Bio treatment Strategies for the Removal of Phthalate Esters from Asa River using Chlorella vulgaris (MG257915) and Nannochloropsis sp (MG257918) as Biodegradation Tool

Lawal, Ramat Idowu1; Adewoye, Solomon Olayinka2 ; Adenigba, Victoria Olaide3*; Ajao, Alfred Abolade1; Adeyinka, Gbadebo Clement4

1Department of Environmental Health Science, Kwara State University Malete, P.M.B. 1530, Kwara State, Nigeria.
2Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, P.M.B 4000, Oyo State, Nigeria.
3Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomoso, P.M.B 4000 Oyo State, Nigeria.
4Environmental Pollution and Remediation Research Group, Department of Chemical Engineering, Mangosuthu University of Technology, Durban 4031, South Africa.

*Corresponding Authors: Adenigba Victoria Olaide, Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomoso, P.M.B 4000 Oyo State, Nigeria.

Abstract: Phthalate esters (PEs) is one of the compounds that are known for their endocrine disrupting potential and they are a class of emerging contaminant that are found associated with plastics and other materials. Their presence in the environment is a great environmental concern and hence attention is focused on their reduction, transformation or removal. This study focused on the quantitative evaluation and phycodegradation of PEs in Asa river water sample using a marine (Nannochloropsis sp) and freshwater (Chlorella vulgaris) microalgae under different pH (2, 4, 9 and 13) and contact time (24, 48, 72 and 96 h). A total number of seven phthalates: Di-2-ethylhexyl phthalate (DEPH), Butyl Benzyl phthalate (BBP), Diethyl Ester Phthalate (DEP), Dibutyl Phthalate (DBP), Dimethyl Phthalate (DMP), DiHexyl Phthalate (DnHP), and Di-n-octyl phthalate (DnOP) was obtained and the concentration of five of them was higher than the permissible limit of 3 µg/L. At the expiration of 96 hours, it was observed that C. vulgaris was able to remove and thereby reduce the concentration of the PEs more than the counterpart. Removal efficiency of the PEs by C. vulgaris at an optimum pH of 9 ranged from 71.27% to 100% while Nannochloropsis sp at an optimum pH of 9 was between 34.59% - 67.19%. The outcome of this research has present the two microalgae as potential tools for the biotreatment tool in the removal of phthalate.

Keywords: phthalate esters, Chlorella vulgaris, Nannochloropsis sp, biodegradation, Asa River

1. INTRODUCTION

Asa River is a major river within Ilorin Metropolis, Kwara State in Nigeria and it is constantly being fed by different tributaries within the community. Different activities within this environment are suggestive of the presence of phthalate esters (PEs). Industrial effluents, domestic and commercial waste are released into the tributaries which later get washed into the major river. Some of the activities around these areas include indiscriminate dumping of waste especially Poly Vinyl Chloride (PVC) based, farming, washing of cars and plastic chairs, block and pharmaceutical industries are also situated along the tributaries of concern. Some of these activities have been reported to be sources of phthalate esters in the aquatic environment [1]. The high dependence of the community on this river for recreation, fishing and domestic purposes is of a great concern because of the possibility of both direct and indirect contact with PEs. As a result of this unavoidable dependence, it is of great need to evaluate and attempt the degradation of phthalate in order to save the community from the harmful effect of these compounds.
Phthalate esters (PEs) are one of the important chemical in the plastic industries, this is because it aids in the conferment of flexibility, extensibility and also increase the length of durability of plastic based products [2,3]. They are endocrine disrupting compounds which have been reported to be readily available in different environments which include: sediments, water, atmosphere and soil [4, 5]. Phthalate esters are major components of plasticizers and by extension; many plasticizers based products contain high level of PEs. Unfortunately, most of these products are in close contact with human at all levels; for example, products like children toys and materials for packaging foods [6], drugs and medical appliances [7], ink, paper, paints and ceramics [8], cosmetics [9], bottled and sachet water[10].

