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THE EFFECT OF TEMPERATURE AND PHOTOPERIOD ON BIOREMEDIATION POTENTIALS OF MONO AND MIXED CULTURES OF CHLOROPHYTE AND CYANOBACTERIA FOR MUNICIPAL WASTEWATER TREATMENT

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Abstract
Investigation on organic xenobiotics bioaccumulation/biodegradation in green algae is of great importance from environmental point of view because widespread distribution of these compounds in agricultural areas has become one of the major problems in aquatic ecosystem. Temperature, light intensity, amount and type of nutrients, amount of CO_2, and pH are the key factors influencing algal growth. Collections of Wastewater Samples were done according standard method. BG-11 media was prepared for culture of microalgae. Identification and isolation of microalgae was done according standard method and haemocytometer were used for microalgae cell counting. The results indicated that temperature and photoperiod affect the activities of microalgae; temperature at 25°C provide with 12:12h light/dark photoperiods show more significant with increases the number of days for both of the species as well as mixed culture, when compared with the treatments at temperature of 10°C provide with 6/18h light/dark photoperiods. Furthermore, Chlorogonium sp. and Chlorella sorokiniana showed more response during treatments in increase with the number of weeks when compared with Microcystis earuginosa and mixed culture of the species. Therefore, temperature and photoperiod impacts potentials of microalgae in the remediation of wastewater effluents.

Key Words: *Temperature, Photoperiod, Bioremediation, Wastewater and Algal Culture*

Introduction

Microalgae are eukaryotic and prokaryotic microorganisms that grow fast due to their unicellular or multicellular structure, low nutrient requirements and higher photosynthetic efficiency (Becker, 1994; Soha, 2012). The persistence of these chemicals in the environment poses a chronic threat to the health and safety of human and wildlife (Pavlostathis *et al*., 2001). Chemical contaminants present in the aquatic ecosystem may be immobilized and accumulated in sediments or may be subject to transformation and activation processes (Martínez-Jerónimo *et al.,* 2008).

The algae proved to be effective in hyper accumulation of heavy metals as well as degradation of xenobiotics (Suresh

and Ravishankar 2004). Investigation on organic xenobiotics bioaccumulation/biodegradation in green algae is of great importance from environmental point of view because widespread distribution of these compounds in agricultural areas has become one of the major problems in aquatic ecosystem (Jin *et al.,* 2012). Very high or low temperature can have a negative result on the microalgae growth and might cause growth inhibition. Increasing temperature can help algal growth until it reaches the ideal temperature. For example, Munoz *et al.* (2004), reported that, an increase in the temperature from 25 to 30°C could dual the removal efficacy when used a symbiotic microcosm formed by *C. sorokiniana* and *R. basilensis* strain. However, very high temperatures could reduce the growth rate of microalgae particularly in humid climate when evaporation is low. In cold climate, low temperature may limit algae growth but there are some microalgae that can tolerate cold weather such as blue-green algae (cyanobacteria). High light intensity related with low temperature is also another factor that result growth inhibition (Larsdotter, 2006).

The optimal temperature for growth differs from species to species (Goldman and Carpenter, 1974). Some microalgae species can have good growth in high temperature while some species can tolerate very low temperature. Razzak *et al*. (2013) Reported that the decrease of 15°C below the optimum temperature may not affect the growth of most microalgae species.

Materials and Methods

Study Area

Wastewater samples were collected from River Ginzo municipal wastewater of Katsina metropolis. River Ginzois passed along Kofar Durbi, Kofar Marusa and Kofar Sauri within Katsina metropolis (figure 1).

