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THE POTENTIAL OF INDIGENOUS PHYTOPLANKTON IN PHYCOREMEDIATION OF EFFLUENTS IN RIVER GINZO KATSINA METROPOLIS, KATSINA STATE, NIGERIA, UTILIZING THE MESOCOSM TECHNIQUE

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Abstract
Sewage disposal has become a major ecological problem in metropolitan areas and semi-urban communities, with effluents from both residential and industrial activities discharged directly into water bodies, constituting a major cause of water pollution. Twelve mesocosms were set up, each receiving approximately 15 liters of water containing algae. Weekly sampling for physical chemical parameters, phytoplankton biomass, and biochemical components was done for four weeks. The results revealed that 41 species of phytoplankton were identified throughout the course of four weeks, and that pH remained around neutral levels, with increases in dissolved oxygen (DO) as the number of weeks increased. Furthermore, the physico-chemical indicators showed a decrease with an increase in the number of weeks (total dissolved solids, 70.50 %, nitrate 97.44 %, phosphorus 100%, ammonium 92.26%, potassium 91.35%, zinc 96.52%, manganese 98.63%, iron 92.90%, copper 97.56%, nickel 98.66%). While biological components such as fat, glucose, and protein had increased with number of days. These findings prove mesocosm as the optimum and cost-efficient method of wastewater treatment for enterprises before the release of effluents into water bodies that eventually join rivers.

Key Words: *Mesocosm, Phytoplankton, Phycoremediation, Wastewater, Effluent*

Introduction

Algae can also be used to remediate wastewater since they thrive on the nutrients found in the water (Larsdotter, 2006). With advancing technology, there are now microalgae-based wastewater purification approaches that may remove nutrients from domestic wastewater more effectively than older methods (Wang *et al*., 2009). This is a very promising process (Harun *et al*., 2010), as wastewater provides required nutrients for microalgae development, hence a low-cost alternative to contemporary waste water treatments and nutrient removal as well as a potentially lucrative feedstock (Olgun, 2003; Patel *et al*., 2012). At night, algae do not develop well, and the CO_2 cannot be temporarily stored till daybreak. As a result, a more efficient and

inexpensive source of carbon, such as sodium bicarbonate, is required. Microalgae may utilise organic carbon as an energy source, which is beneficial since it reduces the inhibitory effects of seasonal and diurnal light limitations on development in outdoor cultures.

Microalgae can grow in many environments and on different substrates such as wastewater. When growing on wastewater, microalgae assimilate phosphorus and nitrogen, nutrients necessary for their growth. In addition, they can also assimilate heavy metals and pharmaceutical products from wastewater and capture atmospheric carbon dioxide (CO_2). This, as well as favoring bioremediation of wastewater and protecting the environment from the risk of eutrophication, can also favor the removal of dangerous contaminants from wastewater and mitigate the negative effects caused by the excessive concentration of CO_2 in the atmosphere. Lastly, this type of treatment, in addition to recycling water, can also produce microalgae biomass that can be destined for different uses, such as food, energy and other products at lower costs (Ferro *et al.,* 2018; Peralta *et al.,* 2019) In aquaculture systems, the principal route to nitrogen removal is through the consumption of dissolved nutrients by microalgae (Attasat *et al*., 2013; Sirakov *et al*., 2013). Many researchers have looked into using microalgae to clean aquaculture wastewater.

Microalgae production and wastewater treatment in aquaculture systems appear to be extremely promising for microalgae growth combined with biological cleansing (Mata *et al.,* 2010). This research aim's at to demonstrate the potentials of natural indigenous phytoplankton in phycoremediation of effluents using mesocosm experimental design.

Materials and Methods

The experiment was carried out during the dry season (December to January). Samples for physicochemical conditions and phytoplankton analysis were collected at the beginning of the experiment from the sub-surface layer of the water column (15 cm deep). Initial physicochemical and biological characteristics as well as biomass were measured (pH, TDS, phosphorus, nitrate, ammonium, potassium, DO, heavy metals and biomass.

Twelve (12) mesocosms were constructed and situated in the study area; River Ginzo, Katsina metropolis, Katsina state, Nigeria. The mesocosms were constructed using plastic baskets fitted with polyethylene bags, and empty plastic bottles served as floats. The baskets were held together by passing a long rope in-between them at equal distance of 2metres, which was subsequently fastened to poles located at the far ends. Approximately 15 liters of water containing algae biomass were transferred into each of the 12 mesocosms. Sampling was carried out weekly for 4 weeks for physicochemical parameters and phytoplankton biomass. All experiments were carried out in triplicates. Samples were periodically analyzed (every week) for each physicochemical parameter such as pH, TDS, phosphorus, nitrate, ammonium, DO (dissolved oxygen), and heavy metals, using standard methods by APHA (2005).

