrigation, drinking and any other purpose. In the present study, TDS values of stations 3, 5, 6 and seven were high. The alkalinity of natural water is mainly due to the salts of weak acids, although weak or strong bases may also contribute. Water containing more than 200 mg/l of total alkalinity is not considered desirable for drinking purpose (BIS, 1983). In the present study, the alkalinity of the sample collected from station 7 crossed the standard value. So, the water is not advocated for drinking purpose.

The Hardness of water mainly depends upon the amount of calcium or magnesium or both the salts (Sawyer *et al.,* 1994). Although hard water has no known effects on health, it is unsuitable for domestic use. The total hardness value was exceeding more than 300mg/l is not recommended for drinking purpose. In the present study, the hardness level in the stations 5, 6 and 7 was found to be higher than the acceptable limit.

Since fluoride concentration is a substantial aspect of the geochemistry of water, its impact on human health is very enormous. High fluoride content causes fluorosis (Fawell *et al*., 2006). Safe limit of fluoride concentration is from 0.60 to 1.20 mg/l in drinking water. BIS prescribed limit is in between to 1.0 mg/l and 1.5 mg/l. An increased ammonium-nitrogen value in water bodies is due to the decomposition of proteinous and nitrogen-rich compounds present in organic waste discharge. The ammonia level was higher in the stations 4 and five compared to the standard limits.

The sulfate ion is one of the significant anions present in natural water and produces a cathartic effect in human beings when it is present in excess. The higher values of sulfate content may be contributed due to biochemical, anthropogenic sources, and industrial processes. The

sulfate level recorded in our study is within the acceptable limit. Nitrite is the most important source of biological oxidation of organic nitrogenous materials (Peavey *et al*., 1985; Lalitha *et al.,* 2003). In this study, the nitrite level was found to be higher in station 4. Total coliforms in water sample were analyzed using multiple tube tests. According to WHO standards, standard total coliform count for drinking water is 10/100 ml (Table 2 and 3). It is evident that water sample from station 1 had low coliforms, and the highest total coliforms were found in stations 2, 3 and 4 when compared with other water samples.

Table 5. Microbial analysis of water samples from different stations

Sampling Station Code	*Escherichia coli*	*Klebsiella pneumonia*	*Salmonella* sp.	*Shigella* sp.	*Vibrio cholerae*	*Vibrio parahaemolyticus*
S1	-	+	-	-	-	-
S2	+	-	-	-	+	+
S3	+	-	+	-	+	-
S4	-	+	+	+	-	+
S5	+	-	+	-	+	-
S6	+	+	+	-	-	+
S7	+	-	+	-	+	-

+ = Presence; - = Absence

Table 5 presents the observation of coliforms and other pathogens identified during the bacteriological analysis of water samples. Coliforms such as *Escherichia coli* was isolated and identified in EMB agar, *Salmonella* sp. and *Shigella* sp. were identified using *Salmonella-Shigella* agar. TCBS agar was used for the identification of *Vibrio cholerae* and *Vibrio parahaemolytics*. *Klebsiella* sp. was identified by UTI agar and MacConkey agar.

Total viable bacterial counts and total coliforms encompass the standard microbiological examination of water resources. The presence of *E. coli* and *P. aeruginosa* species specify that the drinking water is most possibly polluted with human and animal feces. Three stations reported values of 1.1 and 7.1 CFU/100 mL as its lowest and highest average number or total coliform and fecal *E. coli* respectively. and these numbers exceeded the international endorsed consumption level for drinking water reported by WHO (1996).

The percentages of the main bacterial species isolated from different water sources were *E. coli* 27%, *Klebsiella pneumoniae* 30%, *P. aeruginosa* 23%, *Citrobacter freundii* 14%, *P. fluorescens* 3%, and *Serratia plymuthica* 3% (Al-Aragi *et al*., 2012). The diarrheal disease occurs from consumption of contaminated drinking water with various types of microbial agents especially *E. coli* (Blake *et al*., 1980). Sadeghi *et al*. (2007) reported that some fecal microorganisms such as viruses and protozoa might be more tolerant of management with chlorine than the indicator bacteria. The bacteriological analysis described that even a low level of contamination may be a risk for an outbreak of diarrhea like cholera (WHO, 2004). The polluted water contains a large amount of organic matter that provides an excellent source of nutrition for the growth and multiplication of microbes as suggested by Thomas (2007).

MacConkey agar used for the identification of total coliform bacteria the appearance of small pink colonies (*E.coli*), large pink mucoid colonies (*Klebsiella pneumoniae*), Eosin Methylene and Blue agar plates for *E.coli* with green metallic sheen colonies. The bacteriological analysis in Vaigai River water revealed the presence of *E. coli* and other bacterial pathogens such as *Klebsiella, Enterobacter*, *Vibrio* sp. *Salmonella* and *Shigella Sp*. The people of Madurai district entirely depend upon the water from the Vaigai River for agricultural and domestic activities. Nevertheless, the physicochemical parameters of water were found to be above the standards of the WHO and BIS. There is continuous practice of improper hygiene, drainage system and the release of septic tank water in the river. Also, the results show that the water is surely unfit for drinking purposes without any form of treatment. So it is very much necessary to conduct more research on this river water quality to create awareness among the people.

Conclusion

The order of quality water as analysed from the results is as follows station 1> station 6> station 7> station 5> station 3> station 2> station 4. Biological monitoring should be coupled with physicochemical characteristics monitoring to establish the supply of safe drinking water. There is a need for periodic monitoring, adequate treatment, and management.

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PHYSICO–CHEMICAL CHARACTERISTICS OF ELECTROPLATING INDUSTRIAL EFFLUENT

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Abstract

The electroplating industry generates a large portion of wastewater containing heavy metals. The present study deals with the physicochemical characterization of electroplating industrial effluent and its contaminated soil. The physicochemical parameters like color, odor, pH, electrical conductivity, TDS, BOD, COD, calcium, magnesium, chlorides, sulfates, phosphates, oil and grease, chromium, nickel, and zinc content were above the permissible limits of CPCB and are responsible for groundwater pollution.
Keywords*: Electroplating industrial effluent, chromium, nickel, zinc, CPCB, BOD, COD and physicochemical characteristics.*

Introduction

Contamination of environments is one of the major issues in the world, and it is increasing day by day due to urbanization and industrialization. Over the last few decades, large-scale use of chemicals through various human activities has grown very fast, particularly in a country like India which underwent rapid industrialization to sustain an overgrowing population (Mustafa *et al*., 2010). Only about 30-40% of the metals are effectively utilized during plating processes, making it a grave concern. The rinsing waters used in the electroplating process are contaminated by the remaining percentage of the metals. In accordance with environmental regulations worldwide, the rinse water containing more than 1000 mg/L of toxic heavy metals must be controlled to an acceptable level, before being discharged to the environment (Dermentzis *et al*., 2011). The released heavy metals are one of the crucial factors that exert adverse influences on man and his surroundings causing toxicity to plants and other forms of biota that are continually exposed to toxic heavy metals (Chandra *et al*., 2010).

Although, the wastewater treatment system in Indian industries is advocated to be efficiently installed to meet the wastewater release norms, at present only 10% of the wastewater generated is treated. The rest of unprocessed water is released as it is into nearby water bodies (Mehta and Bhardwaj, 2012). Using industrial effluents for irrigation is a systematic approach of finding an use for the substantial quantities of N,P,K and Ca along with other essential elements present in it (Niroula, 2003).

This is an attempt to study the physicochemical characteristics of electroplating industrial effluent collected in Madurai. According to the guidelines prescribed by CPHEEO/CPCB, the pollution level of the effluent and effluent contaminated soil was compared with that of permissible level.

Materials and Methods

Collection of Effluent and Soil from Electroplating Industries

Soil and effluent samples were collected from electroplating industries located at Jaihindpuram area in Madurai. Samples were collected in pre-sterilized polyethylene containers and brought to the laboratory immediately.

Analysis of Physicochemical Parameters

Physico-chemical parameters like appearance, colour, odour, turbidity, total dissolved solids, electrical conductivity, pH, total alkalinity, total hardness, free ammonia, nitrates, chlorides, fluorides, sulphates, phosphates, BOD, COD, oil and grease, sulphides, cyanide, and the levels of metals like copper, zinc, chromium, nickel, cadmium, silver, calcium, magnesium and iron were analyzed in effluent samples adopting standard methods (APHA, 1998) and the metal content was analyzed using ELICO atomic absorption spectrophotometer. A similar analysis was carried out in soil samples collected from effluent contaminated sites.

