BIS (2-ETHYLHEXYL) ADIPATE DEGRADATION VIA A MICROALGAL APPROACH - A CRITICAL NECESSITY

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By

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CERTIFICATE

This is to certify that this dissertation entitled, **"Bis(2-ethylhexyl)adipate degradation via a microalgal approach - A critical necessity"** submitted by **S. Loorthumini Reg. No. 21APBO07 to St. Mary's College (Autonomous)**, Thoothukudi in partial fulfilled for the award of the degree of "Master of Science in Botany' is done by her under my supervision. It is further certified that this dissertation of any part of this has not been submitted elsewhere for other degree.

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DECLARATION

I do here by declare that this dissertation entitled, **"Bis (2-ethylhexyl)adipate degradation via a microalgal approach - A critical necessity"** submitted by me in partial fulfillment for the award of the degree of "Master of Science in Botany', in the result of my original and independent work carried out under the guidance of Dr. G. Flora M.Sc., M.Phil., Ph.D., Assistant Professor, Department of Botany, St. Mary's college (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

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Contents

INTRODUCTION

Plastic is derived from the Greek word *plasticos*, which means capable of being moulded into various shapes. Plastic materials have found versatile applications in every aspect of modern human life as a result of rapid technological advancement and the geometric progression of global population growth. Different substances are blended in various proportions in plastics to improve performance and reduce costs (Li *et al., 2005).* Plastic is a matter that is hard to destroy and degrade once manufacture that goes in contradiction to natures rule: consequently, it creates a catastrophe for the complete world (Gilmore *et al*., 2018). Plastic pollution has become one of the most wide spread recalcitrant environmental contaminants (Stabinkova *et al*., 2021). Plastic particles are ubiquitous pollutants in the living environment and even in the blood stream of human beings. Polyethylene terephthalate, polyethylene and polymers of styrene were widely encountered in the blood stream (Lesile *et al*., 2022). Plasticizers are among the most important additives needed in the processing of polymer materials, particularly polyvinyl chloride (PVC) plastics, which account for more than 60% of total plastic auxiliary yield (Erythropel *et al*., 2014; Rahman *et al*.,2004). Traditional petroleum-based phthalate plasticizers are the most commonly used around the world. Phthalate plasticizer yield and consumption account for a large proportion of total plasticizer production and sales, but they are gradually being phased out due to potential threats to human health and the environment. Strict environmental and safety regulations have been developed and implemented. A research focus has been on the development of environmentally friendly non-toxic plasticizers and biodegradable bio-based plasticizers to replace phthalates. Non-toxic green plasticizers with high performance, oil resistance, extraction and migration resistance are constantly being developed, produced, and applied in electrical insulation, food packaging, and medical and health products. Plasticizers are functional

additives used to improve polymer flexibility, plasticity, processability, and elongation, particularly in PVC products (Choi *et al*., 2004). Phthalates, also known as phthalic acid esters, are a type of xenobiotic organic compound that is widely used to make plastic goods more flexible. (Kashyap and Agarwal, 2018). They are colourless, odourless, and flavourless, and exist as liquids over a wide temperature range (25°C to 50°C) (Tran *et al.,* 2021). Di(2-ethylhexyl)phthalate (DEHP) was added to plastic polyvinyl chloride (PVC) in the 1930s to improve flexibility and elasticity. OECD (Organization for Economic Cooperation and Development, 2018), phthalate ester (PE) including di(n-butyl) phthalate (DBP) and di (2-ethylhexyl) phthalate (DEHP) is synthetic compound commonly used as a plasticizer to impart flexibility, workability, and durability to polymers such as polyvinyl chloride. Also this compound is used in a wide variety of products such as paints, adhesive in cosmetics (Ling *et al*., 2007; Babu and Jiunn-tzong-wu, 2010). The most commonly used plasticizers, phthalates and adipates, are hydrophobic and lipophilic and can accumulate in the soil. In this regard, an accurate toxicological assessment of these compounds is required. There is some analogy between the physiological and toxicological properties of chemically similar substances. Furthermore, when researching the toxicity of plasticizers, it is necessary to consider the toxicity of the substances that form them. The type of acid that forms, such as the alcohol radical, influences the physiological and toxicological properties of plasticizers. An increase in toxicity is associated with a decrease in the number of carbon atoms in the alcohol radical in the series of phthalic acid esters (Lazarev *et al*., 1976; Vikhareva *et al*., 2021). As a result, PE has become ubiquitously disturbed in the environment (Yuun *et al*., 2008). In 2019 annual plastic production was 368 million tons , and it is expected that is production will increase up-to 33 billion tons by 2050 (Bellesi *et al*., 2020; Plastic Europe, 2020). It is estimated that 76% of total plastics produced are land filled or

spread in the natural environment (Geyer, 2020). When plastic garbage is disposing in landfills, it appears to eliminate waste from the upper surface of land but it actually diminishes agriculture land (Zhang *et al*., 2004). Because natural decomposition takes so long, the landfill area cannot be used for other purpose (Tansel and Yildiz, 2001). In comparison to landfills incineration is a superior solution because as it requires less space and provide better energy recovery (Sinha *et al*., 2010). However it is not without restriction because it produces green house gases, as well as Polychlorinated biphenyls (PCBs) and free radical exposure (Astrup *et al*., 2009). Regardless, landfills and incinerated constraint can be addressed through recycling, though this procedure is costly and its end product is poor (Yamada-Onodora *et al*., 2001). In compare to other approaches, biodegradation is the most effective one. It is inexpensive and does not emit hazardous pollution in to the atmosphere.

The main polymer constituents of microplastics found in water have been identifiedas polyethylene, polypropylene, poly stryrene and polyethylene terephthalate accounting for 70% of the total but polyvinylchloride, polyacrylonitrile, rubber different copolymers are also common (Li *et al.,* 2020). MPS are constantly present in fresh and marine ecosystem and they easily leaching the plasticizers due to physical and chemical factors of the nature. Because some plastic additives are physically bound to the plastic and can easily be released into the environment, they will eventually become available to organisms. Heat and acidic or basic conditions (E.g., bisphenol A) can disrupt the hydrogen bonds of additives that are chemically bound to polymers, releasing the additives into their surroundings (Rani *et al*., 2015; Hermabessiere *et al*., 2017). Plastic additives have been found in varying concentrations and distributions in the biosphere (Hermabessiere *et al*., 2017; Hahladakis *et al*., 2018). As a result, plastic environmental pollution is caused not only by the plastic materials themselves, but also by the

chemicals used in plastic manufacturing to achieve the desired characteristics of each product. Plastic additives have been reported to be potentially toxic to mammals (e.g., endocrine disruptors, contributors to chronic health effects, and cancer risks) (Hermabessiere *et al*., 2017; Hahladakis *et al*., 2018; Groh *et al*., 2019). A lot of micro plastic debris was found even in oceanic surface water (Audrezet *et al*., 2020). It is considered that micro plastic have become a main source of anthropogenic pollution of the oceans (Bowly *et al*., 2021). MPS concentration in highly contaminated rivers could be up to 100mg/L. It is evident that the quantity of micro-plastic will increase over the next decade, so the fate, and biological impact on the environment of this contaminant are in focus of scientific research. Micro-plastic have been also in freshwater (Wagner *et al*., 2014), drinking water (Eerkes-Medrano 2019), soil (Guo *et al*., 2020), as well as in food particle (Rainieri *et al*., 2019). Di Methyle Phthalate (DMP) only has a modest level of toxicity, its metabolic intermediary mono-methyl phthalate (MMP) is not only poisonous but also and endocrine disrupter that can affect how animal and even human developed and reproduced by decreasing sperm counts and testosterone production (Brar *et al*., 2009). Microalgal biotechnology did not really take off until the middle of the last century. Now microalgae were used in many fields including bioremediation, bioaccumulation, etc. Microalgae are well-known pollutant scavengers for a wide range of chemicals emitted by the domestic, industrial, and agricultural sectors. Microalgae are photosynthetic microorganisms found at the base of aquatic food chains. Eco-pollutants are toxic to microalgae and, as a result, have a negative impact on all higher-level organisms in food chains, as well as human surroundings and humans. Metals and metalloids, organic solvents, pesticides, and detergents, as well as pharmaceuticals and personal care products, have all been shown to have a negative impact on micro-algal populations (Maizek and Brozek-Pluska, 2019). Many researchers found that microalgae

have the capacity to reduce the toxicity of plasticizers. Similarly the microalgae also do adsorption and absorption on emerging contaminants. These kinds of information evidenced that the microalgae certainly helps to eliminate the BEHA pollution in the aquatic environment. Hence the present study focused on the potential of microalgae in degradation of (BEHA) bis(2-ethylhexyl) adipate.