Physicochemical Analysis, Microscopy and Cell Count

The biochemical composition from the twelve (12) mesocosms was carefully

mixed and then the pH and temperature of the mesocosm were taken using a multi-parameter water quality portable Hanna instrument (model no. H1991300). Also, 100mL samples from three of the mesocosm out of twelve mesocosm were collected weekly for four (4) weeks. Out of the 100mL, 20mL subsamples were preserved in Lugol's iodine solution for microscopy and identification of phytoplankton using keys provided by Bellinger and Sigee (2010). Phytoplankton cell counts were carried out using the drop and count technique (Chia *et al*., 2012).

Preparation of Samples for Biochemical Analysis

The remaining 80mL of each collected sample was divided into four centrifuge tubes containing 20 mL each. It was then centrifuged at 300rpm for 10 min, the supernatant was discarded and the pellets were re-suspended in pre-cooled phosphate buffer (pH 7.8). These samples were used for the carbohydrate, total lipid, and protein analysis.

Carbohydrate

1mL pellet were diluted in 1mL distilled water and 1mL 5 % aqueous phenol in water was added, and then 5mL concentrated sulfuric acid was applied immediately to the surface of the solution, according to the procedure described by Chaplin, (1986). The absorbance was measured at 490nm in a (Genesys) spectrophometer after 20mins. 5mL of concentrated sulfuric acid was applied immediately to the surface of a blank solution made with 1mL of purified water and 1mL of 5% aqueous phenol solution

Total Lipids

Total lipids were determined by centrifuging 50mL of culture at 3000rpm for 10 minutes, adding 6mL of chloroform and methanol in a 2:1 ratio to harvested pellets, vortexing violently for 15 minutes, and then adding 1.5mL of distilled water. Two layers were formed after centrifugation at 3000rpm for 10 minutes; weigh the empty tube and transfer the lower layer while discarding the higher layer. Reweight the tube after drying it in the oven until all of the solvent has evaporated. Total lipid is the weight of the tube after drying minus the weight of the empty tube

Total Protein

The method of Bradford (1976) was used as 5mL culture was centrifuged at 3000rpm for 10 minutes, supernatant discarded, 1.5mL NaOH added, vortexed and added to re-suspend pellets, and extracts baked at 1000C for 2 hours. Supernatant was collected after centrifugation at 4000rpm for 10 minutes, and 4mL of Bradford reagent was added to 1mL of supernatant and left for 5 minutes. The spectrophotometric reading was at 595nm. 1.5 mL of NaOH and 4 mL of Bradford reagent was used as the blank solution.

Data Analysis

Analysis of variance (ANOVA) and T-test were used to compare physicochemical parameters before and after treatment at7days interval. The $p \leq 0.05$ level of significant of was used. Graphpad statistical software was used for the analysis.