Phthalate esters are highly toxic and the United State Environmental Protection Agency (USEPA) and other similar agencies have regarded them as pollutants of high priority. There are six major PEs that has been listed as priority control pollutants and these include: Dimethyl phthalate (DMP), Diethyl phthalate (DEP), (DBP), (DOP), (BBP), (DEHP). These chemicals have become a major source of environmental and health concern because of its negative impact on both aquatic lives and humans. The health risk from exposure to PEs include endocrine disruption in the cases of testicular damage, Leydig cell hyperplasia, cryptorchidism [11, 12], neuro dysfuntion and memory loss in mice has also been documented [13]. Giulivo [14] also gave a report on the carcinogenic, tetratogenic, and mutagenic potentials of PEs.

When PEs are used as an additive to plastic, the former is not tightly bound to the plastic matrix and the mobility of these compound make it easy for them to leach out of resulting product into the environment. Phthalate esters find their way into the environment during production process where they escape with the industrial effluent or when the products are disposed after use [15]. Considering the health and environmental risk associated with the exposure to PEs, it is important to consider environmental safe and cost efficient way of removing phthalates from the environment especially at the discharge point of the production processes before the wastewater containing phthalate esters are discharged. The use of microorganisms has been considered as one of the efficient ways to degrade phthalate esters.

Some strains of bacteria have been reportedly employed in the efficient degradation of PEs and this is achievable because of their ability to produce the enzyme esterase which is important in the breaking down of phthalate esters [16]. Similarly, Luo [18]also documented the ability of a fungal strain; Fusarium sp to produce esterase intracellular and this enabled it to degrade a PE known as DMP. Apart from bacteria and fungi, microalgae are another type of microorganisms with the ability to degrade PEs. They are primary producers in the aquatic environment and PEs can find their way across the aquatic food chain via microalgae [2].

The fate of PEs in the environment is highly dependent on the activities of the presiding microorganisms. Microalgae serve a dual purpose in the degradation of PEs. First, they produce oxygen during photosynthesis and this aids the heterotrophic bacteria in being able to degrade these pollutants because of the availability of oxygen. Secondly, microalgae can degrade these pollutants directly [19]. Several researches have been done on the use of microalgae especially freshwater microalgae while there have been fewer reports on the use of marine microalgae. However, these reports have focused on degrading of specific PEs in their pure form or a mixture of known PEs.

On the other hand, this work focused on attempting degradation of evaluated PEs in water sample from Asa River. The degradation was done directly on the water samples, although there have been few work on the biodegradation of pollutants especially heavy metals in Asa River [20] but there are rare reports of both quantification and biodegradation of PEs. Also, the biodegradation of phthalate esters in Asa River is being reported for the first time in this study.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted on the Asa River, located in Ilorin, North Central, Nigeria between Latitude 8°28’-8°31’ North and Longitude 4°38’-4°40’ East. Asa River is the major water body within the Ilorin metropolis, its course enters the southern end of the industrial estate from Asa Dam and it runs northwards through residential and commercial areas of Ilorin city. Apart from the containment of
industrial effluents from several manufacturing plants, the river also serves as a recipient of domestic (sewage) wastes and agricultural waste run offs along the bank of the river.

2.2. Sample Collection

Water sample was collected (a 1L water sample) in a sterilized screw-cap amber glass bottles. To avoid possible contamination of phthalates arisen from the plastic lining of the bottle, which may give a false information about the level of phthalates in the samples, the lids of glass bottles were lined with aluminum foil. The water samples was kept cool under ice and transported down to the laboratory where further analysis were carried out immediately.