Massive plastic accumulation has been taking place across diverse environments like aquatic and terrestrial environments due to large-scale plastic production. Now a days, societies struggle with continuously increasing concerns about the subsequent pollution and environmental stresses that have accompanied this plastic revolution. Exposure to weather conditions and environmental micro-flora like bacteria and microalgae can slowly corrode the plastic and release the plasticizers. These are potential sources of negative effect on global food chains. In recent years, several studies have been targeting the utilization of micro algae for remediate the plastic and plasticizers pollution. Hence the bioaccumulation of di(2-ethylhexyl) adipate using *Chlorella sp.* was studied. Through this research the reduction of emerging contaminants will be studied meanwhile the adsorption, absorption and accumulation of Bis(2- ethylhexyl) adipate (DEHA) by microalgae was also established. This research definitely become a boon to our society.

Objectives

The main objectives of this study are as follows;

- To identify the microalgae which degrade plasticizers.
- To estimate the chlorophyll content of the microalgae with and without treatment of bis(2-ethylhexyl)adipate.
- To retrieve the level of degradation of plasticizer using GC-MS.

Micro-plastic pollution is a difficult issue (Windosr *et al*., 2019) with significant environmental and public health repercussions. This pollution problem is a classic transboundary of how land based pollution can spread rapidly, even into remote areas such as virgin mountainous regions, wilderness areas, and the Arctive (Bergman *et al*., 2019; Brahney *et al*., 2020), as well as the ocean's deepest trenches (Jamieson *et al*., 2019). Because plastic pollution is physically apparent, it has piqued the interest of a diverse group of stakeholders including scientists, policy markers, the media, and the general public. This subject has gotten a lot attention, possibly more than any other pollution concern in history of science (Sedlak, 2017).

Food, medicines, cosmetics, detergents and chemicals all employ synthetic polymers in their packaging. Plastics are utilized for packaging applications in about 30% of the world's population. The usage rate is still growing at a rapid rate of 12% each year. Because they offer greater physical and chemical qualities. Such as strength, lightness, resistance to water and most water-borne germs, they have supplanted paper and other cellulose-based products for packaging. Polyethylene (LDPE, MDPE, HDPE and LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), poly(ethylene terephthalate) (PET), poly(butylenes terephthalate) (PET), poly(butylenes terephthalate (PBT), nylons are among the most commonly used plastics in packaging. Plastics are widely used not just because of their advantageous mechanical and thermal qualities, but also because of their stability and durability (Rivard *et al*., 1995). Because of their durability and visibility in litter, plastics (Polymers) have gotten more public and media attention than any other component of

the solid waste stream. In 1993, the global demand for plastics was around 107 million tones, and in 2000, it was anticipated to be above 146 million tones.

The dramatic increase in production and lack of biodegradability of commercial polymers, mainly commodity plastics used in packaging (e.g. fast food), industry and agriculture, has focused public attention on a potentially huge environmental accumulation and pollution problem that could persist for centuries (Albertson *et al.,* 1987). Plastic waste is dispose off through the process such as land filling, incineration and recycling. Because of the persistent presence of wasted plastic in our environment, several communities have become more aware of its negative impacts on animals and the aesthetic characteristic of towns and forests. Plastic that has been improperly disposed of has the potential to damage people by polluting the environment. Furthermore, the combustion of polyvinylchloride (PVC) polymers releases persistent organic pollutants (POPs) such as furans and dioxins (Jayasekara *et al.,* 2005).

Plastics have become an indispensable part of the society due to their lightweight, easy handling, durability, flexibility, resistance to water, and other microbial attacks. However, their extensive use has led to a plastic pollution problem. To avoid long-term environmental damage, degradation of plastic is the most preferred option. Microbial degradation using bacteria and fungi is an emerging strategy to manage plastic waste. This chapter highlights the benefits, concerns, and threats surrounding the use of plastics, including plastic production and plastic waste generation, environmental and health effects of plastic pollution, plastic waste management options, biodegradation of plastic polymers and the mechanism involved, biodegradable plastics, and challenges and constraints of plastic waste biodegradation (Yogalakshmi and Singh, 2020)

Microplastics concentration in the Manas River, china, was explored by a group of researchers (Wang *et al*., 2019). They observed a range of 21±3 to 49±3 items/L in which fibrous microplastics were dominant at all sites with a size range of 0.1 and 1.0 mm, while black and white were the dominant colours. IR spectral analysis showed that the dominant polymers were PP and PET. The study can help to understand the contamination features of microplastics in inland rivers.

Microplastic fibers constitute the largest component of plastics in aquatic environments. (Sait *et al*., 2021) investigated the photodegradation of PET, PA, PAN and their respective chemical profile, along with their potential for additive leaching. PET and PA fibers showed significant morphological changes upon exposure to UV. Chemicals identified in fibers and aqueous leachates include monomers, UV stabilizers and degradated polymers. Bisphenol A, Bisphenol S and Benzophenone-3 were quantified in all fibers and wool at concentrations between 4.3 and 501 mg/g, with wool displaying maximum concentration of BPs and BzPs at 863 and 27 mg/g, respectivetly.

Kosuth *et al*. (2018) investigated microplastic particles in tap water, beer, and commercial sea salt. Microplastics were present in 81% of the tap water samples which composed of fibers (98.3%) of 0.1± 5 mm. the abundance ranged at 0 to 61 particles/L. Likewise microplastics were present in beer and salt. The composition was dominated by fibers (99%). Beer was contaminated with 46.7 to 806 particles/kg. It was estimated that an average person consumed more than 5800 particles of microplastics from tap water, beer and salt. In another study, high and low cost commercial were purchased by Renzi and Blaskovic (2018) to analyse for the presence of microplastics. The microplastics ranged between 1.57- 31.68 items/g. the sizes of particles ranged within 4-4628 µm. the samples were purchase from Italy and Coratia and it was found that all samples from

both the countries had microplastic contaminated but varied in abundance which is dependent on several factors.

Gundogdu and Cevik (2017) reported the distribution of micro-meso plastics in the Northest Levantine coast of Turkey. The average of micro and meso plastics was determined to be 0.376 items/m². The highest value was determined in Mersin Bay at the mouth of the Seyhan river (906) items and the lowest level was found in Station No.4 in Iskenderun Bay (78 items).

Babu and Wu (2010) observed thephthalate esters are widely distributed pollutants that originate from synthetic plasticizer and are known to act as toxicants as well as environmental pheromones in aquatic ecosystems. This study revealed that sixteen species of freshwater algae and cyanobacteria were capable of producing di(n-butyl)phthalate (DBP) or mono(2-ethylhexyl)-phthalate (MEHP) or both. The incubation of the cells in culture medium containing $NaH_{13}CO_3$ confirmed that both phthalates were de novo synthesized by the studied cells.

Chang *et al*. (2021) reported that Phthalate esters (PAEs) are one of the most widely used plasticizers in polymer products and humans are increasingly exposed to them. Epidemiological studies found a consistent association between PAE exposure and a decrease in sperm quality in males and symptom development of ADHD in children. Future studies need to thoroughly perform in large-scale populations to increase the precision of the association and enhance the overall understanding of potential human health risks of PAEs.

Benjamin *et al*. (2015) investigated the phthalates are a group of xenobiotic and hazardous compounds used in plastics to enhance their plasticity and versatility. He

have shown endocrine disruption, hepatotoxic, teratogenic and carcinogenic properties, but usage continues due to their cuteness, attractive chemical properties, low production cost and lack of suitable alternatives. The major phthalates used in industry, routes of environmental contamination, evidences for health hazards, routes for *in situ* and *ex situ* microbial degradation, bacterial pathways involved in the degradation process, half-lives of phthalates in environments.

Ganta *et al*. (2020) reported the bisphenols and phthalates are two known plasticizers that have been found to cause health impairments in various organs and fetuses and newborns. Bioremediation is a low cost and eco-friendly solution that can accumulate and concentrate the toxin to the point of easy disposal. Many living systems or their components can be used as agents for toxin removal among which they have discussed a few bacteria, fungi, and plant biomass for the removal of bisphenols and phthalates.

Gaur *et al*. (2022) described the microplastics have become a major environmental and human health hazard, and microorganisms have evolved to degrade different classes of plastic polymers. Meta "omics" approaches have been used to identify the active microbiota and microbial dynamics involved in the mitigation of microplastic-contaminated sites. Protein engineering approaches have opened new avenues to tackle this alarming situation.