Results

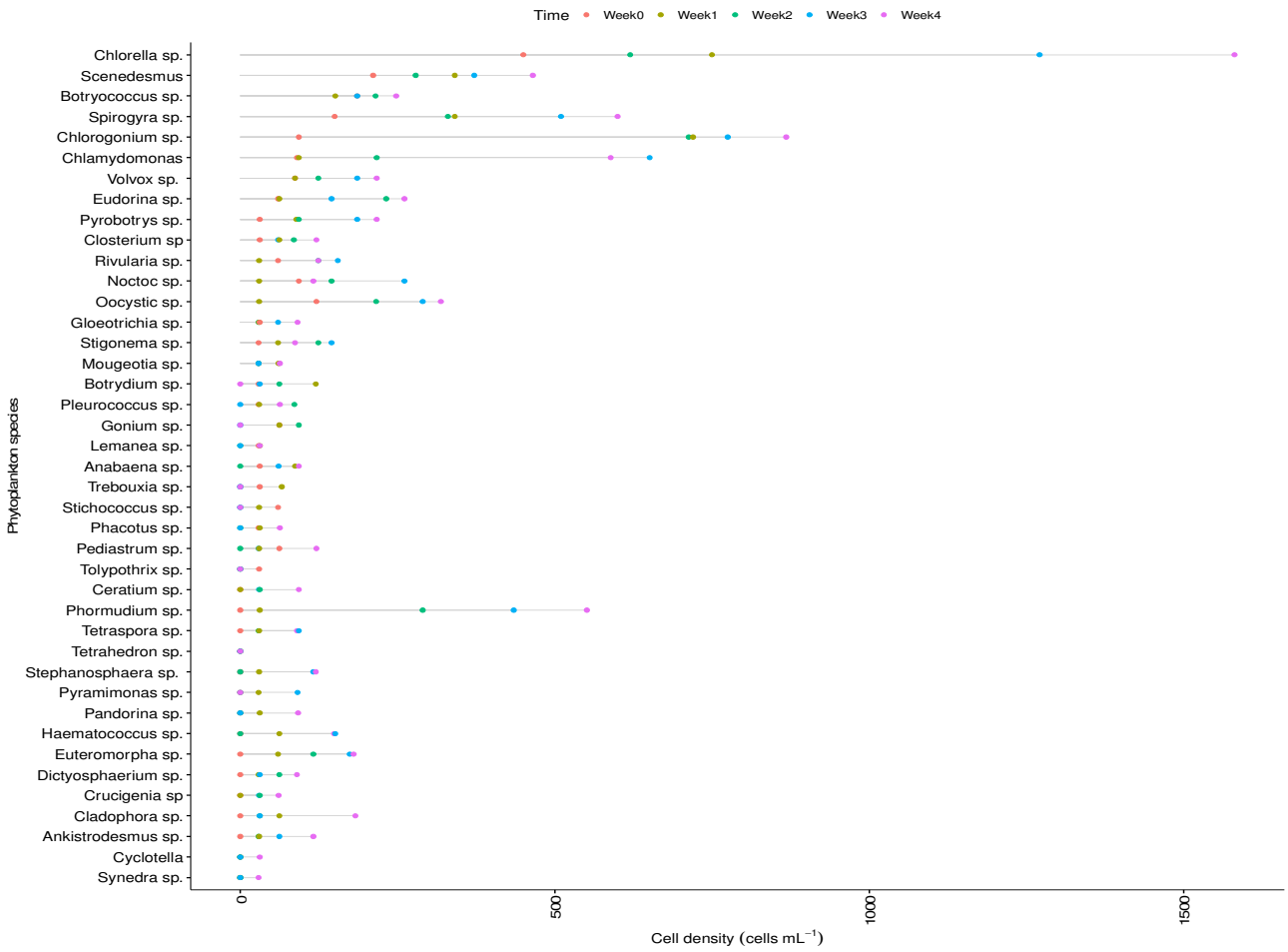

Fig. 1: Presents the number of identified phytoplankton and their cell density in the constructed mesocosm for four weeks