2.3. Quantification of Phthalates in the Water Sample

The chromatographically separated phthalate esters were detected and quantified using an Agilent gas chromatography mass spectrometry (GC-MS). The chromatographically separated phthalate esters were detected and measured with quantified using an Agilent gas chromatography mass spectrometry (GC-MS) (GC-6890-MS-5973 series Germany), with a ZB-5MS fused-silica capillary column of length 30 m (0.25 µm internal diameter and 0.25 µm film thickness). The instrument parameter details adopted in this study followed the method used by Selvaraj et al [21] with little modification. The injection and detector temperatures were set at 270°C and 310°C respectively and the solvent delay was set at 4.00 minute. Oven temperature was set at 150 °C and held for 1 min, raised to 290 °C at 8 °C/min held for 1 min and raised to 300°C at 8 °C/min held for a further 9 min. A 10 µL injection volume was used at approximately 4.6 μl/sec into the injector and was performed in the splitless mode using helium (99.999 % purity) as the carrier gas. MS was operated in full-scan mode from m/z 35–500 and an external calibration method was used for the quantification of Phthalate in the samples. The new method for analysis of Phthalate in water samples using GC/MS was validated according to Skoog [22, 23], and Institute Bachema AG Quality Management Guidelines as certified by ISO phthalate 17025 [24, 25].

2.4. Collection and Maintenance of Microbial Culture

Two microalgae culture (Chlorella vulgaris MG257915 and Nanochloropsis sp MG257918) previously isolated from fish pond water and sea water respectively in our laboratory were maintained in Blue Green Medium (BGII medium) for 7 days before they were used for the biodegradation experiment [26].

2.5. Pretreatment of Wastewater Prior Biodegradation

The water sample was filtered in the laboratory using Whatman filter paper to remove some particulate impurities. The filtered sample was sterilized separately in an autoclave at 121° C for 15 minutes in order to remove any living organisms that may be present in the samples.

2.6. Biodegradation of Phthalate Esters Using Microalgae

Biodegradation of phthalate esters was carried out at different pH ranges (2, 4, 9, and 13) while the contact time was 24, 48, 72 and 96 hours. The inoculation of the phthalate contaminated water sample was done according to the method of Gao and Chi [2]. The microalgae cells were harvested through centrifugation at 4000 rpm for 5 mins using a Centrifuge (AXIOM Centrifuge, 80-2), the pellet containing the cells were washed with sterile distilled water and later used to inoculate 100 ml of the water sample at the various specified pH. The mixture was agitated for the first 4 hours in order to enhance the interaction between the micro algal cells and the PEs in the water sample; the reaction bottles were placed in the laboratory at room temperature. At the expiration of each contact time, the mixture was separated by centrifugation at 5000 rpm for 15 minutes to remove the microalgae biomass and the concentration of the phthalate esters in the solution was determined.

3. RESULTS AND DISCUSSION

3.1. Identification and the Concentrations of Phthalate Esters in Asa River

The water sample collected from Asa River was investigated for the presence of phthalate esters and the concentration of each was also obtained. A total number of seven PEs were obtained, the PE with
the highest concentration was BBP (0.697 µg/L) followed by DEP (0.648 µg/L) and the lowest was DnHP (0.064 µg/L) (Table 1).

### Table 1. Types and concentration of phthalate esters found in the water sample obtained from Asa River

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phthalate ester</th>
<th>Code</th>
<th>Concentration (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Butyl Benzyl phthalate</td>
<td>BBP</td>
<td>0.697</td>
</tr>
<tr>
<td>2</td>
<td>Diethyl Ester Phthalate</td>
<td>DEP</td>
<td>0.648</td>
</tr>
<tr>
<td>3</td>
<td>Dimethyl Phthalate</td>
<td>DMP</td>
<td>0.525</td>
</tr>
<tr>
<td>4</td>
<td>Di-n-octyl phthalate</td>
<td>DnOP</td>
<td>0.443</td>
</tr>
<tr>
<td>5</td>
<td>Dibutyl Phthalate</td>
<td>DBP</td>
<td>0.399</td>
</tr>
<tr>
<td>6</td>
<td>Di-2-ethylhexyl phthalate</td>
<td>DEHP</td>
<td>0.198</td>
</tr>
<tr>
<td>7</td>
<td>DiHexyl Phthalate</td>
<td>DnHP</td>
<td>0.064</td>
</tr>
</tbody>
</table>