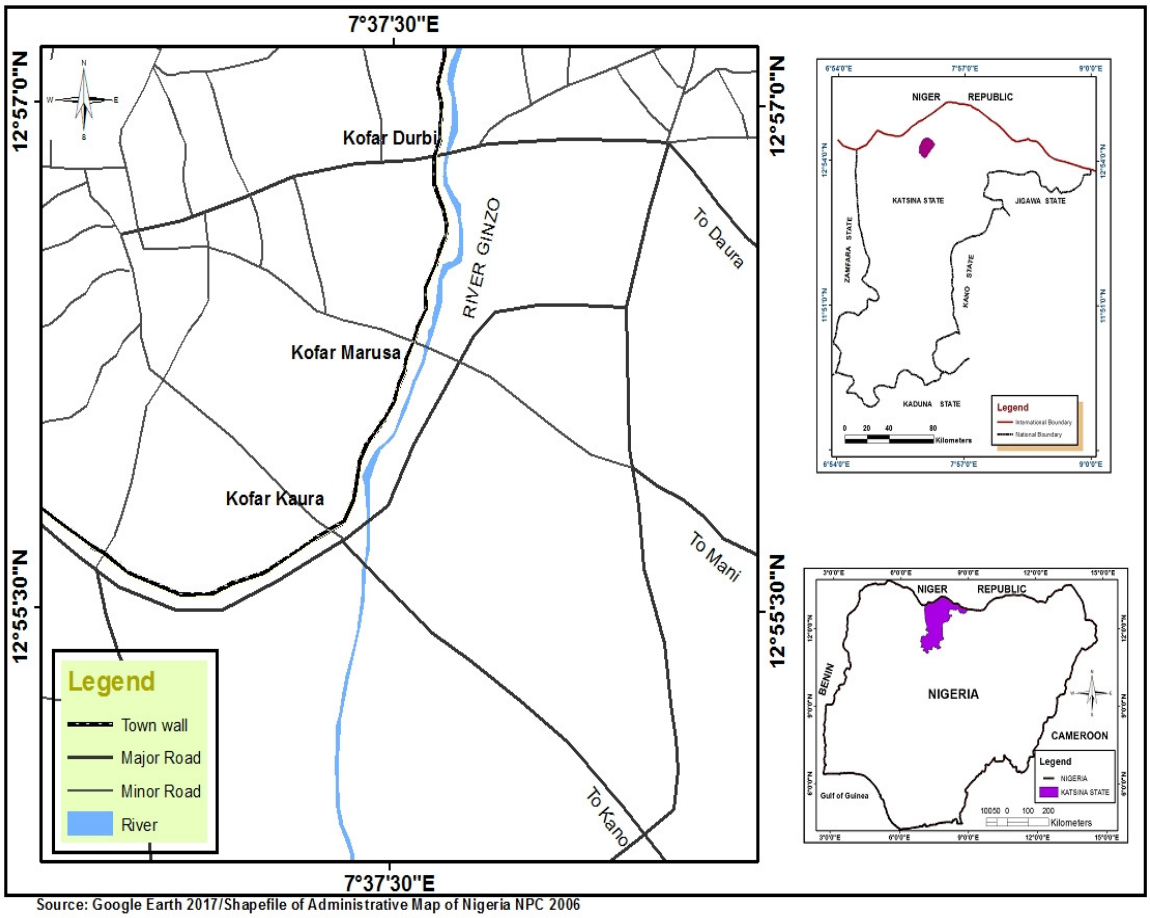

Fig. 1: Map of the study area

Collection of Wastewater Samples

For isolation of microalgae, domestic wastewater samples in this study were collected from sewage municipal wastewater of Katsina metropolis (River Ginzo). Water sample was collected using 2-litre dark brown bottle as described by Indabawa, (2012)

Media Preparation

BG-11 media were prepared

Enrichment of Culture in the Media

Collected wastewater samples were brought to the laboratory and the samples were centrifuge at 2000 rpm for 20 minutes. Centrifugation and washing were repeated six times in order to expel most of the microorganisms present in algal samples and the cells were then inoculated into sterile conical flasks containing media and this was incubated for weeks by providing required environmental condition such as 12:12h light/dark photoperiods and a temperature of 25°C. (*Mohan et al*., 2009, 2010).

Identification and Isolation of Microalgae

Agar plating was used for the isolation of individual species, for isolation, the inoculation loop loaded with the natural water sample were streaked across the agar surface, similar to the microbiological method used for bacteria.

After incubation for few days, the colonies originating on the surface of agar plates was removed with nichrome bacterial loop or using micropipette tip and immersed into the liquid media or onto another agar plate.