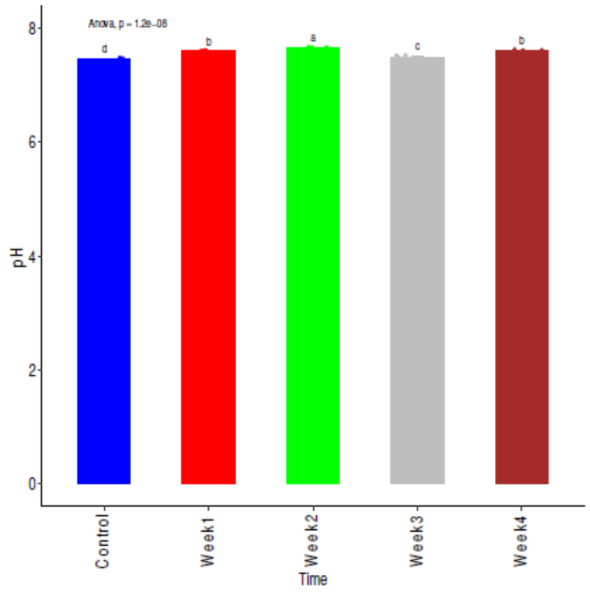

Fig. 2: pH changes in the mesocosm for four week

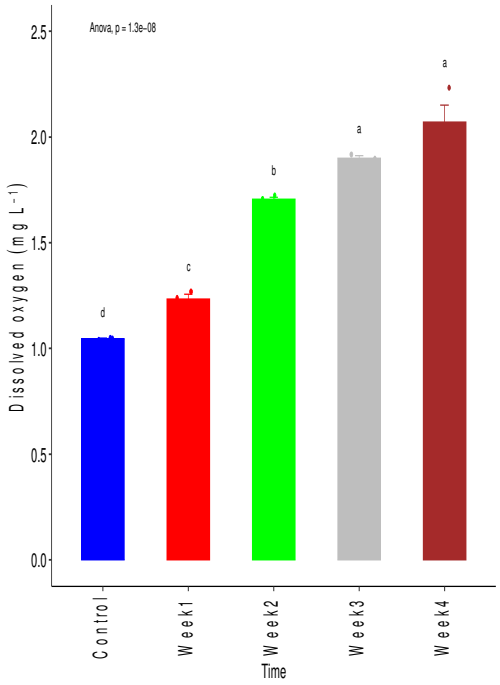

Fig. 3: DO changes in the mesocosm for four weeks

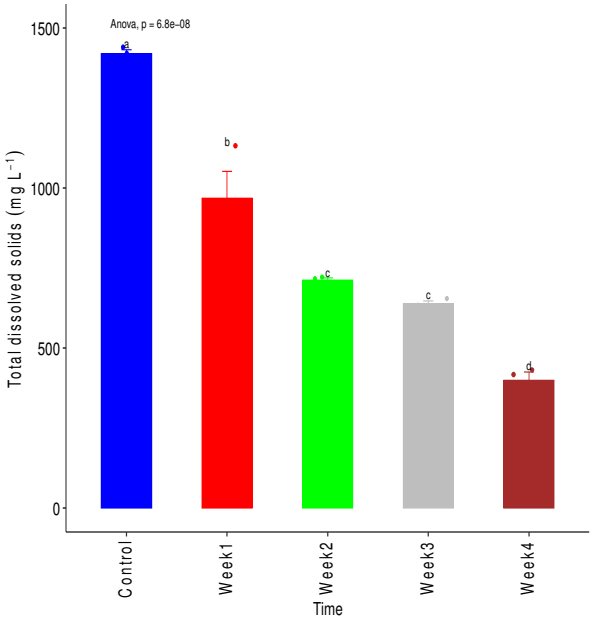

Fig. 4. TDS changes in the mesocosm for four weeks

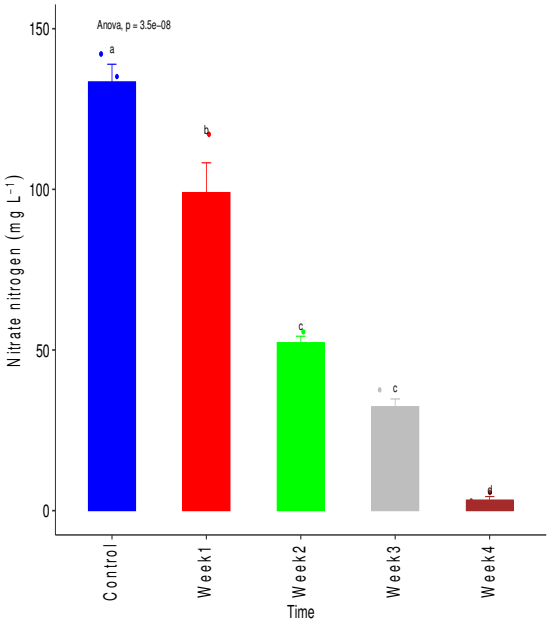

Fig. 5: Changes in nitrate concentrations in the mesocosm for four weeks

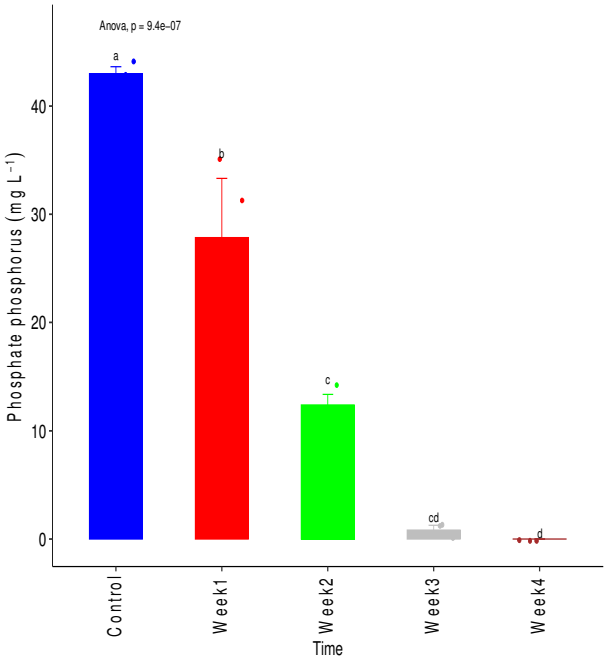

Fig. 6: Phosphorus concentration changes in the mesocosm for four weeks

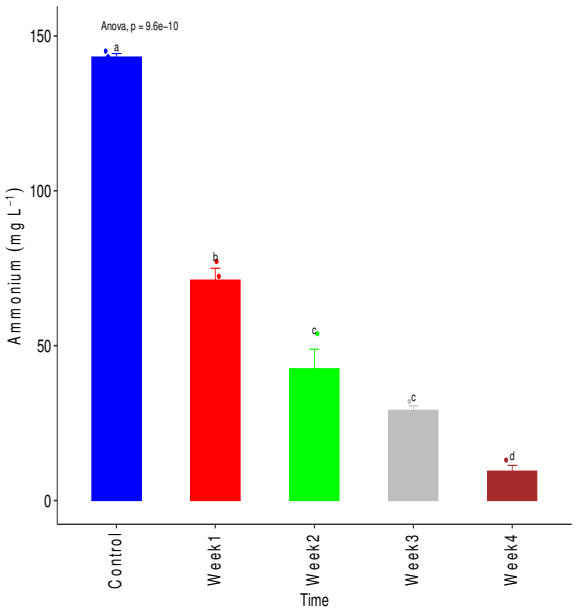

Fig. 7: Changes in ammonium levels in the mesocosm for four weeks

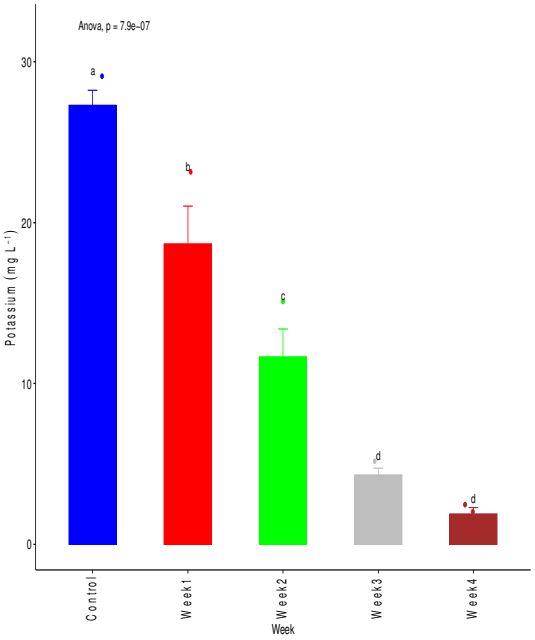