Results and Discussion

Table 1 and 2 show the physical and chemical characteristics of electroplating industrial effluent respectively. The pH of the sample was 6.5 and within the range of recommended pH limits (6.0 – 9.0). The BOD and COD of the samples, 50 and 150 mg/l were exceeding the permissible limits (BOD – 30 mg/l, COD – 100 mg/l) respectively. The measure of total dissolved solids and electrical conductivity were exceeding the standard limits. Whereas, the values of free ammonia and phosphates like 0.5 and 0.5 mg/l respectively were within the range of recommended limits. The amount ofsulfates, chlorides, oil, and grease were exceeding the permissible limit of 800, 6000 and 10 mg/l respectively.

Table 1 Physical Parameters of Electroplating Industrial Effluent

S. No	Physical Parameters (unit)	Values	CPHEEO/ CPCB limits
1	Appearance	Clear liquid	-
2	Colour	Greenish	-
3	Odour	Objectionable	Unobjectionable
4	pH	6.5	6.5
5	Turbidity (NT units)	15	2.5 - 10
6	Total dissolved solids (mg/L)	14490	500
7	Electrical Conductivity (Micro ohm/cm)	20700	-

CPHEEO – Central Public Health Environment Engineering Organization

ii) CPCB –Central Pollution Control Board

Table 2 Chemical parameters of electroplating industrial effluent

S.No	Chemical parameters (unit)	Values	CPHEEO/CPCB Limits
1	Total alkalinity (mg/L)	200	200
2	Total hardness (mg/L)	5000	200
3	Free ammonia (mg/L)	0.5	-
4	Nitrates (mg/L)	30	45
5	Chlorides (mg/L)	6000	200
6	Fluorides (mg/L)	0.8	1
7	Sulphates (mg/L)	800	200
8	Phosphates (mg/L)	0.5	-
9	BOD (mg/L)	50	30
10	COD (mg/L)	150	100
11	Oil & grease (mg/L)	13	10
12	Sulphides (mg/L)	<0.04	2
13	Cyanide (mg/L)	Nil	0.2

Table 3 exhibits the concentration of metals in the effluent sample. The levels of the metals such as zinc, chromium, nickel, silver, calcium, magnesium and iron (787.60, 84, 2599, 0.16, 1200, 480 and 2 ppm) were exceeding the permissible limits (5, 0.1, 3, 0.1, 70, 0.4 and 0.3 ppm) respectively. The concentrations of the metals such as copper and cadmium (1.05 and 0.12) were within the range of recommended (3 and 1 ppm) limits.

Table 3 Metal concentration in electroplating industrial effluent

S.No	Metals	Concentration (ppm)	CPHEEO/CPCB Limits (ppm)
1	Copper	1.05	3
2	Zinc	787.60	5
3	Chromium (VI)	84	0.1
4	Cadmium	0.12	1
5	Nickel	2599	3
6	Silver	0.16	1.2
7	Calcium	1200	70
8	Magnesium	480	0.4
9	Iron	2	0.3

Table 4 displays the physicochemical parameters of electroplating industrial effluent contaminated soil samples. The levels of nitrates, phosphates, and potassium in the soil samples were noted as 64.77, 4.31 and 150 ppm respectively. Table 5 divulges the concentration of metals in the soil samples. Metals like copper, zinc, chromium, cadmium, nickel, silver, calcium, magnesium, iron and manganese were noted as 232, 5360, 372, 30, 11.4, 66, 847.2, 4470, 11.74 and 16.56 ppm respectively.

Table 4 Physico-Chemical Parameters of Electroplating Industrial Effluent Contaminated Soil

S. No	Physico-Chemical Parameters (unit)	Values
1	Texture	Sandy loam
2	pH	6.7
3	Electrical Conductivity (dSm^{-1})	0.60
4	Nitrates (ppm)	64.77
5	Phosphates (ppm)	4.31
6	Potassium (ppm)	150

Table 5 Metal Concentration in Electroplating Industry Soil Samples

S.No	Metals	Concentration (ppm)
1	Copper	232
2	Zinc	5360
3	Chromium	372
4	Cadmium	30
5	Nickel	11.4
6	Silver	66
7	Calcium	847.2
8	Magnesium	4470
9	Iron	11.74
10	Manganese	16.56

The mobilization of heavy metals into the biosphere by anthropogenic intrusions has become an imperative process in the geochemical cycling of these metals. Release of large quantities of heavy metals into the atmosphere and soil from various stationary and mobile sources is evident in urban areas where it exceeds the natural emission rates (Al-Khashman *et al*., 2009). An assessment of the risk to some living species other than human beings or an entire ecosystem requires regulation, handling, and bioremediation of hazardous materials (Adham *et al.,* 2011).

pH is a simple parameter but is very significant, since most of the chemical reactions in the aquatic atmosphere are controlled by any change in its value. The aquatic organisms are susceptible to pH changes, and biological treatment needs pH control or monitoring (Lokhande *et al*., 2011). In the present study, the pH value is 6.5 near neutral within the recommended limit.

In water, the turbidity level increases as a result of interference to the penetration of light. The increase in turbidity will harm the aquatic life and also decrease the quality of surface water. Elevated values of turbidity reduce the filter runs which cause pathogenic organisms to be more dangerous to human life. For this reason the WHO, ICMR, and BIS suggested a maximum range of 2.5, 5 and 5 NTU respectively depending upon the processes used for the treatment of wastewater (Sawyer *et al*., 1994, Burden *et al*., 2002, De, 2003). In the present study, the turbidity was 15 (NTU) which was exceptionally higher than the recommended limit.

In the present work, total dissolved solids (TDS) level exceeded the recommended limit. The results coincided with the levels reported by Sawyer *et al*. (1994). Leo and Dekkar (2000) stated that the increased values of TDS cause destructive effects to health such as the central nervous system, provoking paralysis of the tongue, lips, and face, irritability and dizziness. The optimum range of TDS falls between 500–1500 mg L^{-1} as prescribed by the US EPA (1997), and ICMR, WHO, and BIS.

Also in this study, high conductivity values nearing 20700 Micromho/cm were observed. Increased conductivity may decrease the aesthetic value of the water by imparting mineral flavor. For industrial and agricultural activity, the conductivity of water is a significant parameter to be monitored. Water with higher conductivity may cause deterioration of metal surfaces of equipment. It is also applicable to home appliances such as water heater system and faucets. Excessive conductivity is identified to destroy food-plant and habitat-forming plant species (Jai *et al*., 2010; Katsoyiannis and Zouboulis, 2013; Heydari and Bidgoli, 2012).

In the present study, total hardness of electroplating industrial effluent was found to be higher than the prescribed CPCB limits. Total hardness in the range of 899 - 2727 mg/l was reported by Nagarajan *et al* (2012) in the electroplating industrial effluent. Ahamed and Alam (2003) studied the physicochemical and toxicological properties of industrial effluents in and around Delhi and groundwater quality of some areas in Delhi and reported that the total hardness of electroplating industrial effluent as 512 mg/l. In the present study, the concentration of sulfates was found to be high compared to the CPCB recommended values. Similar results were reported by Chhikara and Dhankar (2008) with the sulfates level as 187 mg/l in the electroplating industrial effluent.

Chlorides are the inorganic compounds resulting from the mixture of the chlorine gas with metals. Chlorine alone as Cl_2 is extremely toxic, and it is often used as an antiseptic. With a metal like sodium, it becomes necessary for life as sodium chloride. Chlorides may reach surface water sources from rocks containing chlorides, agricultural run-off, wastewater from industries, oil well wastes, and effluent from wastewater treatment plants. The taste of food products are affected by corrosion of metals in the cooking utensils by chlorides. They can pollute streams and lakes. Fish and aquatic communities cannot live in high level of chlorides. Hence, water used in industry or that proceeds for any use is treated to decrease chloride level (Kumar and Puri, 2012). In the present work, the chloride levels exceeded permissible limit by 30 folds.

According to WHO (1984) and Indian standard drinking water specification (1991), the acceptable limit of fluorides in drinking water is 1.5 ppm and the desirable limit is 1.0 ppm. Skeletal and dental fluorosis is caused by fluoride concentrations above 1.5 ppm in drinking water. Low concentration of approximately 0.5 ppm can provide defence against dental caries. Among the 23 nations of the world, India is where health problems occur due to fluoride contamination and the amount of fluoride pollution in water varies from 1.0 to 400 mg/l. In India, fluorosis and endemic fluorosis affects approximately 20 to 40 million people respectively (Chinoy, 1991). In the present study, the fluoride levels (0.8 ppm) were within the recommended limit.