The microalgae samples were subjected to microscopic observation for correct identification using compound digital microscope. A standard phycological key described by Edward and David, (2010) was used for the determination and identification of species.

Growth and Maintenance in Media

For the maintenance of algal cultured, broth was prepared and each identified algal species were inoculated into. These were kept for incubation at 25°C provide with 12:12h light/dark photoperiods. The culture was maintained both in slants and broth cultures for future use (*Mohan et al*., 2009, 2010).

Microalgae Cell Counting

A cover slip was used to cover the grids of the haemocytometer and a pipette was used to fill its chamber. The pipette was placed at the tip of the haemocytometer and the sample flows into the chamber by capillary action. Cells were allowed settled and checked under microscope for satisfactory distribution of cells. The grid was divided into 9 large squares, each large square was divided into 25 medium squares and each medium square was further divided into 16 small squares. For essential measurement, the typical number of cells of the centre large square were counted, the procedure was repeated twice. The cell density obtains by multiplying the average cell count for each species by conversion factor for Neubauer ($x10^4$). The X40 objective lens was used to count the cells. The average number of cells was counted weekly for ten weeks.

Collection of Wastewater (Sampling of Wastewater Effluent)

For the treatment of effluent, the domestic wastewater samples in this study was collected from sewage municipal Wastewater River Ginzo Katsina. Water samples were collected in a 2 litre bottles which were washed with 10% HNO_3 for 48 hrs, labelled and few drops of HNO_3 were added to prevent loss of metals using grab sampling techniques (Kaul, and Gautum, 2002). The samples were centrifuge to removed coarse particles and then divided into three replicates.

Physico-chemical Analysis of Water Samples

The initial physico-chemical analysis of water samples was measured before inoculation of algae and at final stage, the total content in each flask were filtered to remove algae and then use for the analysis of various parameters such as; pH, TDS, phosphorus, nitrate, ammonium, potassium, DO (dissolved oxygen) and heavy metals using standard methods (APHA, 2005).

Remediation Bioassay in Laboratory

For each species as well as for each mixed culture, twelve flasks (100ml wastewater samples in 250ml conical flasks) were prepared. Each of the twelve flasks was inoculated with 10ml of cultured individual microalgae suspensions or with multiple species in the case of mixed culture. These was further Incubated under temperature of 25°C provide with 12/12h light/dark photoperiods for a period of four (4) weeks. The second were set at temperature of 25°C provide with 6/18h light/dark photoperiods for a period of four (4) weeks. The third treatment also set at

temperature of 10°C with 12/12h light/dark photoperiods for a period of four (4) weeks.

Samples were periodically analysed (every week) for each physico-chemical parameter such as pH, TDS, phosphorus, nitrate, ammonium, DO (dissolved oxygen), and heavy metals, using standard methods APHA, (2005).

Data Analysis

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

Results

Table 1: Average growth of three different isolated and cultured microalga species for a good ten weeks

	CELLS/mL SD		
Week/ALGAL	*Chlorella sorokiniana*	*Chlorogonium* sp	*Microcystist aeruginosa*
week 1	(6.33±2.31) x10^4	(12.16±5.11) x10^4	(16.83±4.65) x10^4
week 2	(6.87±2.21) x10^4	(13.66±3.51) x10^4	(23.33±4.01) x10^4
week 3	(9.33±4.01) x10^4	(38.33±5.69) x10^4	(23.67±1.53) x10^4
week 4	(15.33±5.50) x10^4	(44.67±4.51) x10^4	(39.5±3.28) x10^4
week 5	(17.00±6.54) x10^4	(51.00±12.21) x10^4	(40.33±3.62) x10^4
week 6	(26.47±4.38) x10^4	(60.5±3.50) x10^4	(73.00±11.36) x10^4
week 7	(27.00±4.36) x10^4	(63.00±6.05) x10^4	(82.00±13.00) x10^4
week 8	(28.33±5.51) x10^4	(66.33±6.03) x10^4	(102.00±5.29) x10^4
week 9	(38.33±11.9) x10^4	(70.00±1.00) x10^4	(115.0±10.44) x10^4
week 10	(53.33±26.60) x10^4	(73.167±3.33) x10^4	(133.50±14.08) x10^4