3.2. Biodegradation of Phthalate Esters by Microalgae

3.2.1. Biodegradation Pattern of Phthalate Esters by Nannochloropsis sp

Biodegradation of phthalate esters was carried out by introducing *Nannochloropsis* sp at different pH, it was observed that at 24 hours, the concentration of DEPH at pH2 reduced with reference to contact time from 0.198 µg/L to 0.161 µg/L, 0.131 µg/L, and 0.127 µg/L at 24, 48 and 72 hours respectively while the concentration at 96 hours was 0.122 µg/L. Similarly, the concentration of DBP at pH 2 was also monitored with time and a decline in concentration was also observed with time as follows: 0.399 µg/L (0 h), 0.351 µg/L (24 h), 0.322 µg/L (48 h), 0.329 µg/L (72 h) and a concentration of 0.32 µg/L was obtained at the 96th hour. At 24 hours, the concentration of BBP at pH 2 also reduced gradually with reference to time from the initial concentration of 0.697 µg/L to 0.605 µg/L at the end of the 96th hour. In addition, the initial concentration of Dimethyl phthalate (DMP) at pH 2, reduced slightly from 0.525 µg/L to 0.424 µg/l at the expiration of 96 hours. The activity of *Nannochloropsis* sp at pH 2 resulted in a decrease in the concentration of DEP from 0.648 µg/L to 0.525 µg/L after 96 hours while that of DNOP also reduced from an initial concentration of 0.443 µg/L to 0.432 µg/L at the end of 96 hours reaction time. The concentration of DnHP which was the lowest out of the PEs present in the water sample also was slightly affected by the activity of the microalgae with a reduction in concentration from 0.064 µg/l to 0.04 µg/l at the 96th hour (Figure 1).

![Figure 1. Biodegradation pattern of phthalate esters present in the water sample by Nannochloropsis sp., at pH 2 for 96 hours](image-url)
Bio treatment Strategies for the Removal of Phthalate Esters from Asa River using *Chlorella vulgaris* (MG257915) and *Nannochloropsis* sp (MG257918) as Biodegradation Tool

respectively. Similarly, the concentration of DMP (0.525 µg/L) reduced with time and was 0.372 µg/L at the 96th hour. The same trend was observed with the remaining three PEs (DEP, DNOP and DnHP) at pH4: at the expiration of 96 hours, DEP reduced in concentration from 0.648 µg/L to 0.529 µg/L; DNOP from 0.443 µg/L to 0.4 µg/L and DnHP from 0.064 µg/L to 0.033 µg/L (Figure 2).

![Figure 2](image)

**Figure 2.** Biodegradation pattern of phthalate esters present in the water sample by *Nannochloropsis* sp., at pH 4 for 96 hours

The initial concentration of DEPH (0.198 µg/L) at pH 9 reduced to 0.13 µg/L after 24 h and gradually reduced to 0.08 µg/L after 96 h. Also, there was a reduction in the concentration of DBP (0.399 µg/L), the longer the contact time the lower the concentration and at 96 h; the concentration had reduced to 0.261 µg/L. At 24 hours, the concentration of BBP dropped from 0.697 µg/L to 0.6 µg/L and a continuous reduction was observed up to 96 h (0.391 µg/L). A similar trend was observed with DMP, DEP and DNOP where the concentration of each Pes reduced with time. The initial concentrations of 0.525 µg/L, 0.648 µg/L and 0.434 µg/L for DMP, DEP and DNOP had reduced after 96 h to 0.278 µg/L, 0.3 µg/L and 0.281 µg/l respectively. In addition, the activity of *Nannochloropsis* sp on DnHP also resulted in a reduction in its concentration from 0.064 µg/l to 0.021 µg/L at the 96th hour. All the above data were represented in Figure 3 respectively.

![Figure 3](image)

**Figure 3.** Biodegradation pattern of phthalate esters present in the water sample by *Nannochloropsis* sp., at pH 9 for 96 hours
Bio treatment Strategies for the Removal of Phthalate Esters from Asa River using *Chlorella vulgaris* (MG257915) and *Nannochloropsis* sp (MG257918) as Biodegradation Tool

The maximum removal of the seven phthalate esters present in the water sample at pH13 was also achieved at 96 hours. DEPH (0.198 µg/L), DBP (0.399 µg/L), BBP (0.697 µg/l), and DMP (0.525 µg/L) reduced to 0.08 µg/L, 0.27 µg/L, 0.435 µg/L, and 0.312 µg/L respectively at the 96th hour. Similarly the concentration of DEP, DNOP AND DNHP also reduced after 96 hours from the initial concentrations of 0.648 µg/L, 0.434 µg/L, 0.064 µg/L, and 0.381 µg/L, 0.29 µg/L, and 0.021 µg/L respectively (Figure 4).