Nas and Berktay (2006) reported that diverse agricultural activities result in the increase of nitrate concentration in ground and surface water. The children under the age of 1 are most affected due to the intake of

water polluted with nitrates. The range of Nitrate-Nitrogen prescribed by ICMR, WHO and BIS are 20, 45 and 45 mg L^{-1} respectively (Nyamangara *et al*., 2013). In the present study, nitrates did not exceed the limit. The level of phosphate was found to be below the recommended values in the current work. Industrial and sewage waste create phosphate pollution which increases the growth of micro-organisms. Elevated phosphate level causes muscle damage, breathing difficulties and kidney failure (Nyamangara *et al*., 2013).

The Biological Oxygen Demand (BOD) test provides an index of organic pollution and also determines the efficiency of sewage treatment plants. It also serves the regulatory authorities for checking the quality of effluents discharged into fresh waters. In the present study, BOD of the electroplating industrial effluent was found to be high, indicating a heavy load of organic compounds in the effluent. Analogous outcomes were obtained by Agarwal and Sachan (2003). The BOD value of electroplating industrial effluent was 50 mg/L while the permissible limit is only 30 mg/L for effluents which remain released to water bodies. The values of Chemical Oxygen Demand (COD) in the present study are well above the CPCB levels. Relative answers were attained by numerous examiners. Chhikara and Dhankar (2008) found the COD levels to be 4566 mg/l in electroplating wastewaters; Narasimhula and Setty (2012) reported a high COD level of 447 mg/l in the effluent of electroplating industry.

In the present investigation, the oil and grease content was high in the effluent samples collected from electroplating industries. It is significant here to observe that oil which forms a surface film on the river can coat plants and animals reducing oxygenation from the atmosphere above. The film of oil that floats over the water body affects the transmission of light through the water body thereby disturbing the process of photosynthesis in aquatic plants. In animals, oil coating can destroy the insulating properties of fur and feathers. Oil bioaccumulates in higher animals and further enters the food chain. Also, petroleum or grease spilled over water produces chemicals that are tremendously dangerous to marine animals (Lokhande, 2011).

Nickel is employed widely in metal processing units, and it is highly toxic in high concentrations. The effluent from metal processing units contains 2599 mg/l nickel as against 3 ppm maximum permissible limit in the effluent. Several studies reported similar results. Chhikara and Dhankar (2008) reported 13.87 mg/l of nickel in electroplating sludge; Orescanin *et al*. (2013) reported 0.532 mg/l of nickel in electroplating industrial effluent and Narasmihula and Setty (2012) reported 13.1 mg/l of nickel in electroplating industrial effluent.

Zinc is an essential trace element which plays a vital role in the physiological and metabolic processes of many organisms. However, at high concentrations, it can prove to be toxic. The main sources of aquatic contamination of zinc are metal processing units, paints, and fungicide factories. The effluents from metal processing units are reported to have zinc in the range of 787.60 ppm in contrast to 5 ppm maximum permissible limit in the effluent. Similar results were reported by many workers. Chhikara and Dhankar (2008) reported 94.8 ppm of zinc in electroplating industrial effluent. Orescanin *et al*., (2013) noticed high Zn levels in electroplating industrial effluent while Narasimhula and Setty (2012) observed 88 mg/L of zinc in the effluent.

The potential source of chromium in the aquatic environment is effluent from tanneries and textile mills. Besides these, Cr is extensively used in chrome plating and as a corrosion inhibitor in cooling tower operations. High concentrations of Cr are toxic to both plants and carcinogenic to animals. The electroplating industry waste contains 84 mg/l of chromium concentration, as against 0.1 ppm maximum permissible limit in drinking water. Different studies reported parallel values. Chhikara and Dhankar (2008) reported 117 mg/l of chromium in electroplating industrial effluent. Narasimhula and Setty (2012) observed 102 mg/l of chromium in the plating effluent. Orescanin *et al.* (2013) noticed 6.3 mg/L of chromium in electroplating effluent.

Rajan and Paneerselvam (2005) studied the drinking water quality in Dindigul city and all the physicochemical parameters such as pH, TDS, Total hardness, magnesium, chlorides, sodium, and potassium were above the permissible limits of BIS and were responsible for groundwater pollution. The effluent was not suitable even for irrigation. The hardness of water is largely associated with the presence of calcium and magnesium ions and is an essential indicator of the toxic effect of poisonous elements (Tiwari, 2001). Based on the results of the present study, TDS, total hardness, magnesium, and calcium levels were above the standard limits, and others were within the recommended limit.

Conclusion

In the present study, physico-chemical parameters of electroplating industrial effluent were analyzed and compared to the standards prescribed by CPCB. The

BOD, COD, total dissolved solids, electrical conductivity, oil and grease, zinc, chromium, nickel, calcium, and magnesium exceeded the permissible limits.

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ASSESSMENT OF POLLUTION BY PHYSICO-CHEMICAL PARAMETERS OF SATHIYAR RIVER IN MADURAI DISTRICT

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Abstract

Improperly treated or even untreated industrial and municipal effluents have been continuing to pollute surface water and ground water sources. Regular monitoring of water bodies would not only prevent the outbreak of diseases and occurrence of hazards, but would check the water from further deterioration.The river flows geographically between latitude 10^0 4'0" and longitude 10^0 4'0". It originates from Sirumalai hills of the Eastern Ghats situated 25km away from Dindigul. A dam has been constructed across this river namely Sathiyar Dam in Palamedu for irrigation. The river is used by the community for multiple activities including agriculture, laundry, drinking, commercial purpose and bathing, watering of crops for raw consumption and in certain areas for swimming by youth without prior treatment. The present study aims at evaluating the physicochemical parameters of water in Sathiyar River.

Keywords*: Physicochemical parameters, Sathiyar River, Pollution, Water quality*

Introduction

Renewable fresh water is an indispensable resource for life. It deserves special attention because it is very impaired and seriously threatened by human activities. Population growth accompanied by rapid urbanization causes many disturbances for natural environments (Kinney, 2002; Renjith *et al*., 2015; Chakraborty *et al*., 2014). Industrialization, the non-rational use of fertilizers and pesticides, and the lack of public awareness of the protection of the environment, lead to an imbalance of the ecosystem and generate pollutants that can affect the physicochemical and biological quality of aquatic receptors (Alayat, 1991; Nath *et al.,* 2014; Diop *et al*., 2015) and also alter the uses of water (Burton *et al*., 2001). For the human and industrial growth, water is necessary. With the increase in population and industrialization, the demand for the freshwater increased in the last decades. This demand would be fulfilled by the rivers which provide the water for human life and agriculture purposes. Due to the waste discharged from human and industrial activities, the quality of river water has deteriorated which affects human as well as aquatic life. According to WHO, CPCB, BIS, and ICMR, the water quality of about 70% river water is contaminated due to pollutants in India and is unfit for human consumption (Ramakrishnaiah *et al*., 2009; Jindal and Sharma, 2010). Assessment of river water quality using various parameters (physicochemical and biological) and the different ways and techniques to protect the river water are reported in the literature (Santosh *et al*., 2008; Yisa and Jimoh, 2010; Shah *et al*., 2015). The present study aims at estimating the physicochemical parameters to predict the water quality in the Sathiyar River.

Materials and Methods

The present study was carried out for Sathiyar River, located in Madurai district. The water samples for the present study were collected by using polypropylene bottles. Collection was done in the morning hours at ten places from upstream to downstream along the length of Sathiyar River from March 2014 to February 2015. The ten selected sampling stations are Sathiyar dam-S1, Sathiyar dam II-S2, Errampatti-S3, Keelachinnampatti Kanmai-S4, Kulamangalam Kanmai-S5, Veerapandi Kanmai-S6, Vepankulam Kanmai-S7, Tagore Nagar Kanmai- S8, Vandiyur-Sundaram Park Kanmai-S9, and Vandiyur Kanmai Shutter -S10. Water temperature, pH, EC and TDS were analyzed immediately on the spot after the collection, whereas the remaining parameters were analyzed in the laboratory. The water parameters such as Temperature, pH, EC, DO, Turbidity, TDS, Nitrates, Sodium, Calcium and Magnesium were determined using the standard procedures (Rain and Thatcher, 1990; Clescrl *et al*.,1993; Jain and Jain, 2007).