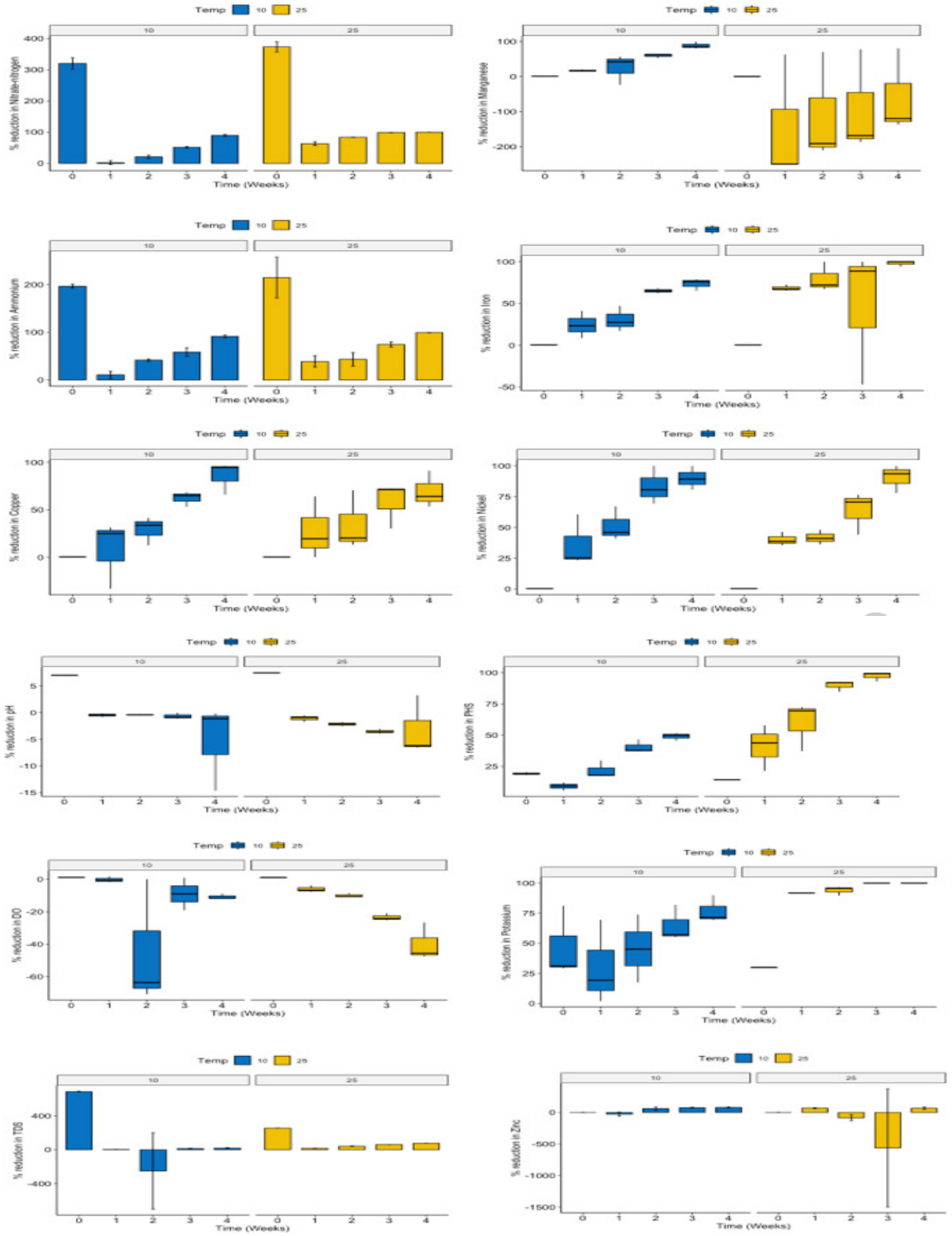

Fig. 1: Comparison reduction percentage on Physico-chemical parameters by *Chlorella sorokiniana* under different temperature of 10°C and 25°C