**Figure 4.** Biodegradation pattern of phthalate esters present in the water sample by *Nannochloropsis* sp., at pH 13 for 96 hours

### 3.2.2. Biodegradation Pattern of Chlorella vulgaris against Phthalate Esters Waste Water

The pattern of phthalate biodegradation by *Chlorella vulgaris* was also monitored at different pH (2, 4, 9, and 13) and contact time (24, 48, 72 and 96 h). Figure 5 shows the pattern of degradation at pH 2 from 24 to 96 h. The reduction in concentration of the PEs was gradual from 24 to 96 h; DEPH (0.198 µg/L), DBP (0.399 µg/L), BBP (0.697 µg/L), and 0.525 µg/L reduced to 0.091 µg/L, 0.281 µg/l, 0.592 µg/L, and 0.312 µg/L respectively at the 96th hour while at the same time duration DEP(0.612 µg/L), DNOP(0.443 µg/L), and DEHP (0.055 µg/L) also reduced to 0.51 µg/L, 0.348 µg/l, and 0.03 µg/L respectively.

**Figure 5.** The pattern of degradation of seven phthalate esters present in the water sample by *Chlorella vulgaris* at pH2 for 96 hours.
The degradation of the seven PEs at pH4 was also contact time dependent, the longer the time of microalgae-phthalate interaction the lower the concentration of the each phthalate. The initial concentration of DEPH, DBP, BBP and DMP was 0.198 µg/L, 0.399 µg/L, 0.697 µg/L, and 0.525 µg/L, there was a gradual reduction up to 96 h and the concentration of each reduced to 0.071 µg/L, 0.251 µg/L, 0.474 µg/L, 0.321 µg/L respectively. Also, there was reduction in the concentration of DEP (0.648 µg/L), DNOP (0.443 µg/L), and DNHP (0.064 µg/L), to 0.418 µg/L, 0.281 µg/L, and 0.055 µg/L respectively (Figure 6).

**Figure 6.** The pattern of degradation of seven phthalate esters present in the water sample by *Chlorella vulgaris* at pH4 for 96 hours.

The biodegradation potential of *C. vulgaris* at pH 9 with an accompanied reduction in concentration of all the seven phthalates at the different time interval is presented in Figure 7. A drastic decline in concentration was observed for all the PEs, the initial concentration of DEPH (0.198 µg/L), DBP (0.399 µg/L), BBP (0.697 µg/L), and DMP (0.525 µg/L) reduced after 96 hours to 0.01 µg/L, 0.047 µg/L, 0.114 µg/L, and 0.112 µg/L respectively. Also, the initial concentration of 0.648 µg/L (DEP), 0.443 µg/L (DNOP), and 0.064 µg/L (DNHP) reduced drastically after 96 hours to 0.091 µg/L, 0.131 µg/L, and 0.021 µg/L respectively.

**Figure 7.** The pattern of degradation of seven phthalate esters present in the water sample by *Chlorella vulgaris* at pH9 for 96 hours.
Bio treatment Strategies for the Removal of Phthalate Esters from Asa River using Chlorella vulgaris (MG257915) and Nannochloropsis sp (MG257918) as Biodegradation Tool

The interaction between C. vulgaris and the phthalate present in the water sample at pH13 showed that there was reduction in concentration at each contact time but the longer the interaction the lower the concentration. Figure 8 shows that there was a decline in the concentrations of DEHP (0.198 µg/L), DBP (0.399 µg/L), BBP (0.697 µg/L), DMP (0.525 µg/L), DEP (0.648 µg/L), DnOP (0.443 µg/L), and DEHP (µg/L) at pH 13 and the concentrations after 96 h were 0.021 µg/L, 0.071 µg/L, 0.387 µg/L, 0.203 µg/L, 0.214 µg/L respectively.