Selvaraj *et al*. (2021) reported the microalgae, especially *Chlorella sp*., have been used for centuries as food and currently their biotechnologicl potential is notable for the presence of several compounds relevant to the market, like PHB. Microalgae are appealing because of the increasing demand for biopolymers like PHB. Therefore, the

present study is aimed to determine the efficacy of biomass production and yield of PHB producing microalgae isolated and identified by phylogenetic analysis**.**

Gobas *et al*. (2020) explored the bioaccumulation behavior of several phthalate esters in aquatic food-webs, concluding that they do not biomagnify in the food-web. Higher molecular weight esters (DEHP, DnOP, and DnNP) show evidence of trophic dilution, which is consistent with findings from laboratory and modeling studies. Bioaccumulation patterns of DBP, DiBP, and BBP indicate no significant relationship with trophic position consistent with a lipid-water partitioning model. Low bioavailability of the high-molecular weight esters in natural waters is the main reason why the BAFs of the higher molecular weight phthalates are below the UNEP criteria.

He *et al*. (2016) studied the removal and biodegradation of nonylphenol (NP) by four freshwater microalgae, including three green algae (*Scendesmus quadriauda, Chlorella vulgaris,* and *Ankistrodesmus acicularis*) and one cyanobacterium *(Chroococcus minutus),* was studied in bacteria-free cultures exposed to different concentrations of NP for 5 days. All four algal species showed a rapid and high ability to remove NP, with *A. acicularis* having the highest NP removal rate (83.77%) at 120 h when exposed to different NP treatments. *C. vulgaris* had the highest NP biodegradation percentage (68.80%).The extracellular NP contents were lower than the intracellular NP contents in all tested algae, with the ratio of the extracellular and intracellular content ranging from 0.04 to 0.85. These results indicate that *A. Acicularis* and *C. vulgaris* are more tolerant to NP and could be used for treatment of NP contaminated aqueous systems effectively.

Chi *et al*. (2007) investigated the influence of major nutrients (N, P) on the biodegradation and bioconcentration of dibutyl phthalate (DBP) and di-2-

ethylexylphthalate (DEHP) by *Chlorella vulgaris* in lake water. It found that nutrient addition had a significant effect on biodegradation rate constants and BCFs of DBP and DEHP, with P addition being less pronounced than N addition due to N-limitation status of phytoplankton, while addition of both N and P more greatly affected biodegradation than addition of N or P. BCFs decreased with increasing algal exudate as measured by dissolved organic carbon (DOC) and a strong correlation between BCFs and DOC was obtained. This suggests that DOC plays an important role in the bioconcentrate of DBP.

Touliabah *et al*. (2022) proposed a new bioremediation method based on the diverse functionalities of algae that is more ecologically friendly and environmentally sustainable than prior methods with other bacteria. Algae-based wastewater treatment systems are becoming increasingly popular due to their environmental sustainability and lack of secondary pollutants. Phytoremediation is a cost-effective alternative to conventional treatments for degrading organic contaminants and can be an important part of the bioenergy value chain. They focuses on microalgae and cyanobacteria species, which may remove many organic contaminants from water systems.

Liu *et al*. (2022) investigated the plastics and microplastics are difficult to degrade in the natural environment due to their hydrophobicity, covalent bonds, and functional groups. In nature, they are more likely to attract other substances, which can be toxic and harmful. Degradation is an effective way to eliminate plastic pollution, but there are no mature and effective methods that can be applied in engineering practice or widely used in nature. There is an urgent need for research on the degradation of (micro)plastics.

Tan *et al*. (2023) reported the nonphthalate plasticizers (NPPs) are increasingly used for industrial needs, but knowledge is limited on their environmental occurrences,

fate, and human exposure risks. They investigated 45 NPPs along with major PAEs in house dust from five regions in the Asia-Pacific region and the US. The median total concentrations of NPPs ranged from 17.8 to 252 μg/g, while the mean ratios of ΣNPPs to ΣPAEs ranged from 0.19 (Hanoi) to 0.72 (Adelaide). Potential exposure risks cannot be overlooked due to lack of toxic threshold data, additional exposure pathways, and possible cocktail effect.

Palah *et al*. (2020) explored the potential of *Chlorella vulgaris* and Pretreatment to remediate plastic waste. Results showed that Pretreatment had a marked effect on the cracking and alteration of plastic polymer, which helped to grow microbial species on the cracked surface. GCMS analysis revealed that the microbial specie could produce biodegradation products such as alkanes ester, fatty acids, benzoic acid, and aromatics. The most toxic product of biodegradation is Bis (2-Ethyl hexylphthalate), which is the biodegradation product of toxic ingredient of plastics.

Moog *et al*. (2019) reported the biological degradation of plastics is a promising method to counter the increasing pollution of our planet and develop eco-friendly recycling strategies. *Ideonella sakaiensis*, a bacterium possessing the ability to degrade PET and use the degradation products as a sole carbon source for growth, was isolated in 2016. It expresses a key enzyme responsible for the breakdown of PET into monomers, PETase, which has potential for the development of biological PET degradation and recycling processes as well as bioremediation approaches of environmental plastic waste.

Khoironi *et al.* (2019) investigated the polyethylene terephthalate (PET) and Polypropylene (PP) are the most widely used plastics in manufacture of packaging, fibres, and drinking bottles. This study evaluated the interaction between microalgae

Spirulina sp. and microplastics in a 1 L glass bioreactor for 112 days. The results showed that the tensile strength of micro plastic PET decreased by 0.9939 MPa/day while the decreasing carbon in PET was higher than PP. The CO_2 evolution of cells imposed by PET microplastic was higher than imposed by PP. Biodegradation has important role in the degradation process of plastic.

Barone *et al*. (2020) studied the plastic accumulation has been taking place across diverse landscapes since the 1950s, leading to increasing concerns about pollution and environmental stresses. Degradation of used plastics is highly time-consuming and causes volumetric aggregation. Exposure to weather conditions and environmental microflora can slowly corrode the plastic structure. Cyanobacteria (e.g., *Synechocystis sp.* PCC 6803, and *Synechococcus elongatus* PCC 7942), which are photosynthetic microorganisms and were previously identified as blue-green algae, are currently under close attention for their abilities to capture solar energy and the greenhouse gas carbon dioxide for the production of high-value products. Microalgae are also suitable for environmental and biotechnological applications based on the exploitation of solar light. In recent years, several studies have been targeting the utilization of microorganisms for plastic bioremediation. Wild-type or engineered cyanobacteria may represent an interesting, environmentally friendly, and sustainable option.

Zhang *et al*. (2018) investigated the combined effects of UV-B irradiation and di-(2-ethylhexyl) phthalate (DEHP) on photosynthesis and antioxidant system of *Scenedesmus acuminatus*. Results showed that UV-B radiation decreased chlorophyll a fluorescence yield, photosynthetic activity (Fv/Fm), pigment content and superoxide dismutase activity, while DEHP increased ROS production and soluble protein and malondialdehyde contents. The highest degradation rate was 89.9% at an initial DEHP

concentration of 10 mg L−1 within 6 h. This result may be attributed to the regulation of ROS generated by *S. acuminatus*, and the addition of high DEHP concentration aggravated cell damage.

Gu *et al*. (2017) investigated the acute toxic effects and underlying mechanisms of dibutyl phthalate (DBP) at different concentrations (0–20 mg L−1) on two typical freshwater algae (*Scenedesmus obliquus* and *Chlorella pyrenoidosa*). The growth of both algae was inhibited by DBP exposure, and the 96-h median effective concentration values (96h-EC50) were 15.3 mg and 3.14 mg. The increased production of intracellular reactive oxygen species and malondialdehyde content was linked to oxidative stress and lipid peroxidation in both algae, as well as increased activity of antioxidant enzymes such as superoxide dismutase and catalase. The findings will contribute to the understanding of toxic mechanisms in PAEs and the evaluation of environmental risks for primary producers in aquatic ecosystems.

Li *et al*. (2020) reported the microplastics are ubiquitous in aquatic ecosystems, but knowledge on their impacts on phytoplankton, especially freshwater microalgae, is limited. To investigate this issue, microalgae *Chlamydomonas reinhardtii* was exposed to polystyrene (PS) microplastics with 4 concentration gradients and the growth, chlorophyll a fluorescence, photosynthetic activities, Fv/Fm, contents of malondialdehydes (MDA), soluble proteins, extracellular polymeric substances (EPS) and settlement rate were measured. Results showed that the density of microalgae decreased as the increase of PS microplastics concentrations, and the highest inhibitory rate (IR) was 45.8% on the 7th day under the concentration of 100 mg/L. The high concentration (100 mg/L) of microplastics also inhibited the content of EPS released by microalgae into the solution. The scanning electron microscope (SEM) images showed

that microplastic beads were wrapped on the surface of the microalgae and damaged their membranes, which could suggest the reduction of photosynthetic activity and the increase of soluble proteins and MDA content.