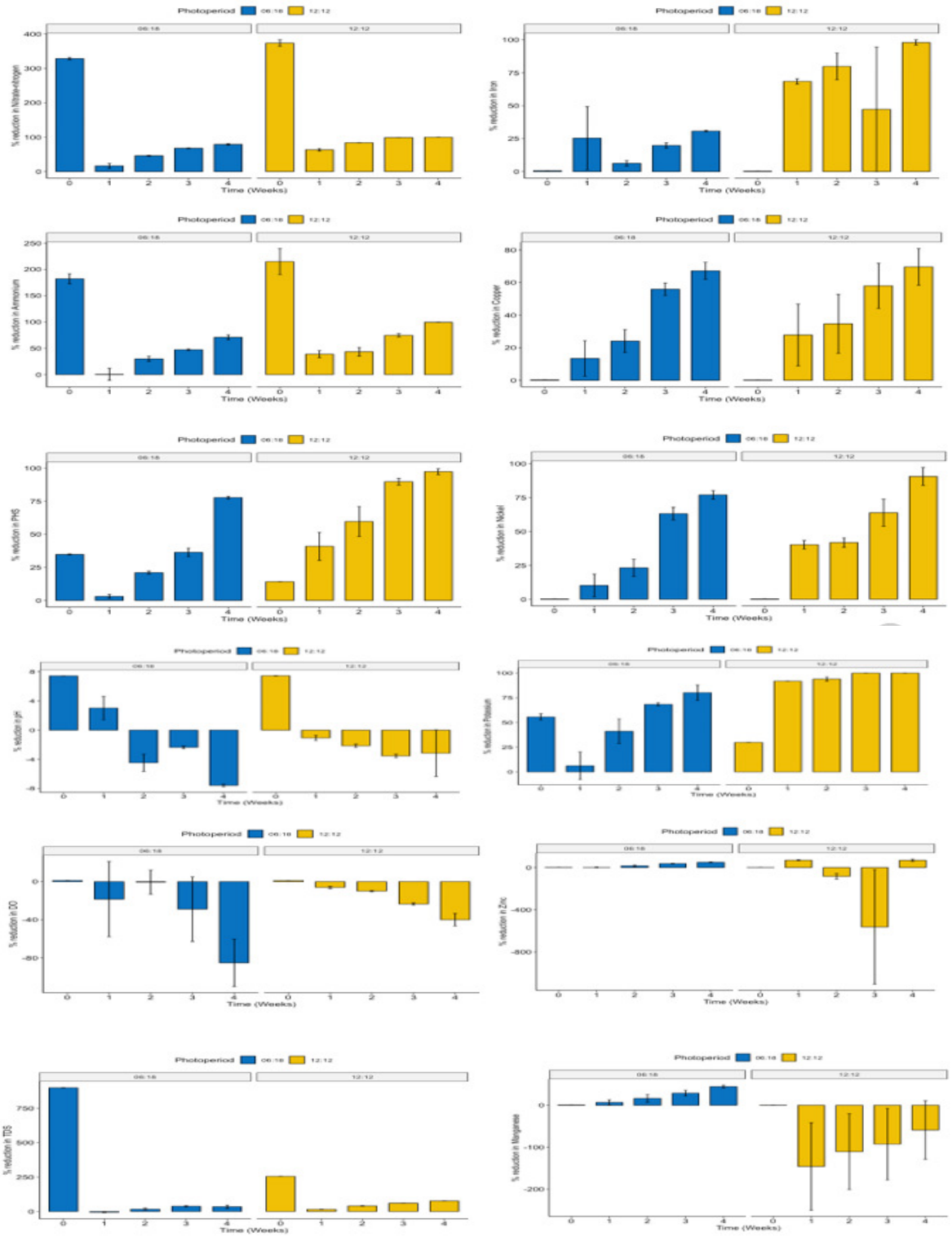

Fig. 2: Shows comparison reduction percentage on Physico-chemical parameters by *Chlorella sorokiniana* under different photoperiod of 06/18 and 12/12h light/dark photoperiods