Results and Discussion

Table 1 divulges the results of physical and chemical parameters of Sathiyar River water samples in the ten stations during the study period. Aquatic organisms are affected by changes in pH, because most of their metabolic activities are pH dependent. Optimal pH range for sustainable aquatic life is 6.5-8.2. pH of an aquatic system is an important indicator of the extent of pollution in watershed areas (Kumar *et al*., 2011). It was observed that among the ten samples, pH ranged from 6.1±0.24 to 8.1±0.33, and maximum pH was in station 10 and minimum in station 1. Shah *et al*. (2015) observed pH in the range of 5.9–8.00 and 6.5–8.9 in Netravathi, and Sabarmati River respectively.

The Turbidity of water causes the reduction in transparency due to particulate matter such as clay or silt, finely divided organic matter, plankton and other microscopic organisms. Turbidity of water influences light penetration inside water and thus affects life (Verma and Saksena, 2010; Tambekar *et al.* 2013). Akuskar and Gaikwad (2006) observed higher turbidity during monsoon period and minimum turbidity during winter season at Manjara River. The present investigation showed the turbidity levels ranging from 0.63±0.01 NTU at sampling point S1 to 2.36±0.18 NTU at sampling point S10. The turbidity ranges of Sathiyar River are within the desirable limit as per BIS, and just below the WHO recommended permissible limit of 5 NTU.

Alkalinity is the function of bicarbonates and carbonates. The salts get hydrolyzed in solution and produce hydroxyl ion. It is also used as an indicator of productivity (Jhingran, 1982; Hulyal and Kaliwal, 2011). Natural water bodies in tropics usually show changes in their total alkalinity due to geography and season. In the present study, the alkalinity levels ranged from 11.42±0.54 mg/l at S1 to 37.49±2.9 mg/l at S10. These results in Sathiyar River showed alkalinity within the WHO permissible limit of 100mg/l.

The average temperature for all stations in Sathiyar River for the sampling period 2015 March to 2016 February shows the minimum of 14± 0.7 ºC at station S1 and maximum of 27± 0.81ºC at station S9. Even though a small fluctuation occurs, the temperature of Sathiyar River remained normal. Higher temperatures reduce the solubility of dissolved oxygen, decreasing its concentration and thus its availability to organisms. Thripathaih *et al.,* (2012) and Shyamala *et al.,* (2008) reported the range of temperature as 24.75 to 28.5ºC and 26.3 to 27.2ºC respectively.

TDS represents the amount of solid materials dissolved in water. High TDS values cause harmful effect on health such as the central nervous system, provoking paralysis of the tongue, lips, and, face, irritability and dizziness. The presence of synthetic organic chemicals even in small concentrations imparts objectionable and offensive taste, odor and color to fish and aquatic plants (Chang, 2005). The permissible level recommended for TDS is 500 mg/l as prescribed by IS 10500, BIS, and FAO. In the present work, the TDS lies between 57.14±4.57 mg/l at S1 and 171.05±10.2 mg/l at S10. Chittora *et al*. (2017) reported the total dissolved solids varying between 5.50 and 152 mg/l in different lakes of Udaipur city. Kumari *et al*. (2013) found TDS of 136–360 mg/l in Narmada River.

Electrical conductivity (EC) is the ability of an aqueous solution to conduct the electric current. The source of EC may be an abundance of dissolved salts due to poor irrigation management, minerals from runoff, or other discharges (Patra *et al*., 2011). The mean electrical conductivity of the water samples in the present study ranged from 367.63 ± 22.05µs/cm at S1 to 766.41±45.98 µs/cm at S10 which is above the standard limit of 300µs/cm. Thus the water has very high electrical conductivity, implying low level of ionic species. Weldemariam (2013) also reported the range of electrical conductivity of the Gudbhari River from 382 to 1090 µs/cm. The conductance of water increases which might be due to organic conducting species from soaps and detergents of the bathing places. Dissolved oxygen determines whether the biological changes are brought about by aerobic or anaerobic organisms. It reflects the physical and biological processes happening in water. The oxygen present in water can be dissolved from atmosphere or produced by photosynthetic organisms. Oxygen is generally reduced in water due to respiration by biota, decomposition of organic matter, rise in temperature, oxygen demanding wastes and inorganic reductants such as hydrogen sulphide, ammonia, nitrites and ferrous iron (Singh, 2012). A high pollution load may also decrease the DO levels. In the present work DO values ranged from 3.31±0.29 mg/l at S8 to 7.93±0.23 mg/l at S4. The minimum range observed in Sathiyar River exhibited a slight decrease than the desirable level but within the range of agreed concentrations. The low DO was due to waste discharges being high in organic matter and nutrients near the river and due to increase in microbial activity occurring during the degradation of organic matter (Yisa and Jimoh, 2010). Sharma *et al*. (2008) reported a DO value of 6.5–15 mg /l which is under the prescribed

limit as per WHO. Nitrate content in water is reported as either nitrate-nitrogen (NO_3-N) or nitrates (NO_3^-). Nitrate-N being the most common form of nitrogen present in natural waters is the end product of aerobic decomposition of organic matter (Rai, 2010; Agca *et al.,* 2014). In the present study, the nitrate concentration was between 11.46±0.34 mg/l at S10 and 4.04±0.20 mg/l at S1. Shah *et al*., (2016) reported nitrate concentration in Gaughat water in Lucknow ranging from 13.45 to 26.25 mg/l. From these results, it is inferred that all the nitrate values were within the maximum permissible limits recommended by WHO (2011) and the nitrates in all sampling sites in the Sathiyar river were within safe limits for drinking and irrigation purpose.

Calcium and magnesium exist in water mainly as carbonates and bicarbonates. The main source of magnesium is sewage inflows and minerals generated from soil erosion. They are important for enzyme activation, chlorophyll and phytoplankton (Ramesh and Seetha, 2013; Verma *et al*., 2012). In the present work calcium levels were from 70.80± 5.66 mg/l at S1 to 96.39±7.71mg/l at S9. These results indicated that calcium levels in the Sathiyar River were higher than the permissible limit of 75 mg/L recommended by WHO (2011), as it received pollutants from agriculture fields. The magnesium concentrations were from 2.89±0.17 to 6.96±0.27 mg/l. The highest level of magnesium was noticed at sampling point S9 and the lowest at S1. In all the stations magnesium levels were within the limit of drinking water standard recommended (30 mg/L) (ICMR, 2011) and WHO permissible limit of 50 mg/L. This indicates that the water is safe for drinking and irrigation purposes.

Conclusion

The physical and chemical parameters of the Sathiyar River fell within the WHO permissible limits for most of the stations. Exceptions were the EC, DO and Ca levels. Regulatory bodies have to monitor industries to ensure that they treat their wastes before disposal and regular check has to be carried out to find out the state of the water bodies from time to time as they still serve as major supply to people living around them.

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Table 1 The mean values of physico-chemical parameters of Sathiyar River in ten stations from March 2014 to February 2015

Station No	pH	Temperature (^{0}C)	EC (µs/cm)	Turbidity (NTU)	TDS (mg/l)	Alkalinity (mg/l)	DO (mg/l)	Ca (mg/l)	Mg (mg/l)	Nitrates (mg/l)
S1	6.3±0.31	14±0.7	367.63 ±22.05	0.63±0.01	57.14± 4.57	11.42±0.54	4.07± 0.12	70.80± 5.6	2.89± 0.17	4.04±0.2
S2	6.1±0.24	17±0.8	378.79 ±15.15	1.1±0.07	62.82± 3.76	11.84±0.8	4.68± 0.14	77.11± 3.85	2.76± 0.19	4.42±0.13
S3	6.2±0.49	18.0.2	386.4 ±19.3	1.16±0.04	58.32± 3.4	12.57±0.37	7.43± 0.52	74.97± 0.42	3.35± 0.23	4.17±0.2
S4	6.43±0.49	18±0.7	391.42 ±23.48	1.20±0.07	68.57± 4.11	23.57±1.4	7.93± 0.23	83.54± 2.50	6.43± 0.45	4.43±0.35
S5	6.1±0.42	19.±2	404.05 ±12.12	1.72±0.08	71.43± 6	26.78±2.14	4.22± 0.12	87.82± 3.51	6.19± 0.31	7.52±0.52
S6	6.4±0.32	19±4	597.74 ±15.9	1.78±0.14	80.13± 7.21	25.70±2.31	3.69± 0.25	91.04± 5.46	5.78± 0.34	7.71±0.61
S7	6.5±0.23	20±0.2	680.73 ±40.8	1.9±0.13	84.56± 4.22	29.9±2.09	4.06± 0.24	84.61± 4.23	5.99± 0.17	8.29±0.74
S8	6.8±0.19	21±0.4	685.4 ±34.27	1.67±0.15	111.73± 8.93	33.2±1.66	3.31± 0.29	90.37± 7.22	6.15± 0.4	9.10±0.72
S9	7.8±0.62	27±0.81	690.79 ±41.4	2.04±0.16	113.24± 5.66	34.27±2.05	3.61± 0.25	96.39± 7.71	6.96± 0.27	9.54±0.57
S10	8.1±0.33	26±0.2	766.41 ±45.98	2.36±0.18	171.05± 10.2	37.49±2.9	3.37± 0.20	80.33± 5.62	6.26± 0.48	11.46±0.34