Gao and Chi, (2015) investigated the biodegradation of diethyl phthalate (DEP) by three marine algae. The first-order biodegradation rate constants of DBP in algal solutions were in the order of *Cylindrotheca closterium* > *Dunaliella salina* > *Chaetoceros muelleri*. When singly existed, DEP was degraded more quickly than in a mixture with DBP, indicating that DBP had inhibitory effect on the biodegradation of DEP. The degradation trends of DEP and DBP in both extra- and intracellular crude extracts were similar, and DEP was largely in water phase while DBP remained in both water phase and algal phase. It was concluded that algal extracellular enzymes played key roles in the degradation of DBP.

Zhang *et al*. (2016) investigated the biodegradation characteristics of dimethyl phthalate (DMP) by three freshwater unicellular organisms. All three organisms were capable of metabolizing DMP, with PCC7822 achieving the highest degradation efficiency. Phthalic acid (PA) was detected to be an intermediate degradation product of DMP and accumulated in the culture solution. The optimal initial pH value for the degradation was 9.0, which mitigated the decrease of pH resulting from the production of PA. After 72 hours' incubation, no more than 11.8% of the residual of DMP aggregated in Cyanobacteria cells while majority of DMP remained in the medium. Esterase was induced by DMP and the activity kept increasing during the degradation process, suggesting that esterase could assist in the degradation of DMP.

Ji *et al*. (2014) investigated the toxicity and cellular stresses of bisphenol A (BPA) to *Chlamydomonas mexicana* and *Chlorella vulgaris* and its

biodegradation/bioaccumulation by both microalgae. The 120-h EC50 of BPA was 44.8 and 39.8 mg L−1, respectively, and the dry cell weight and chlorophyll a content decreased with increasing BPA concentration. The highest rates of BPA biodegradation, 24 and 23% respectively, were achieved at 1mg L−1 BPA. Total nitrogen (TN) and total phosphorous (TP) removal was higher in C. vulgaris. Both microalgae were more tolerant to BPA and could be used for treatment of BPA contaminated aqueous systems.

Gao *et al*. (2021) reported that the marine diatom (*Phaeodactylum tricornutum*) was exposed to different concentrations of dimethyl phthalate and diethyl phthalate (DEP) for 96 h to investigate their toxicities. Results of this study showed that the diatom could remove DMP and DEP effectively with removal rates of 0.20–0.30 and 0.14–0.21 mg L−1 h−1, respectively. However, the two PAEs significantly inhibited photosynthesis and chlorophyll biosynthesis. Additionally, reactive oxygen species (ROS) level and antioxidant enzymes (SOD and POD) activity increased with the increase of PAEs concentrations. The results of this study help to understand the toxic mechanisms of PAEs and provide strong evidences for evaluating their ecological risks in the marine environment.

Chenchenshen *et al.* (2019) revealed di-2-ethylhexyl phthalate (DEHP) poses a great threat to aquatic ecosystems, with known hazards to aquatic species. This study investigated growth inhibition, oxidative damage and antioxidant enzyme activities in *Chlorella vulgaris* under DEHP treatment. Results showed that DEHP reduced superoxide dismutase and glutathione peroxidise activities, increased hydrogen peroxide level and MDA content in a concentration-dependent way, indicating that DEHP could have biochemical and physiological toxic effects in *C. vulgaris.* These findings helped to

understand the toxicity mechanisms of DEHP and the environmental risk assessment of primary producers of aquatic ecosystems.

Yan *et al*. (1995) studied the *Chlorella pyrenoidosa* has an ability to accumulate and biodegrade phthalate esters, with maxima of 162 at 24 h, 205 at 12 h, and 4,077 at 12 h. Average biodegradation rates of DMP, DEP, and DBF per day were found to be 13.4, 7.3, and 2.1 mg/L, respectively. A second-order kinetic equation was formulated as - dC/dt = KNr, with a factor r indicating the rate of algal growth.

Chi *et al.* (2005) investigated the microalgae have the ability to degrade and accumulate organic pollutants, but little is known about the biodegradation and accumulation of di(2-ethylhexyl) phthalate(DEHP). This study studied the accumulation and biodegradation kinetics of DEHP in *Chlorella vulgaris*. The initial concentration of DEHP was approximately 0.4 mg/L, and the alga was able to accumulate and degrade DEHP significantly. The amount of DEHP accumulation by the alga reached maximum of 107.5 mg/g dry weight at 0.5 h and bioconcentration factor reached maximum of 3.67Ã—105 at 6 h. First-order biodegradation constant was 0. 0021 h-1.

Rabet *et al*. (2018) described the effects of two plastic-derived chemicals, Bisphenol A (BPA) and di-2-ethylhexyl phthalate (DEHP), on the abundance and physiological responses of the marine toxic dinoflagellate *Alexandrim pacificum* were assessed during 7 days of exposure. Results showed that *A. pacificum* was highly sensitive to these contaminants, with a decrease in biomass and photosynthetic activity. However, recovery of contaminated cells activity depending on exposure time and BPA and DEHP contamination could be related to an adaptation to induced stress. Hu *et al.* (2022) described the *Gordonia sp.* GZ-YC7 is a new phthalate esters degrading strain isolated from soil of plastic film mulch culture. It exhibited the highest di-(2-ethylhexyl)

phthalate degradation efficiency under 1000 mg/L and the strongest tolerance to 4000 mg/L. Comparative genomic analysis revealed that there exist diverse esterases for various phthalates, which may contribute to its broad substrate spectrum, high degrading efficiency, and high tolerance. It has potential for bioremediation in polluted soil environments.

Kumar *et al*. (2017) investigated the conventional methods of polyethylene degradation are lethal to the neighboring environment, and a better solution is needed. It is also investigated the biological treatment of domestic polyethylene bags from three different sites in Chennai, Tamil Nadu, India. Microalgae like green algae, blue-green algae and diatoms were isolated from the polyethylene sheets and selected for the biological treatment. The most dominant microalgae were *Scenedesmus dimorphus* (Green microalga), *Anabaena spiroides* (blue-green alga) and *Navicula pupula* (Diatom). The scanning electron microscopical study revealed that the degradation was evident in the treatment of LD polyethylene sheet using the microalga *Anabaena spiroides*, which was found to grow feasibly rather than the other microalgae.

Bate *et al.* (2019) investigated the increasing incidence of cyanobacterial blooms in Southern African aquatic systems is raising concern about the potential for these microorganisms to contaminate potable water with toxic secondary metabolites. They focused on two lakes, an estuary and the sea in a small catchment in Maputaland, northern KwaZulu Natal, South Africa, fed by groundwater impacted by sewage effluent. Analysis of the microalgae in two freshwater lakes showed that cyanophytes made up over 88% of the phytoplankton in the larger Lake Mgobezeleni and over 50% in the smaller Lake Shazibe, raising concerns about the potential health risk to communities using this water for domestic, agricultural and recreational purposes.

Generation Sequencing analysis of bacterial 16S rRNA genes showed that cyanobacterial taxa closely related to species that are known to produce cyanotoxins were detected in all the water bodies sampled. These results highlighted the importance of identifying water systems at risk of experiencing cytotoxic cyanobacterial bloom and monitoring such vulnerable systems to ensure the safety of surrounding community.

Susanti *et al*. (2021) reported that the microalgae are oxygenic photosynthetic microorganisms and closely related to the heterotrophic oraganism. They have a variation in morphological features, biochemical composition, reproduction, cell organization, plastid structure, and habitat. Currently, algae are divided into 10 groups composed of either prokaryotic or eukaryotic. In terms of application, algae biomass is utilized as a valuable natural product worldwide, but biomass productivity needs to be enhanced using complementary growth media usage. Research during the COVID-19 pandemic about the utilization of anaerobically digested dairy manure wastewater as an alternative media for algal biomass production will also be reported.

Sandeep *et al*. (2018) studied the microalgal diversity and dynamics of a tropical estuarine ecosystem (Muttukadu, of Indian south east coast) and applies tools like isolation of useful species to utilize in aquaculture and conserve native strains. Selected diversity indices (Simpson index, Dominance index, Shannon- Weiner index, Pielou's evenness index and Margalef richness index) were used to describe trends of diversity in the estuary during the study period. Sixty three species of microalgae belonging to Chlorophyceae were identified, with Bacillariophyceae forming the dominant flora with twenty six species. The species diversity was increased after the flood during December-2015 in south east coast of India. Nutrient profiling of isolates revealed the presence of essential fatty acids (EPA & DHA) in high percentage in some of the isolates.