![Figure 8](image.png)

**Figure 8.** The pattern of degradation of seven phthalate esters present in the water sample by Chlorella vulgaris at pH 13 for 96 hours.

3.3. Optimum Percentage Biodegradation of the Test Micro Algae

It was observed that throughout the biodegradation period of 96 hours at pH 2, 4, 9 and 13 respectively the phthalate esters were biodegraded by the test microalgae. The biodegradation efficiency of *Nannochloropsis* sp at pH 9, pH 13, pH 4 and pH 2 against DEHP was 60.0, 55.56, 41.41, and 38.38% respectively, DBP was 34.59, 32.33, 29.57, and 19.80% respectively, BBP was 43.90, 37.59, 15.35, and 13.20% respectively. Similarly, at pH9, pH13, pH4 and pH2 against DMP was 47.03, 40.57, 29.14, and 19.24% respectively, DEP was 55.82, 43.89, 22.09, and 22.68% respectively, DnOP was 38.38, 36.40, 12.28, and 5.26% respectively, and the efficiency against DnHP was by 67.19, 54.67, 48.44, and 37.5% respectively (Figure 9A).

Similarly, the biodegradation efficiency of Chlorella vulgaris at pH 2, 4, 9 and 13 for 96 hours was as follows: 54.04, 64.14, 94.95, and 89.39% respectively for DEHP, 88.22, 82.21, 37.09, and 29.57 respectively for DBP, 15.06, 31.99, 83.64, and 44.48%, 31.99 respectively for BBP, 29.52, 38.86, 78.67, and 61.33% respectively for DMP, 24.89, 38.44, 86.60, and 68.48 %, respectively for DEP, 23.68, 38.38, 71.27, and 47.15% respectively for DnOP, and 53.13, 70.31, 100, and 58.75% respectively (Figure 9 B).
esters in this stream has a potential risk to the environment. The resultant effect to the aquatic lives is a concern that requires attention. Xueping [27] documented the risk caused to a 72 h old fish embryo when exposed to a cocktail of PEs at 0.50 ppm, the interaction resulted in extreme toxicity and also induced toxicity in further development of the embryo. The PE with the highest concentration in this study was 0.697 µg/L from BBP; this phthalate has been documented for its toxicity to aquatic life with its potential as an endocrine disrupting compound [28]. Five out of the seven phthalates present in the samples taken from the study area have been marked as priority pollutants [29] and their elevated concentration in this study showed that the river has potential risk to both the aquatic lives and human who depend directly or indirectly on the stream for several activities.

Considering the potential risk of the samples, an attempt was made to biodegrade the phthalate present in the water sample by selecting the sample with the highest concentration of the PAEs for biodegradation. Two microalgae, one marine (Nannochloropsis sp) and the other freshwater (Chlorella vulgaris) were employed as biodegradation agent at different pH. Microalgae have the biodegradability potential to break down organic pollutants which include polycyclic aromatic hydrocarbons, pesticides, phthalate esters and even petroleum [19, 2]. Microalgae are photosynthetic organisms and they are able to convert some environmental pollutant into a source of carbon which they can use for their metabolic activities.

The lowest percentage removal of each of the phthalate esters by Nannochloropsis sp at the end of the experiment was achieved at pH 2 while the highest removal efficiency was obtained at pH 9. Biodegradation of all the phthalates couldn’t be achieved with Nannochloropsis sp at the pH 2, 4 and 13, while at pH 9 there was degradation of the PAEs with a consequential reduction in concentration below the set USEPA criterion for fishes and aquatic organisms. Although at pH 4 and 13, the concentration of DBP AND DnOP were reduced to a permissible concentration while BBP, DMP and DEP were still above the acceptable limit of 3 µg/L. However, the concentration of the phthalates at pH 2 was still higher than 3 µg/L except for DEHP and DnHP, whose concentrations were lower than 3 µg/L right from the onset of the experiment.