Fig. 8: Changes in potassium levels in the mesocosm for four weeks

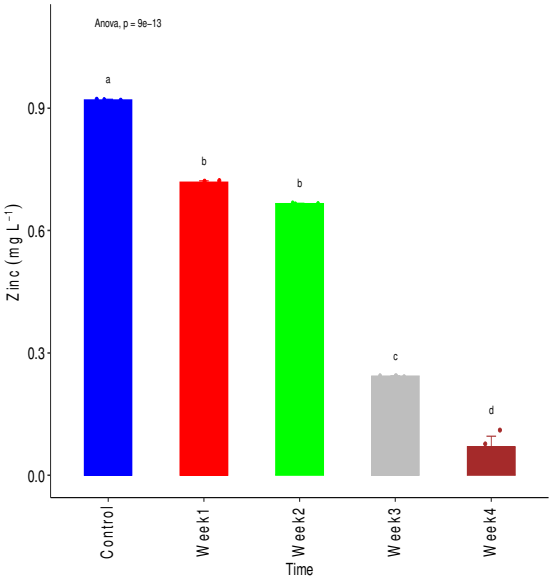

Fig. 9: Variations in zinc concentrations in the mesocosm for four weeks

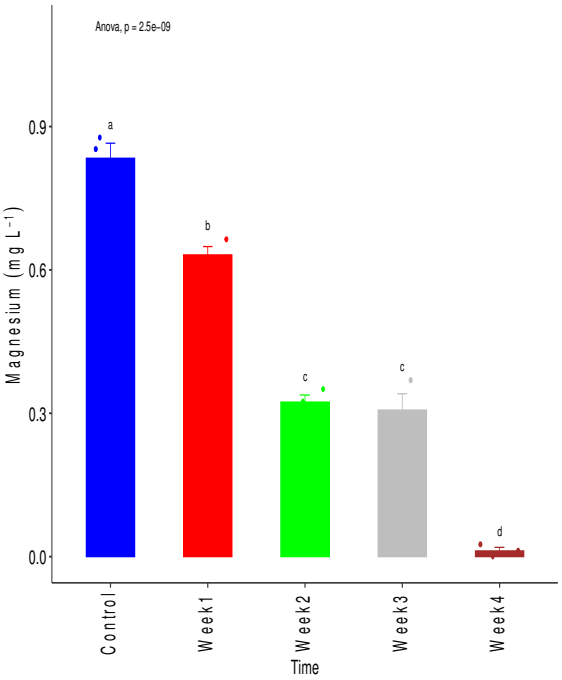

Fig. 10: Changes in manganese concentrations in the mesocosm for four weeks

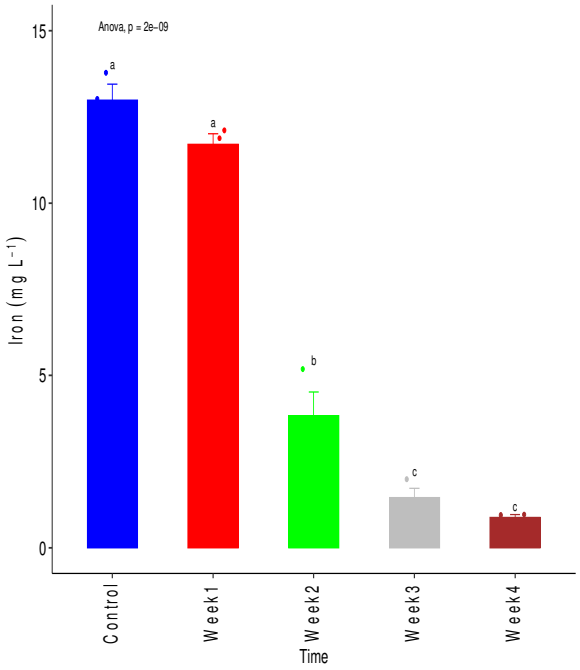

Fig. 11: Iron level variation in the mesocosm for four weeks

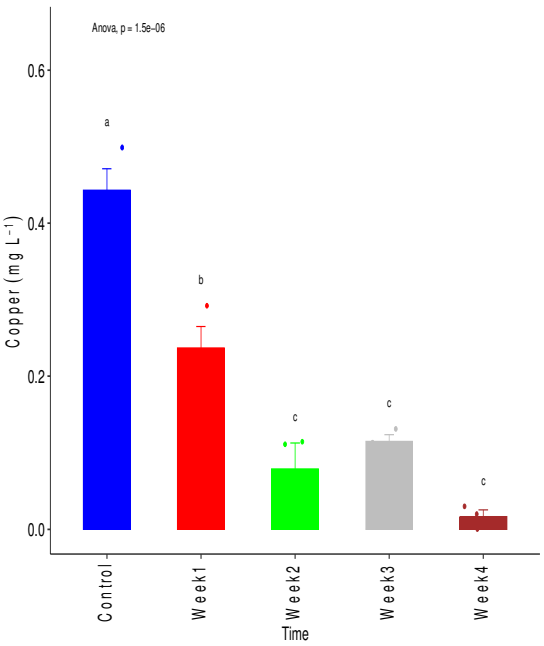

Fig. 12: Changes in copper concentrations in the mesocosm for four weeks

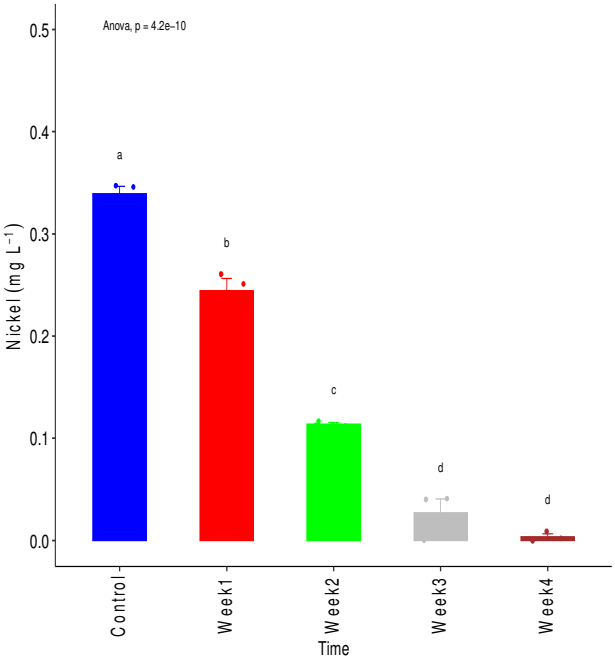

Fig. 13: Changes in nickel concentration in the mesocosm for four weeks

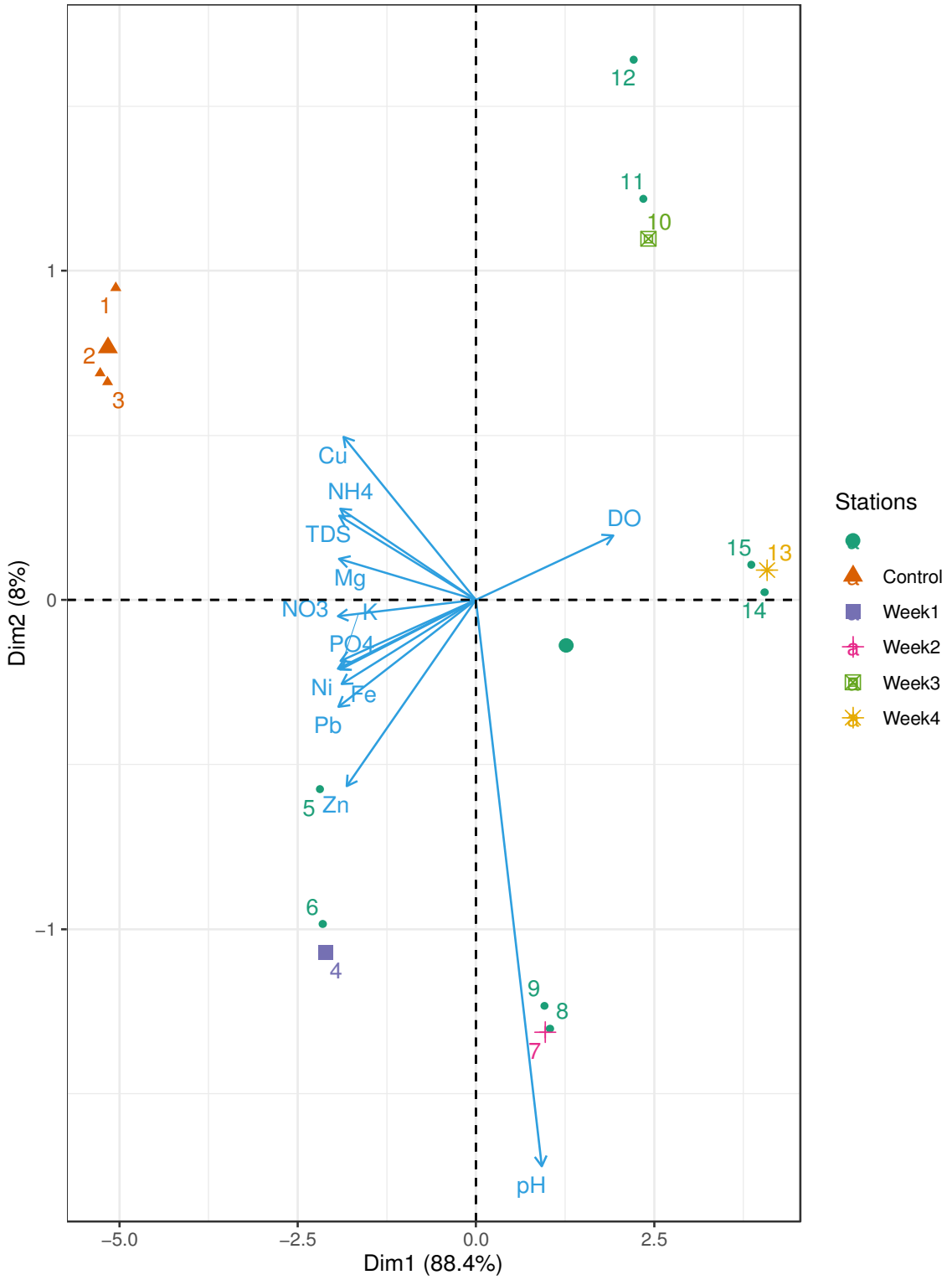