Rampinelli *et al*. (2022) isolated and identified the diatoms found in the Paranaguá Estuarine Complex (PEC). The diatoms were purified and analyzed with light and scanning electron microscopy for morphological identification, while DNA sequences were used for molecular identification. The two best-selected strains were identified as belonging to two genera, *Nitzschia* and *Navicula*. The rbcL region was found to be the most informative for species identification.

Arsad *et al*. (2022) aimed to assess the diversity of microalgae in several different sub-habitats at Siwil Beach and Sempu Island. It used a quantitative descriptive method with data collection techniques, incorporating the purposive sampling method, and non-metric multidimensional scaling. The results showed that the composition of the microalgae species was dominated by Bacillariophyceae, with a total abundance of 5,423,073 cells/cm2, while the highest abundance in Sempu Island was 1,986,252 cell/cm2. Factors that mainly affected the abundance were environmental, as evidenced by the measurement of water quality.

Trivedi and Mitra, (2021) revealed the pelagic environment of the ocean supports two basic types of marine organisms: plankton and nekton. Phytoplankton are free floating tiny floral components that require sunlight, nutrients or fertilizers, carbon dioxide gas and water for growth. Nekton are freefloating animals that are strong enough to swim against independent of water movements. Marine phytoplankton was the dominant producers in the ocean, and their role in the marine food chain is of paramount importance. Approximately 4000 species have been described, and they exhibit remarkable adaptations to remain in floating condition in the seawater. These adaptations include their small size and general morphology, colony or chain formation, and ionic

regulation. A field study was conducted in September, 2017 in the Thakuran River to identify 73 species in a salinity range between 12psu to 18psu.

Ge *et al*. (2022) examined the phytoplankton alpha and beta diversity using investigation data in May (springtime), August (summer) and November (autumn) 2009 in China's Jiulong River estuary, where it was easily polluted due to human population and low self-purification capacity. Potential influencing factors were explored, including dissolved oxygen, salinity, nutrients, nutrient ratios, geographic and hydrologic distance, and so on. Results showed that Shannon's index (H') and Pielou's index (J) decreased from the estuary's upper to middle and then increased from middle to lower reaches, Simpson's (D) observed the opposite trend and species number (S) gradually increased. Beta diversity also showed a gradual decrease trend from the upper to lower reaches. Nutrients and nutrient ratios were characterized by excess nitrogen (N) and silicon (Si) and limited phosphorus (P), which could potentially cause diatom blooms. This study advocates for the protection of the entire estuary system with particular emphasis on its upper reaches, and greater attention should also be paid to impacts associated with N input and nutrient ratio trade-offs to the prospective watershed management of this estuary.

Maltsev and Maltseva (2021) studied the possibility of obtaining commercially valuable products from microalgae stimulates scientific research in this direction. Fatty acids (FAs) are involved in the metabolic pathways of formation and conversion of most lipid classes, and their composition largely determines their properties and practical use. They summarizes information on the diversity of the fatty acid composition of microalgae and cyanobacteria, taking into account their rare and unusual categories. It is formed by 135 FAs, distributed into several groups based on the length of the

hydrocarbon chain, its structure and the presence of substituents. There are both saturated and unsaturated FAs with different numbers of double bonds, rich in omega-3 and omega-6 fatty acids. They also considers the use of fatty acids as an industrial resource, as well as a biomarker.

Hasan *et al.* (2022) studied the Pasur River estuary (PRE) provides vital fishery resources and supports millions of livelihoods in the southwestern coastal region of Bangladesh. This research focused on phytoplankton community assemblages, alpha diversity indices, and the seasonal succession of major phytoplankton species in relation to physicochemical parameters in the tidal mangrove creeks of the PRE. Spatial and temporal variations were assessed by water sampling at 17 stations in the study area from January to December 2019. The mean salinity level was significantly higher during the dry season than during the wet season, and no significant variation was observed in the dissolved inorganic nitrogen and dissolved inorganic phosphorus. Spatially, no significant variation in the alpha diversity was observed, but significantly ($p < 0.05$) varied temporally. The study classifies the study areas as highly diversified zones, and the succession from diatoms (dry season) to blue-green algae (wet season) is attributed to changes in the physicochemical and nutrient parameters depending on seasonal environmental parameter fluctuations.

Petal *et al*. (2021) examined the phytoplankton abundance and diversity from site 1 (downstream) and site 2 (upstream) of the Auranga Estuary (20˚63' N and 72˚82' E). A total of 44 species were recorded, 35 species from downstream and 24 species from upstream. *Dinophyceae* and *Chrysophyceae. Nitzschia, Coscinodiscus* and *Ceratium* were abundant genera at site 1 and *Spirogyra, Microcystis, Chlorella* and *Oscillatoria* were abundant at site 2. Spatially, downstream had higher species diversity and

abundance than upstream, while winter season was favorable for plankton growth compared to summer and monsoon. The Shannon diversity index was 1.417 and 1.268 for downstream and upstream, respectively, indicating less diversity level in this estuary.

Darmarini *et al*. (2023) aimed to evaluate the diversity and abundance of phytoplankton in mangrove habitats at Lubuk Damar, Aceh Tamiang Regency, via plankton net with a 20-micron mesh size. Results showed that in August 2017, the diversity was higher than in January, with an Index Diversity of 1.24-2.83 and an Index Dominance of 0.17-0.48. In January, *Chaetoceros sp*. was dominant in water, followed by *Bacillaria sp*, and *Biddulphia sp.* In August, *Leptocylindrus sp*. was the dominant in diversity, and diatoms were dominant in abundance.

Susanti *et al*. (2014) observed the phytoplankton role in aquatic ecosystems and is one of the bioindicators used to describe conditions, quality, and environmental changes. Jakarta Bay is polluted by various wastes originating from industry, domestic, and sea transportation for fishing activities, which causes a decrease in water quality. This study revealed the composition of phytoplankton and its relationship with the physical and chemical parameters of the waters that empty into Jakarta Bay. The study was conducted in September 2021 at three sampling stations in estuary areas. Station 3 was the most polluted area from industrial waste, but only found the group of Cyanophyta (61.57%), Euglenophyta (38.43%), Chrysophyta (39.74%), and Chlorophyta (19.21%). Station 2 had the highest concentration of chlorophyll-a, but the water quality of the two sampling stations greatly affected the composition and abundance of phytoplankton.

Varghese *et al.* (2022) collected the phytoplankton samples from ten stations in the Kadalundiestuary during July 2018 to June 2019 were studied. 87 species were

recorded, 43 belonging to the Phylum Bacillariophyta (Diatoms) and 24 belonging to Miozoa (Dinoflagellates). *Tripos furca* contributed maximum with 7%, followed by *Trieres chinensis* (6%), *Skeletonema costatum* and *Tripos muelleri* (5% each). An average density of 25130 cells/m3 was recorded from the study area with a maximum of 25% in September and a minimum of 1.7% in July. Station wise concentration varied from 9% to 11%.

Balakrishnan *et al*. (2018) reported the halophilic microalgae were collected from the salt pans of Tuticorin, Southeast coast of India, of which 3 were Bacillariophyceae, 4 were Chlorophyceae and 6 were Cyanophyceae. The species diversity decreased sharply when the salinity of the water increased, with *Dunaliella sp.* being the most prominent species in the crystallizing area forming orange-red patches on the salt crystals. Most of the species failed to grow except *Oscillatoria sp*. and *Nitzschia sp*. in the hypersaline region.

Viji *et al*. (2018) studied the physio-chemical properties of water samples collected from various ponds in Tuticorin District, Tamil Nadu. The results revealed that overall water quality was unfit for drinking and irrigation purposes due to industrialization and population expansion. To prevent these problems, an understanding of fundamental water chemistry and other physical parameters is necessary.

Elumalai *et al*. (2013) reported is used Fourier transform infrared spectroscopy (FTIR) and GC-MS to identify and quantify lipids in freshwater microalgae from Cement factories, Ariyalur district. The lipid fractions were extracted from the biomass through different solvent extractions and analyzed for biodiesel. Results of this study showed that eight Microalgal groups produced SFA in high percentage; seven groups had high yields of PUFA and only one group of microalgal contain MUFA.