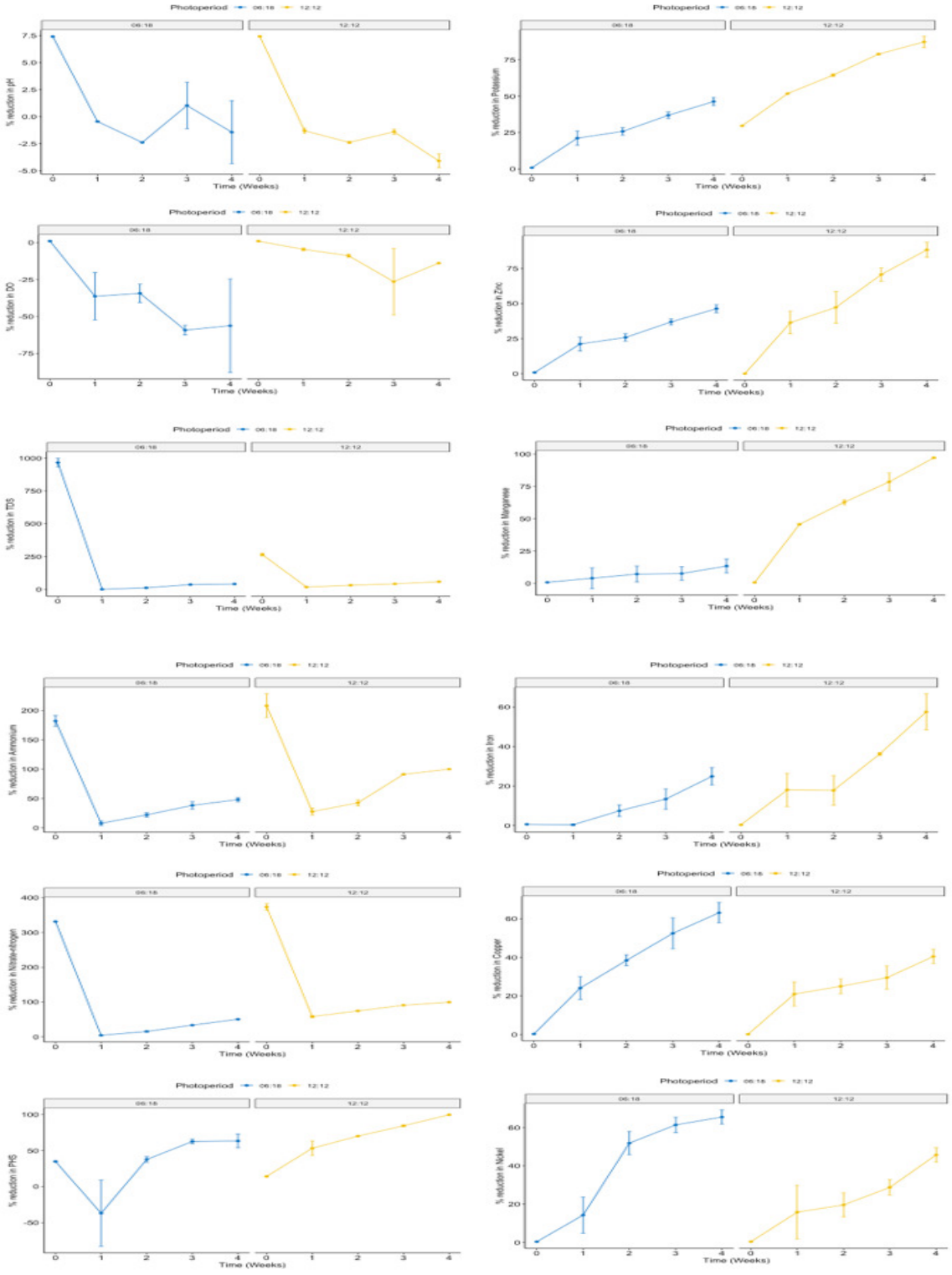

Fig. 3: Shows comparison reduction percentage on Physico-chemical parameters by *Chlorogonium* sp. under different photoperiod of 06/18 and 12/12h light/dark photoperiods