INFLUENCE OF HERBAL EXTRACT ON THE HISTOPATHOLOGICAL CHANGES INDUCED BY CADMIUM IN THE OVARY OF FEMALE WISTAR RATS

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Abstract

Cadmium (Cd) is an environmental toxicant and a key element of Ni-Cd Batteries. Cadmium affects reproductive organs. Its action may be either direct, affecting the gonads and accessory organs. In Indian ethnobotanical studies, a mixture of 17 herbal products (Prasava Rasayanam) is used to fortify the reproductive system of women after parturition. In this investigation, we considered the effect of Cadmium chloride and PR on adult female Wistar rat's reproductive system. Two different doses of cadmium (50 and 200 ppm) were given to Wistar rats aged 45 and 65 days. Prasava Rasayanam was administered orally every day at a dose level of 200mg/kg of body weight to the rats exposed to cadmium. In the present study, a histological examination of the ovary of rat showed that Cd had induced apoptosis, in the ovarian cells, hyperplasia, hypertrophy, follicular atresia, oviduct malignancy, and abortive oogenesis. An administration of PR along with Cd prevented the histopathological inflictions in the ovary.

Keywords: *Cadmium, Prasava Rasayanam (PR), Ovary, Histology, Rats*

Introduction

Cadmium (Cd) is an ecological threat factor with diverse effects in animals and humans. Exposure to this toxicant is generally the outcome of environmental corruption by waste from human activities, such as the residues found in mining waste, those released by the burning of fossil fuels and industry, and the runoff from agricultural soil (Martelli *et al.,* 2006; Nordberg *et al.,* 1992). Cadmium is usually used in industry such as an anticorrosive agent, stabilizer in PVC products, color pigment, a neutron-absorber in nuclear power plants and the fabrication of nickel-cadmium batteries. Phosphate fertilizers also contain large amounts of cadmium (Larz, 2003). Tobacco smoke is one of the extensive sources of Cd pollution in the general population (Zenzes *et al.,* 1995), with the estimated assimilation of 0.2–1.0 µg Cd/cigarette (Satarug and Moore, 2004). Cd has an enormously elongated biological half-life of 15–30 years, principally because of its low rate of excretion from the body, and accumulates over time in the blood, kidney, and liver, where it has several undesirable effects on health. Also, an extensive spectrum of deleterious effects on reproductive tissues has been explained (Henson and Chedrese, 2004). Cd is detrimental to both male and feminine gonads in adults. Such gonadal modifications have been reported in a range of animal models, particularly rats and sheep. Male rats have been shown to extend hasty and long-term damage in the testes after administration of high doses of Cd (El-Ashmawy and Youssef, 1999). Rats show dose and age-dependent toxicity in the ovaries, uterus, and cervix. Cd administration profoundly alters ovarian steroidogenesis associated with a reduction in progesterone secretion (Zhang *et al.,* 2008). Similarly, exposure of cultured human and rat ovarian granulosa cells to Cd causes a decline in progesterone production (Paksy *et al.,* 1997a; Zhang and Jia, 2007). Cadmium induces estrogenic responses and breakdown in the hormone related activity like oxidant-antioxidant equilibrium. The overload production of reactive oxygen species (ROS) due to Cd stress damages the reproductive functioning through a disruption in female sex hormones like estrogen, progesterone, FSH, and LH. In the current study, an attempt has been made to study the effect of Cd on the ovarian function and oxidative stress and the

influence of Cd on endocrine functions (Dailiah Roopha and Padmalatha, 2013).

Herbal medicine is tranquil the stronghold of about 75 - 80% of the world population, mainly in the developing countries, for primary health care. The utilization of herbal drugs is on the flow, and the market is growing step by step (Kamboj, 2000). The herbal products today symbolize shelter in disparity to the synthetics that are regarded as unsafe to human and environment. It has been predicted that in developed countries such as the United States, plant drugs constitute as much as 25% of the total medicines, while in fast-developing countries such as China and India, the involvement is as much as 80% (Joy *et al.,* 1998). In southern India, quite a lot of herbal products had been reported to strengthen the reproductive physiology of women and to moderate oxidative stress due to ROS in the gonads after parturition (Uboh *et al.,* 2010). In traditional South Indian health care system, Prasava Rasayanam (PR) is used to protect uterine functioning after parturition. So a novel idea has been conceived to find out whether the administration of PR can remediate the ovarian tissue that got damaged due to Cd-induced oxidative stress and endocrine dysfunction. Hormonal assay confirmed a recurring effect of PR in the tissue that got damaged due to cadmium (Dailiah Roopha *et al.,* 2013). A histological investigation is a valuable tool for detecting the changes occurring in the ovarian tissue after cadmium treatment and cadmium treatment supplemented with PR; histological work was carried out in the present study.

Materials and Methods

Preparation of Plant Extract

The preparation of extract was carried out the following protocol of Uboh *et al*. The plant materials viz., *Zingiber officinale* (rhizome), *Piper nigrum* (fruits), *Trachyspermum ammi* (seeds), *Brassica nigra* (seeds), *Ferula asafoetida* (resin from rhizome), *Curcuma longa* (rhizome), *Anethum graveolens* (seeds), *Hemidesmus indicus* (root), *Vernonia anthelmintica* (seeds), *P. longum* (fruits and root), *Crataeva religiosa* (bark), *Allium sativa* (bulb), *Spilanthes calva* (flowers), *Alpinia calcarata Roscoe* (rhizome), *Nigella sativa* (seeds), *Acorus calamus* (rhizome) and *Trianthema decandra* (root) were collected and shade dried.

Two hundred grams of the ground powder was soaked in 1.0 l of distilled water for 48 h at room temperature. The mixture was filtered into a 500-ml conical flask with a Whatman filter paper (No. 1). The filtrate was dried at a temperature of 30°C for ten hours to produce a gel-like extract, which weighed 20.5 g. An appropriate concentration of the extract was then subsequently made by dilution with distilled water into 200 mg/kg body weight and administered to the animals.

Experimental Design

Ninety-day-old female albino rats of Wistar strain (*Rattus norvegicus*) obtained from the National Institute of Nutrition, Hyderabad, weighing 140±10 g were used for the present investigation. The rats were maintained in a temperature controlled animals' quarter with 12 h dark: 12 h light schedule and were fed standard rat pellet diet (Broke Bond, Lipton India Ltd., India) and drinking water ad libitum. The animals were dewormed with albendazole (Bendex-400, Cipla Ltd., India) (10 mg/kg body weight, orally), before the initiation of the experiment. The females are mated with males at a ratio of 2:1. Cohabitation began at approximately 16.30 h on each mating day. On the following morning, the females were removed from the mating cages and smeared individually for the presence of sperm in the vaginal lavage. The presence of sperm in the vaginal lavage is indicative that the females mated and those were selected for further studies. The pregnant animals were then allowed to give birth. The mother animals with female pups were divided into the following groups:

Group I: control

Group II: 50 ppm

Group III: 200 ppm

The minimum (50 ppm) and maximum effective doses (200 ppm) of Cd were selected. The mother rats along with female pups were treated with Cd in the type of cadmium chloride, and the Prasava Rasayanam mixed in drinking water from 0-day post-parturition (pp) to 65 days pp.