Oyewumi *et al*. (2018) collected the water samples from ponds at the Federal University of Technology Akure, Nigeria (FUTA) and a pond in Oda Road, Akure in Ondo State, Nigeria. Microalgae were cultured and identified in the laboratory using a microscope, identification keys and algae compendium. Seven (7) microalgae were identified and the growth rates estimated were observed to increase from day 6 to 10 with the maximum peak at day 8. The pH and temperature values on microalgal growth ranged from 7.0 to 8.29 and 23^{o}C to 32^{o}C respectively.

Aragaw *et al*. (2017) investigated the production of microalgae (mixed culture) in photobioreactor configurations using two different media formulations in batch cooperation. The predominant co-cultured freshwater microalgae species *Scenedesmus sp., Chlorella sp., Synedra sp.* and *Achanthidium sp*. were investigated in batch culturing media and the effect of culturing media (BB Medium and BG-11 Medium) for effective algal growth was determined. The maximum biomass concentration was found 0.608 g/L in the Bolt basalt medium and 0.5624 G/L in BG-11 medium for 15 day cultivation time. The amount of time required to adapt the culturing environment is not significantly different and PH range has an effect on mass productions of algae. The optimum pH for high productions of mixed culture microalgae was investigated at pH 8.

Mohanapriya *et al.* (2014) identified microalgae are the most widespread microorganism in freshwater environments and play a vital role in nutrient recycling. Water pollution due to industrialisation has led to the extinction of some species, while eutrophication has caused some microalgal species to overgrow and form algal bloom. They examined the isolation and identification of microalgae from freshwater samples collected from different regions of Noyyal River. 35 green algae, 10 blue green algae, and 4 brown algae were isolated and described.

The present study was carried out in the Korampallam freshwater channel of Puthukottai village, Thoothukudi. Pudukottai is a juction of two main cities (Tuticorin, Thirunelveli and other nearby villages).It is a best commercial and residential place with all facility. Pudukottai is a small Village/hamlet in Thoothukudi Block in Tuticorin District of Tamil Nadu State, India. It comes under Kumaragiri Panchayath. It is located 13 KM towards west from District head quarters Thoothukudi. 11 KM from Thoothukudi Rural. 627 KM from State capital Chennai It is near to bay of bengal. There is a chance of humidity in the weather. The samples were collected from the freshwater channel located nearby bridge.

I. Water Analysis

Physical parameters

The taste, colour, odour and temperature of the water sample were studied.

Chemical constituents

Test for pH

Warm up the instrument for 15min.Calibrate the instrument with the known buffer solutions. (Calibration is done by a buffer solution whose pH is close to that of the sample). Immerse the electrode in the unknown sample, stir for 3min and note the pH.

Test for Alkalinity

Pipette 50ml sample to a Erlenmeyar flask and add two drops of phenolphthalein indicator. If a slight pink color appears, titrate with acid, titrant to a colorless end point and note the reading as 'P' (ml of titrant used for phenopthalein alkalinity). Now add two drops of methyl orange to the same flask and continue to titrate further till the color changes from yellow to orange. Note this reading as 'T' Titrant value of the titrant used for both the titration

Test for Chloride

Pipette 50ml of the water sample (if sea water, take 0.5ml of sample) into a conical flask. Pipette into it, 0.5ml of K_2CrO_4 indicator. This gives yellow color to the sample. Titrate the solution with shaking, against standard silver nitrate solution till the appearance of reddish brown color perform a duplicate titration in an identical

manner.Carry out a blank titration using 50ml of delonized, chloride free water and 0.5ml of the indicator. Subtract this titre value from that obtained for the water sample.

Test for Nitrite

Take a known volume of sample in a 50ml volumetric flask. To it add 1ml of sulphanllamide reagent and mix well. Make up the contents to 50ml by adding distilled water. Shake throughly and measure the absorbance at 543nm against a distilled water blank. Pipette out known concentrations from the standard solution (10 to 100mg). Add reagents as above and draw a standard graph. From the standard graph deduce the amount of nitrite content.

Test for Inorganic phosphorus

Pipette 10ml of the water sample to a test tube. To it add 2ml of mixed reagent and make it up to 15ml with distilled water. Vortex the contents. After 10min. measure the absorbancy at 882nm in a spectrophotometer. Estimate the amount of inorganic phosphorous from the standard curve.

Test for Ammonia

To the sample add 0.4ml of phenol reagent (6) and 0.4ml of nitroprusside reagent (4) and mix well. To it add 1ml of the oxidizing reagent (5) and stopper the tubes immediately. Vortex and incubate for 1hr at room temperature in the dark. Measure the absorbancy at 640nm in spectrophotometer. Prepare a standard graph using different dilutions of the standard graph using different dilutions of the standard solution (1). [Conc. 1 to 10µg] From this find out the ammonia concentration of the sample.

Test for Total phosphorous

Sample digestion for total phosphorous

Take 50 mL of the sample and heat the contents until the volume is reduced to 15ml. Add 1ml of perchloric acid and heat it until the volume is reduced to 5ml. Add 2ml of phenopthalein indicator solution. Then add saturated NaOH solution drop by drop until the solution is turned to pink colour. Make the solution to 50ml with distilled water. Use this sample for the estimation of total phosphorus.

Pipette out known volume of stand and solution to test tubes. (10 to 100µg). Then add 2ml of mixed reagent followed by 2ml of potassium per sulphate reagent and mix well. Incubate for 10mins at room temperatures and read the absorbance of the solution 882nm. Pipette out a known volume as above and measure the absorbance. Fine out the concentration of phosphorous of the unknown sample from the standard curve.

Test for Sulphide

To 7ml of acetate buffer (3.5pH), add 3ml of phenanthroline monohydradte (0.1%) solution. To it add 10ml of water sample. Then make up to 25ml with distilled water and incubate at $25^{o}C$ for 1hr. Run parallel experiment with standard sulphide solutions (10 to 50mg). Read the developed colour at 510nm using suitable blank. Calculate the amount of sulphide by using standard curve drawn, with sodium sulphide.

Test for Calcium and magnesium

Pipette 5 ml of water sample to a 250ml conical flask. To this add 5ml of ammonium buffer and dilute to 100 ml with distilled water. Add a pinch of Erichrome black T and warm the solution to $60^{0}C$. Titrate against EDTA until the red colour turns to blue. Note the end point ‘B’.

II. Degradation of bis(2- ethylhexyl) adipate

1. Media preparation

Chu's medium No. 10 was used to grow the microalgae. The media composition is as follows.

Chemicals	**g/L**
Calcium nitrate	0.232
Dipotassium hydrogen phosphate	0.01
Magnesium sulphate	0.025
Sodium carbonate	0.02
Sodium silicate	0.044
Ferric ammonium citrate	3.5 mg
Citric acid	3.5 mg
Trace metal- 1 mL	
Boric acid	2.4
Manganese chloride	1.4
Zinc chloride	0.4
Calcium chloride	0.02
Copper chloride	0.1
Distilled water	1000 mL

The pH of the medium should be 7.1. The prepared media was autoclaved and stored in glass containers for future use.

2. Identification of microalgae

The microalgal samples were collected from the study area and maintained at refrigerated condition for short term storage. The collected microalgal samples were kept on the glass slide and covered with cover slip and studied under light microscope at 10X, 40X and 100X magnification. The diameter of the microalgae were measured and identified using Floras, and research articles.

3. Isolation of microalgae

The microalgae samples were cultured on agar plates containing Chu's medium No. 10 using spread plate method. The culture plates were incubated at incubation room at 25±2°C with 16 hours light and 8 hours dark cycle provided by cool white fluorescent lights. After 5 to 7 days the grown cultures were seen under microscope and picked the pure colonies. The picked colonies were again kept on agar plates. Repeated this procedure till the pure culture was obtained. The pure cultures were grown massively using Chu's broth medium.

4. Culture condition

The freshwater microalgae *Chlorella sp.* was isolated from the study area.Initially isolated pure colonies were transferred into 10 mL sterile Chu's medium No. 10 and kept inside the incubation room at 25±2°C, under a 16:8 light:dark cycle provided by cool white fluorescent lights. After 3 days of incubation, the algal cells attained sufficient growth; then transfer the whole broth culture 50 mL of fresh broth medium and incubate. After 5 days of incubation, transfer to 100 mL of fresh broth medium and incubate till well growth of algae. Use this as a mother culture and sub-cultured at regular intervals.