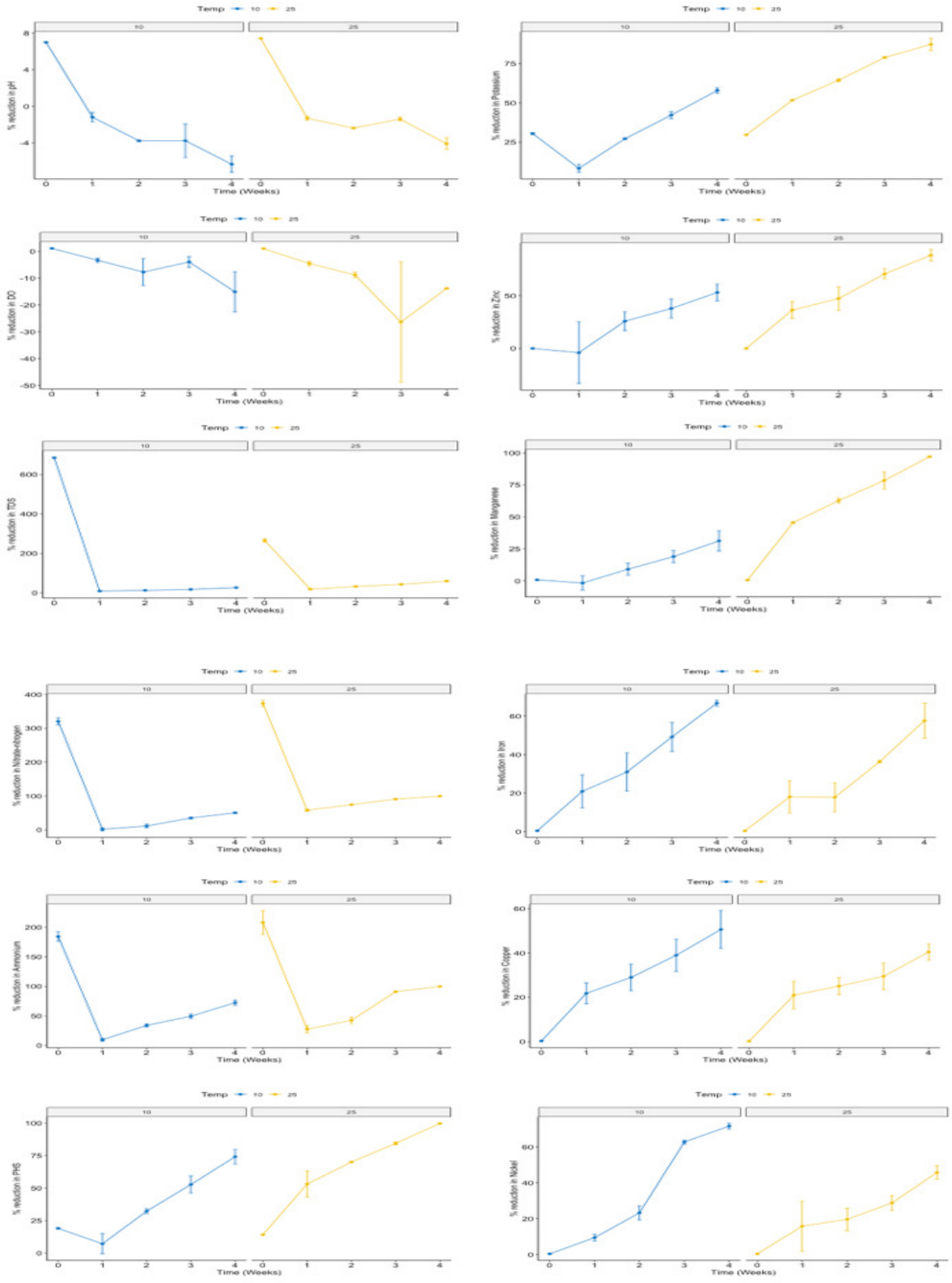

Fig. 4: Shows comparison reduction percentage on Physico-chemical parameters by *Chlorogonium* sp. under different temperature of 10°C and 25°C