Subgroup I: 45-day-old rat—puberty occurred

Subgroup II: 65-day-old rat— full growth of ovary

From the beginning of day 35, all females were examined daily for the vaginal opening. The different stage of the estrous cycle was studied on the vaginal smear by Long and Evans in 1933. The fluid collected by lavage was

placed on a slide and evaluated by light microscopy. Cycle stages were determined with the following criteria:

1. The cornified epithelial cells are present in the estrous.
2. The leucocytes are occurred in the diestrus.
3. The cornified nucleated epithelial cells are there in metaestrus.
4. The nucleated epithelial and cornified cells were found in the proestrus.

After 45 and 65 days of cadmium exposure and PR, the body weights of the animals were measured. Ovaries were dissected out, weighed and fixed in fixative for further studies

Fixation

Immediately after dissection, the ovary was removed surgically and rinsed with cold physiological saline. To fix the tissue Zenker's fixative was used. The tissues were set in the fixative for 12 hours and washed in the running tap water overnight and dehydrated in the ascending grades of isopropyl alcohol. The tissues are placed 1% celloidin dissolved in methyl benzoate. The celloidin in filtered tissues was left in toluene till they became translucent. After removing toluene, the tissues were embedded in wax. The sections were cut at a thickness of 6µ in a rotary microtome. The sections were allowed to expand by gently warming the slide on a hot plate maintained around 50°C. When the sections became flat and expanded, the water was drained. The slides were left overnight on the hot plate maintained at a constant temperature of 45°C. The sections were stained by haematoxylin and analyzed under Nikon research microscope.

Statistical Analysis

All the data are presented as means standard error of the mean (SEM). Statistical significance was calculated using Student's t test to test the significance of individual variations. The value of probability was gained from the degree of freedom by using standard table value, given by Fischer and Yates (1948). The level of significance was assessed at $p<0.05$.

Results

Body and ovary weight

Cd exposure significantly decreased the body weight and ovary weight in 45 and 65 days of Cd-treated rats. But the PR treatment increased the body weight and ovary weight (Table 1 & 2).

Table 1 Effect of Cd and PR on Body Weight in Developing Rats

Body Weight (g)					
Age (Days)	Control	50ppm (cd)	PR+50ppm (cd)	200ppm (cd)	PR+200ppm (cd)
45	0.57±0.05	0.6±0.02	0.51±0.008	0.32±0.02	0.40±0.124
65	0.98±0.05	0.83±0.09	0.88±0.02	0.62±0.06	0.71±0.0.04

Each value represents the mean and SEM of 30 female rats (from 6 mothers). Statistical significance of difference among groups at $P < 0.05$;

Table 2 Effect of Cd and PR on Ovary Weight in Developing Rats

Ovary Weight (mg)					
Age	Control	50ppm(cd)	PR+50ppm (cd)	200ppm (cd)	PR+200 ppm (cd)
45	0.73±0.01	0.56±0.01	0.66±0.008	0.43±0.01	0.56±0.005
65	0.94±0.01	0.73±0.17	0.84±0.008	0.71±0.10	0.81±0.007

Each value represents the mean and SEM of 30 female rats (from 6 mothers). Statistical significance of difference among groups at $P < 0.05$;

Vaginal Opening

In the present study cadmium (50 & 200 ppm) delayed the onset of vaginal opening in Wistar rats. In the animals treated with cadmium (both 50 ppm & 200 ppm doses) the attainment of puberty was delayed (45&65 days) compared to control animals. But the delay in the achievement of puberty was altered in rats, administered with cadmium and Prasava Rasayanam (PR) (Fig 1).

Estrous Cyclicity

In the present study, cadmium delayed the attainment of puberty and lengthened estrous cycle of experimental rats compared to control. The delayed attainment of puberty was altered by Prasava Rasayanam (PR) (Fig 2).

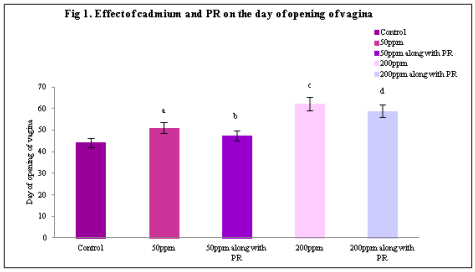

Fig 1. Effect of lactational exposure to cadmium (Cd) along with PR on pubertal onset in developing rats. Each bar represents the mean, and the vertical line above denotes the SEM of 30 female rats (from 6 mothers). Statistical significance of difference among groups at P < 0.05; a control vs 50ppm, b 50 vs PR, c control vs 200ppm, d 200 vs PR.

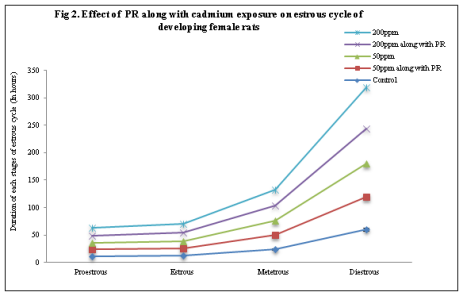

Fig 2. Effect of lactational exposure to cadmium (Cd) along with PR on estrous cyclicity in developing rats. Each line represents the mean, and the vertical line above denotes the SEM of 30 female rats (from 6 mothers). Statistical significance of difference among groups at P < 0.05;

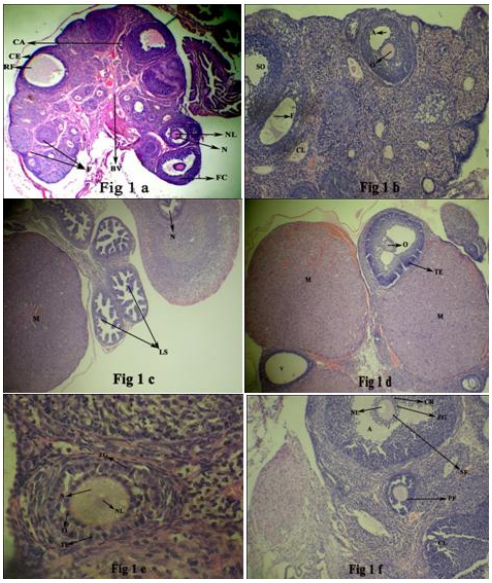

Plate 1a-f represents the ovarian histoarchitecture of control, 50ppm Cd-treated, 200ppm Cd-treated, 5oppm (cd)+PR treated, 200ppm (cd)+PR treated at 45 days. **1 a** The normal ovary showed surface cuboidal epithelium (CE), The cortex of the ovary in various stages of development (F), Blood vessels of the ovarian medulla (BV), nucleus (N) dark nucleolus (NL) and ruptured follicles after ovulation is seen(R). **1 b** showed the Secondary oocyte (SO) was also abnormal. Primary follicle with oocyte and a large antrum (A) was noticed. The follicles are irregularly shaped (F). **1 c** & **d** showed necrosis (N) and weighty growth of labyrinthine structures (LS), the degenerated follicles with a dense mass of tissue (M). The theca externa of the follicle (TE) has developed gaps in several regions. Follicular atresia was common. Maturation of the oocyte was affected, and vacuolation (V) was prominent. **1 e** showed the ovarian follicle with distinct theca interna (TI), and theca externa (TE) are seen. The ovum is well developed with prominent nucleolus (NL) and nucleus (N). A multilayered zona granulosa (ZG) cells around the oocyte are observed. **1 f** showed the primary follicle, secondary follicle, tertiary follicle, and pre-ovulatory graffian follicle are seen. Antrum (A), corona radiate (CR), zona glomerulosa (ZG) and corpus luteum (CL) development were noticed.