5. Experimental setup

The experiment was carried out in triplicate manner in 250 mL Erlenmeyer flasks containing 100 mL of Chu's Medium No. 10 which inoculated with 10% of the actively growing culture of *Chlorella sp.* cells. When the culture attained optical density 0.2 in the absorbance wavelength 750 nm, then it was treated with different concentration of DEHA (20 mg L^{-1},60 mg L^{-1}, 100 mg L^{-1}). A control and solvent control flasks were also maintained. Flasks were manually shaken thrice a day to avoid the adherence of the cells

to the surface of the flasks. Totally 15 days incubation period was given to the culture. Morphological changes in the cells were observed under light microscope (Pancha *et al*., 2013).

6. Growth analysis

Microalgal growth was monitored at regular intervals (0 day, 3rd day, 6th day, 9th day, 12th day and 15th day) by measuringoptical density at 750 nm using UV-vis spectrophotometer.

7. Pigment estimation

For the analysis of pigments content 2ml culture was centrifuge at 10000 rpm for 5 minutes, the supernatant was discarded and 2 ml of absolute methanol was added to the pellet. The content was mixed properly and incubated at 45^0C for 24 h in the dark. The absorbance of the supernatant were read at 470, 652.4, and 665.2 nm and corrected for the turbidity by substracting the absorbance at 750nm. The pigments contents were calculated using the equation (Pancha *et al*., 2013).

$$Chlorophyll\ a(mgL^{-1}) = 16.72\ A_{665.2} - 9.16\ A_{652.4}$$

$$Chlorophyll\ b(mgL^{-1}) = 34.09\ A_{652.4} - 15.28\ A_{665.2}$$

$$Carotenoid\ (mgL^{-1}) = \frac{1000A_{470} - 1.63\ Chlorophylla - 104.9\ chlorophyllb}{221}$$

8. Liquid- liquid extraction

Take 100 ml of water sample in a separating funnel of 2L capacity. Add 10g of NaOH and shake till it get dissolved. Add 50ml of n-Hexane and shake well for 10 minutes. After 30 minutes, discard the n-Hexane layer (impurities) and collect the water

phase. Adjust the pH of water phase to 2 by adding 6M HCl. Add 50 ml of n-Hexane and shake well for 10 minutes for extraction and wait for 30 minutes. Separate n-Hexane layer and stored it. Again add 50ml n-Hexane to the water phase and shake for 10minutes and wait for 30 minutes and separate n-Hexane layer and pool with the previous hexane extract. Add 3g of Na_2SO_4 (anhydrous) into n- Hexane layer for dehydration and leave undisturbed for 20 minutes. Transfer the n-Hexane layer to condensation flask. Concentrate the extract to 5ml by Rotary Evaporator at 35°C and clean up the sample in silica gel column using n-Hexane. Condense elute to 50 ml using rotary evaporator and further to 1ml by passing nitrogen gas. Collect the final extract in a glass vial and store at 4°C prior analyses. Inject 1µl of the sample into GCMS using auto injector for qualitative and quantitative analysis of intermediates of DEHA degradation.

9. GC-MS analysis

The samples were analyzed using a DB 5 MS column (30 m x 0.32 mm ID x 0.25 µm film thickness) using GC–MS. The initial oven temperature was set at 130°C for 5 min, then increased to 200°C at a rate of 8°C per minute. After maintaining at 200°C for 2 min, the temperature was increased to 280°C at a rate of 5°C/min and maintained for 15 min. The injector port and the detector temperatures were 240°C and 250°C, respectively. The peaks were tentatively identified based on the library search report.

RESULT AND DISCUSSION

Physical and chemical constituents of water were essential one to study the quality of water. The survivability of living organisms depends on the physio-chemical composition of water. Water analysis helps to estimate the mineral components of water that is essential to the growth of microalgae. The present study was done in Korampallam channel of Puthukottai, Thoothukudi. The water sample was collected and studied its constituents before the study of phytoplankton. The physical parameters like temperature, odour, taste, and colour were studied. The collected sample was odourless, colourless, tasteless liquid and temperature was 29^{o}C in December, 2022. The pH of the sample was 7.3 and salinity was 13ppt (Table 1). Chemical parameters like chloride (40.9 mg L^{1}), nitrite (5-24 mg L^{-1}), inorganic phosphorous (27.3mg L^{-1}), ammonia (16.7 mg L^{-}), sulphate (0.145 mg L^{-1}), shulpide (21.9 mg L^{-1}), calcium (440 mg L^{-1}), and magnesium (150 mg L^{-1}) were studied and the experiments confirmed that the presence of all above mentioned chemicals but at different ratios. Ammonia, nitrate, and phosphate are nutrients that support the fertility of water, one of the factors that determine water quality. Enrichment of nutrients in the waters causes an increase in the population of phytoplankton (Gypens *et al.,* 2009; Jones-Lee and Lee, 2005), reduces the concentration of dissolved oxygen, decreases biodiversity, and sometimes increases the potential for the growth of harmful phytoplankton species (harmful algal blooms) (Susanti *et al.,* 2022; Jones-Lee and Lee, 2005).

The microalgal strains of the study area were studied. The algal samples was collected by scraping rocks, fine gravel, waste clothes, moist soil, pebbles, and twigs. Totally 13 genera was identified by using light microscope. Of these identified microalgae 8 were from Basillariophyceae, 4 from Chlorophyceae and 1 from Cyanophyceae (Plate 1).

Table 1. Physio-chemical parameters of freshwater sample collected from Korampallam channel of Pudhukottai

Physical parameters	
Colour	Transparent to pale yellow in colour
Taste	Tasteless
Odour	Odourless
Temperature	28^{o}C
Chemical parameters	
pH	7.3
Salinity	13 ppt
Alkalinity	92 mg/L^{-1}
Sulphide	21.9 mg/L^{-1}
Ammonia	16.7 mg L^{-1}
Nitrite	5.24 mg L^{-1}
Inoranic phosphorous	27.3 mg L^{-1}
Sulphate	0.145 mg L^{-1}
Chloride	40.9 mg L^{1}
Calcium	440 mg L^{-1}
Magnesium	156 mg L^{-1}

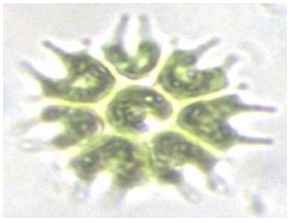
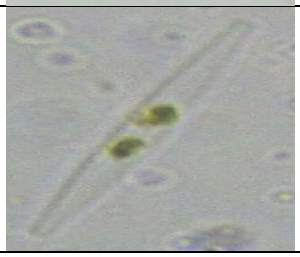
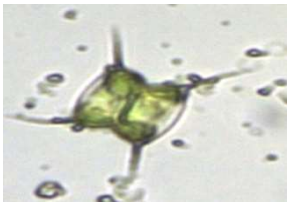
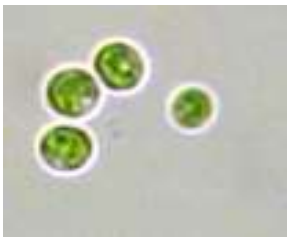
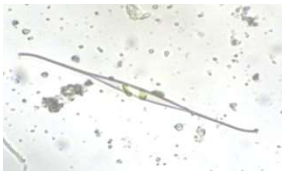
Cyclotella sp.		*Gomphonema sp.*	
Cymbela sp.		*Nitszchia sp.*	
Navicula sp.		*Ocilatoria sp.*	
Naviculv sp.		*Pediastum sp.*	
Syndera sp.		*Scenedesmus sp.*	
Amphora sp.		*Chlorella sp.*	
Closterium sp.			