Fig. 14: Shows the positive and negative relationship among physicochemical parameters of mesocosm for four weeks

Table 1: Concentration of Carbohydrate at 2nd and 4th weeks

2nd µg/mL	4th µg/mL
0.889±0.13[a]	1.847±1.21[b]

Table 2: Concentration of Protein at 2nd and 4th weeks

2nd µg/mL	4th µg/mL
0.417±0.06 [a]	0.997±0.31 [b]

Table 3: Concentration of Lipid at 2nd and 4th weeks

2nd µg/mL	4th µg/mL
0.728±0.05 [a]	1.966±0.12 [b]

Discussion

In this research (forty-one)41 species of the phytoplankton were able to identified throughout the research period, which include*; Anabaena* sp., *Ankistrodesmus* sp., *Botrydium* sp., *Botryococcus* sp., *Ceratium* sp., *Chlamydomonas* sp., *Chlorella* sp., *Chlorogonium* sp., *Cladophora* sp., *Closterium* sp., *Crucigenia* sp., *Cyclotella sp., Stephanadiscuc sp., Dictyosphaerium* sp., *Eudorina* sp., *Euteromorpha* sp., *Gloeotrichia* sp., *Gonium* sp., *Haematococcus* sp., *Lemanea* sp., *Mougeotia* sp., *Nostoc* sp., *Oocystic* sp., *Pandorina* sp., *Pediastrum* sp., *Phacotus* sp., *Phormudium* sp., *Pleurococcus* sp., *Pyramimonas* sp., *Pyrobotrys* sp., *Rivularia* sp., *Spirogyra* sp., *Stephanosphaera* sp., *Stichococcus* sp., *Stigonema* sp., *Synedra* sp., *Tetrahedron* sp., *Tetraspora* sp., *Tolypothrix* sp., *Trebouxia* sp., *Volvox* sp., and *Scenedesmus* sp.