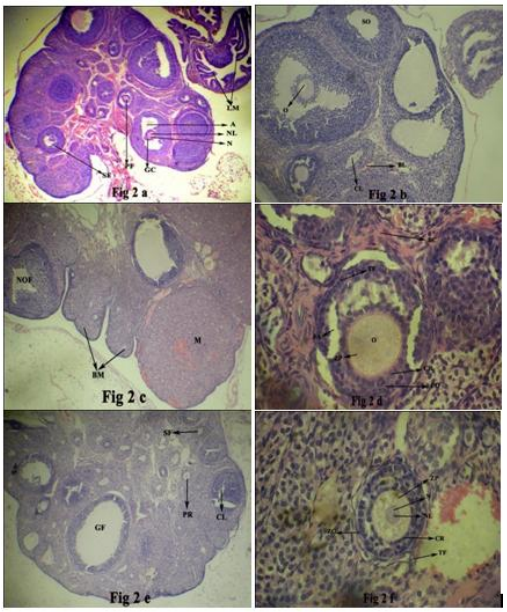

Plate 2 a-f represents the ovarian histoarchitecture of control, 50ppm Cd-treated, 200ppm Cd-treated, 50ppm (cd) +PR treated, 200ppm (cd) +PR treated at 65 days. **2 a**

showed definite antrum (A), many layers of granulosa cells (GC), nucleolar of a nucleus (NL) are seen. A labyrinthine mucosa (LM) of the oviduct got ingresses into the lumen of the oviduct. A normal primary follicle (PF) and secondary follicle (SF) are seen. **2 b** showed degenerated primordial follicle and irregular and abortive oocyte (O), Corpus luteum (CL). It also shows blood clots (BL). **2 c** showed a blunt mass of (BM) outer growth. It was showing a necrotic ovarian follicle (NOF) with arrested oogenesis. **2 d** showed the primary oocyte and zona pellucid (ZP). The zona granulosa (ZG) and corona radiata (CR) were normal. Blood circulation (BC) was normal. The theca externa was not affected and, a large central cavity was formed in the follicular antrum (FA). The primary oocyte is eccentrically positioned within the tertiary follicle and resided within a round of granulosa cells called the cumulus oophons (CO) that protrudes into the antrum. The granulosa cells immediately adjoining the oocyte is expressed as the corona radiate (CR). **2 e** & **f** showed primary follicle (PF), the secondary follicle (SF), the graffian follicle (GF) and corpus luteum (CL). It also showed an oocyte with a distinct nucleus (N), nucleolus (NL), corona radiata (CR), zona pellucida (ZP), theca follicle (TF) and zona glomerulosa (ZG).

Discussion

Cadmium (Cd) is one of the toxic chemicals responsible for tumor formation, mutagenesis and reproductive failure in animals. Cadmium (Cd), ubiquitous food contaminant of health concern, is proposed to be an endocrine destructor by inducing estrogenic responses *in vivo* (Imran Ali *et al.,* 2010). Cd is also a broad spread nephrotoxic food contaminant, which accumulates with a for the most part of the long biological half-life in the liver and kidney (Amzal *et al.,* 2009). Cadmium is an endocrine disruptive agent, which alters the estrogenic responses (Johnson *et al.,* 2003). Effects of cadmium included increased uterine weight, luminal epithelium height, hyperplasia, hypertrophy of the endometrial lining induction of uterine progesterone receptor expression and increased formation of side branches and alveolar buds, in the mammary gland (Alonso-Gonzalez *et al.,* 2007; Zhangand *et al.,* 2007; Hofer *et al.,* 2009). Akesson *et al.,* 2008 observed an association between long-term dietary Cd exposure and endometrial cancer incidents.

Herbal plants have been famous from ancient times among people and in recent years, a multilateral approach was appeared on using herbal medicines along with medical care they get from their health care provider (Modares *et al.,* 2008; Mohajeri *et al.,* 2009). Many plant extracts have been reported to change fertility in rodents. The methanolic extract of *Rumex steudelii* reduced the number of implantation sites drastically. It was also showed that the extract of this plant did not influence the serum estrogen-progesterone ratio (Gebri *et al.,* 2005). The *Hibiscus rosa – sinensis* flowers have antifertility, abortifacient activity, and exhibit anti-estrogenic activity as judged by an increase in uterine weight. Reproductive capabilities of herbal products have been revealed an overall improvement in fertility, reproductive health, and development of animals (Nivsarkar *et al.,* 2005). *Tribulus terrestris* can improve some aspects of male sexual behavior by increasing testosterone levels and muscle strength by raising blood levels of another hormone, i.e., luteinizing hormone and enhance spermatogenesis in rats (Park *et al.,* 2006). *Withania somnifera* (Ashwagandha) plant indicated that it could influence the endocrine system (Visavadiya and Narasinhacharya, 2007). *Telfairia occidentalis* plant is used for treating anemia of pregnant women in Nigeria (Caili *et al.,* 2006). The ginger root (*Zingiber officinale*), an inhibitor of prostaglandin synthesis, has been used for thousands of years for its anti-inflammatory properties and Dong quai (*Angelica sinensis*) demonstrates uterine tonic activity, causing an initial increase in uterine contraction followed by relaxation (Srivastav and Mutafa, 1992). Reports have shown that antioxidants like vitamin C and Vitamin E have shown protection against cadmium-induced toxicity in different animal models (Ognjanovic *et al.,* 2003; Beytut *et al.,* 2003). One study demonstrated that treatment with *Apium graveolens L* extracts significantly reduces reproductive toxicity induced by Sodium valproate (VPA) in male albino rats by normalizing sperm count, sperm motility and the histopathological recovery (Charles *et al.,* 1990). *Nigella sativa* increases fertility, a weight of reproductive organs, numbers of mature Leydig cells in male albino rats (Mohammad *et al.,* 2009). The improving action of *N. sativa* in the fertility index may be due to its antioxidative and hypolipidemic effects (Bashandy, 2007). The protective effect of garlic is maybe caused by the sulfur

compounds combining with the heavy metals in the body and promoting excretion through bile to the feces (Cha, 1987). Several other studies have confirmed a protective effect for garlic against cadmium and mercury poisoning in rats (Koch and Lawson, 1996).

The Cd inhibited the action of enzymes in the scavenging activity of ROS in the female Wistar rats. However, the inhibited antioxidant enzyme levels were elevated considerably due to the action of the bioactive principles present in the herbal antioxidant restoration mixture (PR) (Dailiah Roopha and Padma Latha, 2012).

Conclusion

A natural neutraceutical called PR (Prasava Rasayanam) was administered to protect the ovary function from Cd-toxicity. The Cd treatment had induced apoptosis. In the rat supplemented with PR, Cd could not impair the functioning of the reproductive system and associated hormonal activities. An administration of PR along with Cd prevented the histopathological inflictions in the ovary by rejuvenating the cellular function, diminishing oxidative stress and enhancing endocrine functioning. The treatment of PR indicated a significant histopathological preventive role in the ovary.

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COMPARATIVE STUDY OF THE PHYSICO-CHEMICAL CHARACTERISTICS OF WATER SAMPLES COLLECTED FROM DIFFERENT PONDS OF TUTICORIN DISTRICT, TAMIL NADU

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Abstract

Aquatic ecosystems are essential components of the global environment. In addition to being fundamental suppliers to biodiversity and ecological productivity, they also provide a variety of services for human populations, including water for drinking and irrigation, recreational opportunities, and habitat for economically important fisheries. The problem of pollution in the freshwater system in our country is mainly due to industrialization and population expansion. Water quality is regularly unnoticed in pond management, and poor water quality can direct to common troubles, such as excessive algal blooms, overgrowth of plants, noxious smells, or dead and dying fish. To prevent these problems, an understanding of fundamental water chemistry and other physical parameters is necessary. So the study was conducted to analyze the physio-chemical properties like color, turbidity, odor, TS, electrical conductivity, pH, alkalinity, total hardness, phosphates, nitrates and chlorides of water samples collected from various ponds of Tuticorin District, Tamil Nadu. The results can be concluded that the parameters of study are above the permissible limit of drinking and irrigation standards (WHO). The results reveal that overall water quality was found unfit for drinking and irrigation purpose.

Keywords: *Environment, Pollution, Pond, Water quality, Physico-chemical parameters*

Introduction

Water is the most precious commodity in life. It is one of the vital desires for sustaining human life. But all manner of human activities have negatively affected water qualitatively and quantitatively. The different water sources are available on earth is saline in nature; only small resources are fresh water in nature. India receives about 1400-1800mm of rainfall annually. About 96% of this water are used for agriculture, 3% of domestic use and 1% for industrial activity. Ponds are wetlands positioned in and around human habitations as they are generally seminatural ecosystems constructed by man in landscape suitable for water stagnation. Ecosystem services rendered by these wetlands are immeasurable including tangible and nontangible ones. Besides acting as a source of fresh water, they decline the ambient temperature, raise the water table, enlarge the diversity of flora and fauna, and supply ambiance. Due to unrestrained increase in human population and development of township at large, these freshwater bodies are under incredible pressure owing to their overuse on one hand and fortification due to nutrients and organic material on the other, leading to the enriching eutrophication (Pandey and Pandey, 2003; Gupta and Shukla, 2006; Patil and Tijare, 2001; Singh and Mathur, 2005). Industrial, sewage and municipal wastes are continuously added to the water reservoirs distressing the physicochemical quality of water (Dwivedi and Pandey, 2002).