Plate 1. Microalgal diversity of Korampallam channel of Puthukottai under light microscope with 100 X magnification

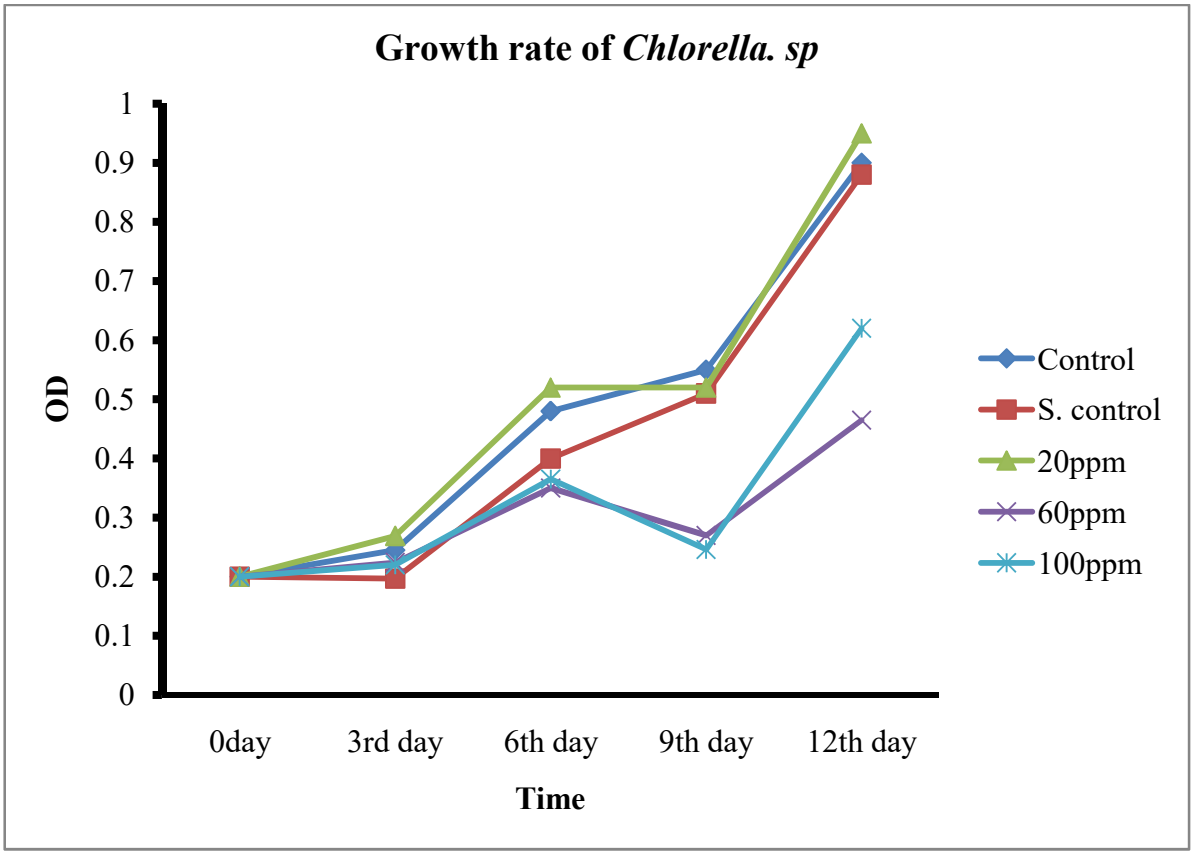


Fig. 1 Determination of growth rate of *Chlorella sp* after BEHA treatment

Table 2. Estimation of pigments from bis(2-ethyl hexyl)adipate treated *Chlorella sp.*

Samples	Chlorophyll a	Chlorophyll b	Carotenoid
3rd day			
Control	3.11±0.98	6.54±3.02	1.56±0.50
Solvent control	2.98±1.19	3.24±1.79	0.84±0.25
20 mg L^{-1}	2.84±1.32	4.54±1.08	0.76±0.05
60 mg L^{-1}	2.28±0.06	4.45±0.28	0.87±0.01
100 mg L^{-1}	2.24±0.68	4.961.84	1.06±0.45
6th day			
Control	4.92±0.77	10.07±0.32	2.10±1.11
Solvent control	3.18±0.54	6.45±2.17	0.72±0.69
20 mg L^{-1}	2.55±1.61	5.67±2.38	1.47±1.36
60 mg L^{-1}	2.03±0.49	3.81±1.09	0.84±0.31
100 mg L^{-1}	2.51±0.16	4.46±0.31	0.79±0.12
9th day			
Control	3.35±0.79	3.72±0.78	0.67±0.40
Solvent control	2.32±0.94	4.34±1.78	0.71±0.39
20 mg L^{-1}	4.6±2.15	5.79±3.62	1.04±0.27
60 mg L^{-1}	2.09±0.54	2.70±1.54	1.12±0.79
100 mg L^{-1}	1.05±0.75	3.36±0.75	0.72±0.34
12th day			
Control	6.33±2.18	4.79±2.19	0.79±0.14
Solvent control	2.30±1.87	6.58±3.49	1.45±1.92
20 mg L^{-1}	3.90±2.45	6.07±0.44	0.69±0.76
60 mg L^{-1}	0.86±0.89	4.8±1.28	1.27±0.65
100 mg L^{-1}	1.64±0.14	3.80±0.16	0.74±0.34

Similar results were obtained by Mohanapriya and Geetharamani, 2014 and Selvaraj *et al.,* 2021. The microalgal strains were cultured using Chu's medium No. 10 for isolation of pure colonies. *Chlorella sp.* was isolated through spread plate method. The isolated pure culture was grown in broth media for further uses. Totally 39 genera of 70 species were identified by Narchonai *et al.,* 2019 and they resulted that, most of the species fall under Chlorophyceae followed by Cyanophyceae and Bacillariophyceae. Halder *et al.,* 2019 reported that the members of Chlorophyceae and Cyanophyceae were dominant in winter season than in late summer. Hence based on the seasons the species richness may change.

Plasticizers are the emerging contaminants present in aquatic environments. The main aim this current investigation was to degrade the plasticizer using microalgae. Hence the microalgae like *Chlorella sp.* was isolated as a pure strain from the study area and treated with different concentrations (20mg L^{-1}, 60 mg L^{-1} and 100 mg L^{-1}) of bis (2 ethylhexyl) adipate (BEHA) and incubated for 12 days (Plate 2). The growth rate and pigments were estimated at regular intervals and the data revealed the tolerance of *Chlorella sp.* on BEHA pollution. 20 mg L^{-1} concentration of BEHA showed good and consistent growth throughout the incubation period while other shows less growth (Fig. 1). The pigment content of BEHA treated *Chlorella sp*. showed different amount of Chlorophyll a, b and carotenoids. The leaching and production of pigments were based on the toxicity. 20 mg L^{-1} BEHA treated *Chlorella sp.* showed high chlorophyll a (4.6 µg ml^{-1}), chlorophyll b (5.79 µg ml^{-1}), and carotenoids (1.04 µg ml^{-1}) content than control, solvent control and all other concentrations at 9 days incubation shown in Table 2. Similar results were obtained by Chi *et al.,* 2019. According to them, the DEP and DBP degradation was more quickly done by a microalgae *C. closterium* than by *C. muelleri* and *D. salina*. The concentration of the plasticizer should be relevant to the

environmental concentration (0.1 mg L^{-1}) of pollutant. The removal of organic pollutants involved a rapid initial passive physiochemical adsorption followed by active absorption, accumulation and degradation (Gao *et al.,* 2011). The degradation of plasticizer revealed that the microalgae were one of the best agents to remove the contaminants from the aquatic environment. Therefore, understanding of metabolic pathway of pollutants by algae is useful for risk assessments. In this work, freshwater microalgae were applied for the investigation of biodegradation pathway of BEHA in aquatic environment. When BEHA was treated with the *Chlorella sp*, high removal rate was observed. It was detected that cellular uptake was the predominant mechanism for the depletion of BEHA by *Chlorella sp*, while biotransformation accounted for the elimination of BEHA by the other species. The GCMS analysis revealed that *Chlorella* sp. have the capability to degrade the toxic plasticizers to less toxic compounds and it also release methane, CO_2 during degradation process. The microalgae either involve bioaccumulation, or biodegradation of plasticizer during the incubation period and also using the chemical constituents of BEHA as a energy source. Due to this effect, the toxicity of the chemical was reduced meanwhile the microalgae was benefitted.

SUMMARY AND CONCLUSION

The increased usage of plastic materials has led a severe threat to aquatic environment. The micro and nano-plastics are one of the major pollutants arose from macroplastics, meanwhile the additives of plastics especially plasticizers were leached out from macroplastics. The negative impacts of these materials are eliminated using remediation approaches. But the degradation of such emerging contaminants was challenging one. Hence the ultimate aim this present study to degrade the BEHA using microalgae. Initially the water sample from the study area was analyzed and found that the presence of nutrients that were essential for microalgal species richness. After that the microalgal strains (13 species) were identified through this study and pure *Chlorella sp.* was also isolated. Further study was done with BEHA at various concentrations with *Chlorella sp.* and discovered that the selected strains have the ability to degrade BEHA at 20 mg L^{-1} concentration, whereas beyond this level the toxicity of BEHA helped to bleach the algae. The pollutants generally enter into different bio-cycles hence broken into threat less compounds due to the interference of microorganisms. This research summarizes that the microalgae has the potentiality to degrade the emerging pollutants present in aquatic environment while at higher concentration the algal culture windup due to heavy toxicity. Furthermore research should be needed to understand the large scale degradation of BEHA and genes responsible for degrading BEHA.

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