Also six (6) phylum were identified; Cyanobacteria, Chlorophyta, Heterokonta, Dinophyta, Bacillariophyta and Rhodophyta. The most prevalent specie in this finding is *Chlorella* sp. (with the total number of 1581cell/mL at week four) and the most common phylum in this research is Chlorophyta (with 22 species). This research indicated that biomass increased with the increasing number of days (weeks). the finding of this research correspond with the finding of Chan, (2011) who cultivated microalgae in wastewater from a fish farm and established that they could promote the growth of *Chlorella* sp. and they obtained a 90% growth rate during the experimental period. Changfu *et al.* (2013) also noted that microalgae development is primarily influenced by the nutrients contained in the wastewater or media, which is consistent with our findings.

The pH of the mesocosm during treatment raises toward neutral values as the number of weeks increases. This implies that the pH readings have changed and that the pH has remained neutral. Aarti *et al.* (2008) obtained comparable findings. In this finding *Chlorella* are the most common phylum due it high tolerance and resistance under different effluents. According to Makareviciene *et al.* (2011) and Mostafa *et al.* (2015), *Chlorella* grows at its fastest when the pH is between 6.0 and 9.0

DO increases as the number of days increases because microalgae species undergo photosynthesis and produce O_2

(oxygen) as a by-product. This finding is similar to that of Oswald *et al*. (2003), who found that using light as an energy source, microalgae uptake CO2 from the environment as a vital carbon source to synthesize sugar for biomass growth and produce O_2 as a by-product.

The drop in TDS in the mesocosm with reduction of (70.50 %) is due to the use of nutrients by algae species. These findings are comparable to those of Mostafa *et al.,* (2015), who found that algal treatment reduced TDS in water samples considerably. This reduction in TDS could be due to algae's use of various nutrients.

Nitrate, phosphorus, ammonium, and potassium levels all drop significantly at $p \leq 0.05$ with the following percentages (nitrate 97.44 %, phosphorus 100%, ammonium 92.26%, potassium 91.35%,) as the number of weeks increases. Azab (2002) made a similar observation, stating that the use of algae for wastewater treatment resulted in varying percentages of mineral.

Heavy metals: As the number of days increases, the concentration of heavy metals decreases with the following percentage (zinc 96.52%, manganese 98.63%, iron 92.90%, copper 97.56%, nickel 98.66%)). This study's findings are consistent with those of Chan et al. (2014), who found that microalgae removed up to 81.7 percent Cu, with a final concentration of 7.8 ppb after 10 days. After ten days, Zn levels had dropped by up to 94.1.

The concentration of biochemical components (carbohydrate, lipid, and protein) in mesocosm indicates rapid buildup in algal cells as the number of weeks increases. A physiological adaptation to stress has been found in phytoplankton accumulation and storage biomolecules (Chia *et al*., 2015; Liefer *et al.,* 2019). Carbohydrate accumulation is induced by inhibited algal cell growth, in which the cells invest in carbohydrate accumulation instead of growth to retain surplus fixed carbon created by imbalanced carbon and nitrogen metabolism (Chia *et al*., 2015). Furthermore, carbohydrate production is linked to signaling triggers under high levels of reactive oxygen species, as well as specialized carbohydrate production.

Protein from table two above, indicated that, it concentration increase with increase with the number of weeks. In terms of quantity, several species of microalgae are reported to possess very high concentrations of protein; (Milovanovic *et al.,* 2019). From the table three above it shows that Lipid is significantly increase with increase with the number of weeks at $p \leq 0.05$. General speaking, most microalgae are rich in polar lipids in the exponential phase of growth, and they accumulate triacylglycerols under stress conditions, which is typically during the stationary phase when nutrients are limited (Rodolfi *et al.,* 2009).

Conclusion

It concluded that, mesocosm is the best method of wastewater treatment to adopt by industries before discharging the effluents into open drains which finally join rivers. The present result showed that, algal species had very good potentials to remediate the toxic level of all physico-chemical parameters. It also concluded that, at the end of mesocosm treatment there is concomitants accumulation of biomass which can be used for other researches as biochemical components shows increasing ie (lipid, carbohydrate and protein).

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