Physico-chemical parameters have significant in determining the trophic status of aquatic habitats (Swati and Kanungo, 2013). Quality of water is a critical alarm for the welfare of the society since it is directly linked to the health of living beings (Rajaram *et al.,* 2013). Without adequate quality and quantity of freshwater, the sustainable development will not be possible (Arya *et al.,* 2011). The dynamic and heterogeneous relationship achieved physical, chemical and biological elements in the aquatic ecosystem, which can be recorded by regular monitoring to maintain the integrity and conserve the ecosystem (Ramachandran *et al.,* 2002). Hence, a challenge was completed to investigate the water quality status of Perungulam, Vanaramutti and Kallurani Ponds in Tuticorin district.

Materials and Methods

Sample Collection

For the collection and analysis of pond water, three ponds located in three cities were chosen, i.e., Vanaramutti, Kallurani, and Perungulam. Pond water was collected using the plastic container of one-liter capacity. The bottle was rinsed with pond water before collection. During sampling, containers were dipped and filled at a depth of 30cm below the surface of the pond. The samples were labeled and elated to the laboratory.

Physico-chemical Analysis

The collected samples were analyzed for the different physic-chemical parameters such as pH, TDS, Electrical Conductivity, alkalinity, chloride content, phosphate content, nitrate content and total hardness as per standard methods. The experimental results were compared with the permissible limit of drinking and irrigation water quality standards (WHO).

Results and Discussion

All the physicochemical characteristics of different water are shown in Table 1.

Table 1 Physico-Chemical Parameter recorded in different Ponds of Tuticorin District, Tamil Nadu

Parameters	WHO	Observed Value Kallurani	Observed Value Vanaramutti	Observed Value Perungulam
Physical Examination				
Appearance	-	Coloured	Coloured	Coloured
Color (Pt.Co-Scale)	-	Brownish	Greenish	Brownish
Odor	-	None	None	None
Turbidity NT Units	5	4	10	10
Total Dissolved Solids (mg/L)	500	124	680	313
Electrical Conductivity (Micro mho/cm)	750	182	999	460
Chemical Examination				
pH	6.5-8.5	7.77	7.76	7.86
Total Alkalinity as $CaCo^3$ (mg/L)	120	60	250	120
Total Hardness as $CaCo^3$ (mg/L)	300	70	480	192
Ca (mg/L)	100	20	92	40
Mg (mg/L)	120	5	60	22
Na (mg/L)	-	10	15	15
K (mg/L)	-	3	5	5
NH_3 (mg/L)	-	32.00	12.80	32.00
NO_2 (mg/L)	45	0.02	0.05	0.02
NO_3 (mg/L)	1	1	2	1
Cl (mg/L)	250	15	150	60
SO_4 (mg/L)	250	9	53	24
PO_4 (mg/L)	0.1	1.03	0.65	1.10
Tidys Test 4 hrs as O^2 (mg/L)	5-7	0.16	0.16	0.16

Color

Naturally, the water has no color. In the Vanaramutti pond water is greenish. It is due to the environmental factors and agriculture effluent discharge. On that, the Kallurani and Perungulam pond show the brownish color. It means, the water contains a high amount of dissolved solids as mud or the presence of dead plant materials.

Turbidity

Muddy or turbid pond water is frequently only an aesthetic problem. It was caused by runoff from distressed areas in the region of the pond or bottom-dwelling fish and muskrats. In established ponds, muddy water can almost always traced to a preventable source. Vanaramutti and Perungulam ponds show the high turbidity of 10 NT. The lowest turbidity value was found in Kallurani pond at 4 NT. These values are within the acceptable limit of WHO set for drinking and irrigation purposes (WHO 2008).

Total Dissolved Solids

The permitted limits of TDS in water were shown in Table 1. All values lie within permissible limits suggested by WHO (i.e., 500-1000 mg/l) (WHO 1984). Henceforth it is suitable for drinking purpose and other domestic and commercial uses. Present research showed that the recorded values of TDS from Vanaramutti pond, Perungulam pond, and Kallurani Pond are found to the 680, 313 and 124 mg/L respectively.

Total Hardness

Total hardness of Vanaramutti, Kallurani, and Perungulam pond was calculated as 480, 70 and 192mg/L respectively. Due to an addition of sewage and large-scale human use, this force have caused elevation of hardness (Dakshini and Soni, 1997; Kumar, 2000).

Dissolved Oxygen (DO)

Dissolved oxygen is an important parameter in water quality assessment and reflects the physical and biological processes of aquatic life. In the present study, DO content was in the range of 0.16 mg/L. The permissible limit is 5-7 mg/L (WHO, 2008).

pH

The pH affects most of the biological processes and biochemical reactions in the water body (Arya *et al.,* 2011). Present research showed that the recorded values of pH from Vanaramutti, Kallurani, and Perungulam pond are found as 7.76, 7.77 and 7.86 respectively.

Electrical Conductivity

Electrical conductivity (EC) is a numerical expression of the capacity of an aqueous solution to bring electric current. The highest EC was recorded in Vanaramutti (999 *µmhos cm*-1), and lowest in Kallurani and Perungulam pond (182 and 460*µmhos cm*-1). Natural waters usually have EC values of 20 to 1500 *µmhos cm*-1 (Boyd, 1978). The EC value depends on several factors like the presence of ions, their concentration, mobility, and temperature of measurement. Sastry *et al.* (1999) found the concentration of dissolved solids to be proportional to the ionic strength and proposed that the increase in conductivity may be due to leachate infiltration from the soil. Electrical conductivity showed a significantly negative relationship with chlorinity and salinity.

Total Alkalinity

Alkalinity is a measure of capacity of water to neutralize the strong acid. Total alkalinity is the sum of hydroxides, carbonates, and bicarbonates. Presence of hydroxides was not recorded in any pond; bicarbonate was the major ion responsible for alkalinity in all the ponds. The maximum bicarbonates were present in Vanaramutti (250 ppm) followed by Perungulam (120 ppm). Kallurani had minimum bicarbonates (60 ppm). According to Durrani (1993), removal of CO_2 from bicarbonates for photosynthesis by algae may amplify total alkalinity. Total alkalinity may be used as a tool for the quantity of productivity.

Chlorides

Chloride content ranges from 150mg/L to 60mg/L in Vanaramutti and Perungulam pond whereas Kallurani pond has 15mg/L chloride content. The reason for chloride is the high amount of contamination with organic wastes (Munawar. 1974). Chlorides are toxic to most plants and, hence they should be checked for irrigation water.

Nitrates and Nitrites

Nitrates and nitrites are chief nutrient factors in aquatic ecosystems and generally, water bodies polluted by organic matter exhibit higher values of nitrates (Shanthi *et al.*, 2002). The present findings showed low level nitrate values ranging in between 1 to 2mg/L. This level prevented algal growth and kept water body free of eutrophication. The nitrite profile of the water samples varied from 0.02 to 0.05mg/L throughout the study area.

Calcium and Magnesium

The full hardness of water is due to the total concentration of Ca and Mg ions expressed as carbonates. In this study maximum Calcium value was found in Vanaramutti pond as 92mg/L. The minimum value was found in Perungulam (60mg/L) and Kallurani pond (20mg/L). Similarly, values of Magnesium ions for Vanaramutti, Perungulam, and Kallurani ponds are 60mg/L, 22mg/L and 5mg/L respectively. Vanaramutti pond shows the maximum values of Calcium and Magnesium ions.

Sulphates

It is one of the chief anions in natural waters and is contributed by industrial and household discharges as a contaminant from tanneries, textiles, etc. This will in effect decreases pH of pond water and increases bacterial load, i.e., sulphate reducing bacteria. Present research showed that the recorded values of Sulphates from Vanaramutti, Perungulam and Kallurani pond are found 53mg/L, 24mg/L and, 9mg/L respectively.

Phosphates

Phosphate values varied from 1.10, 1.03 and 0.65mg/L for Perungulam, Kallurani and Vanaramutti ponds respectively. These cahanges might be due to the discharge of sewage into ponds.

Conclusion

In the present investigation results of physicochemical parameters of Vanaramutti, Perungulam and Kallurani ponds clearly show that the water is unfit for drinking purpose without treatment and these ponds struggle for their existence. Vanaramutti pond was highly polluted because of sewage discharge. So there is a necessity for proper management and restoration for humans and the environment. There is a need for awareness among the local people to maintain the ponds. Regular cleaning of these ponds may be helpful to retain water purity.

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