The acidic medium didn’t favour the removal of phthalates to an acceptable concentration: the concentrations of BBP DMP, DEP and DnOP were still above 3 µg/L at pH 2 while the same was observed at pH 4 except for DnOP which reduced along with DEHP. Chlorella vulgaris in basal medium was able to degrade the phthalate esters to an acceptable concentration except for BBP which was still higher than 3 µg/L at pH of 13 while the percentage removal for all the phthalates at pH 9 ranged between 71.3% and 100%.

The discrepancy in the biodegradability of the two microalgae could be as a result of their metabolic and physiological requirement. Nannochloropsis sp is a marine microalgae previously isolated from seawater while C. vulgaris was also isolated from pond water [30]. The habitat of the two microalgae used in this study defers and this can contribute to their physiological makeup and tolerance in different environment. This submission is in agreement with the report of Gao et al. [31] that the difference in the ability of different microalgae species in removing PAEs could be as a result of the characteristics of the cell in terms of their physiology and metabolic features which include cell wall composition and structure as well as the lipid profile of the cell. There are few reports on the use of microalgae in the degradation of phthalate esters, although there are numerous reports on their activity in the adsorption of heavy metals and some other inorganic compounds.

Chi et al. [32] reportedly employed three marine microalgae (Dunaliella salina, Cylindrotheca closterium and Chaetoceros muelleri ) in the removal of DEP and DBP from solution. Removal efficiency of DBP by the microalgae was 40.0%, 93.1% and 47.1% respectively while that of DEP was 32.3%, 81.2% and 26.3% respectively. Also, Chlorella vulgaris was adopted in the removal of DBP at varying concentrations (5, 10, 20, 50 and 100 mg/L); The highest removal efficiency of DBP at 50 mg/L in 2 days was 31.69 % and it was also observed that high concentration of DBP had a negative influence both on the cell density and lipid profile of the microalgae. This confirms why the C. vulgaris used in this work was able to remove DBP with an efficiency of about 88% in 48 hours since the toxicity of the phthalates is dose dependent and here the concentration was lower than that reported by the authors.

The length of the chain of phthalate esters determines their level of toxicity, the longer the chain the more toxic they are to algal cells. Sun et al. [17] confirmed this when he attempted the removal of
Bio treatment Strategies for the Removal of Phthalate Esters from Asa River using Chlorella vulgaris (MG257915) and Nannochloropsis sp (MG257918) as Biodegradation Tool

four PAEs (DMP, DEP, DAP and DPrP) using Karenia brevis, the authors reported that the longer the chain the higher their toxicity and their bioaccumulation in Karenia brevis; the order of toxicity was DMP<DEP<DAP<DPrP.

4. CONCLUSION

The concentration of five out of the seven phthalates (DEPH, BBP, DEP, DBP, DMP, DNHP DNOP) evaluated in Asa River water sample were higher than the USEPA limit set for fishes and aquatic organisms; this was why an attempt was made on removing the PEs using two microalgae species (Nannochloropsis sp. and Chlorella vulgaris) under different pH and contact time. After 96 hours, it was observed that C. vulgaris was able to remove and thereby reduce the concentration of the PEs more than the counterpart. Removal efficiency of the PEs by C. vulgaris at an optimum pH of 9 ranged from 71.27% to 100% while Nannochloropsis sp at an optimum pH of 9 was between 34.59% - 67.19%. This study has presented both C. vulgaris and Nannochloropsis sp as biodegradation tools which can be used to target the removal of phthalates under some controlled environmental conditions.

REFERENCES

Bio treatment Strategies for the Removal of Phthalate Esters from Asa River using *Chlorella vulgaris* (MG257915) and *Nannochloropsis* sp (MG257918) as Biodegradation Tool


**Citation:** Adenigba Victoria Olaide, e.i.al., “Bio treatment Strategies for the Removal of Phthalate Esters from Asa River using *Chlorella vulgaris* (MG257915) and *Nannochloropsis* sp (MG257918) as Biodegradation Tool”, *International Journal of Research in Environmental Science (IJRES)*, vol. 9, no. 1, pp. 1-11, 2023. Available: DOI: http://dx.doi.org/ 10.20431/2454-9444. 0901001

**Copyright:** © 2023 Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.