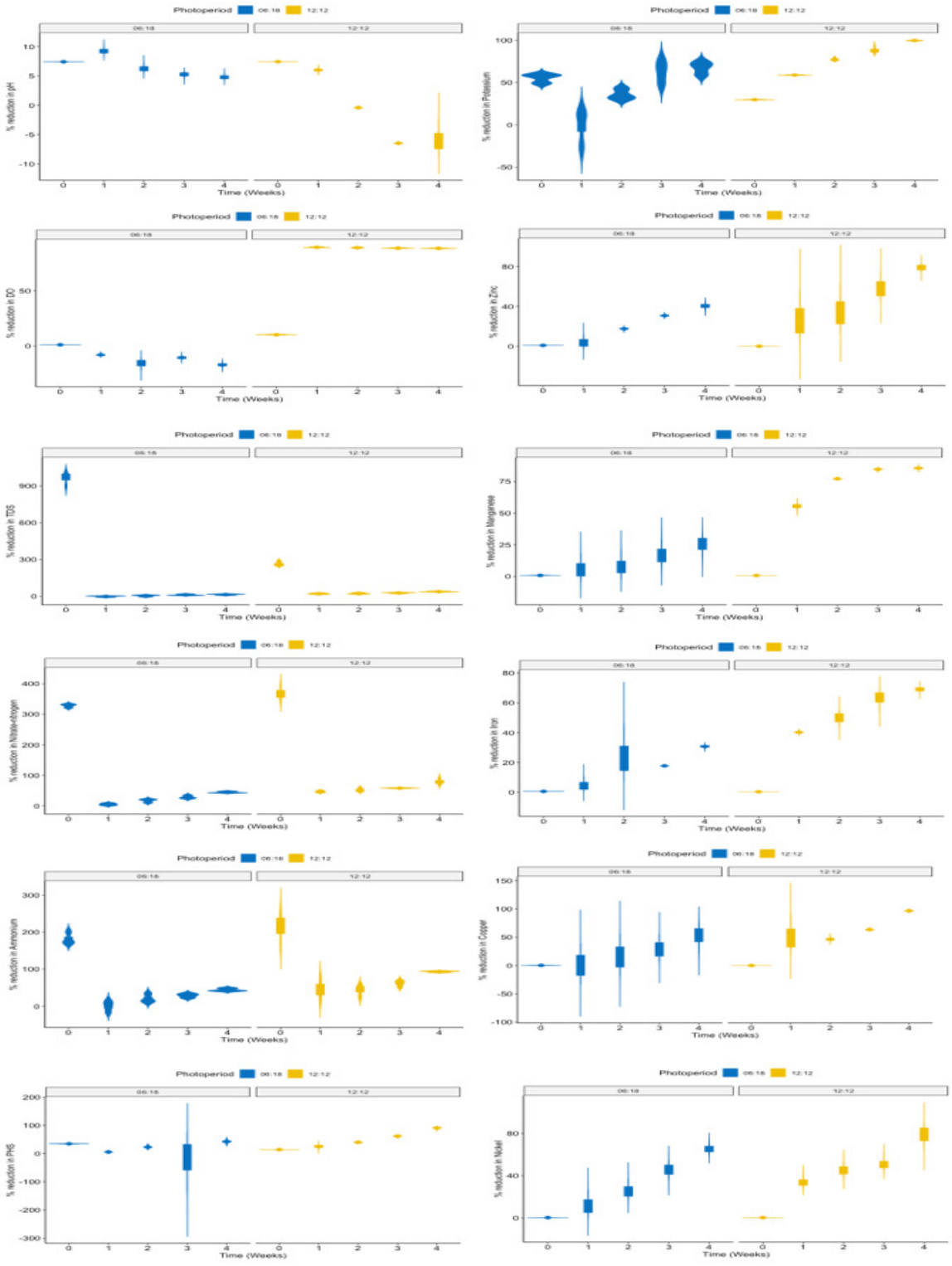

Fig. 5: Shows comparison reduction percentage on Physico-chemical parameters by *Microcystis aeroginosa* under different temperature of 10°C and 25°C

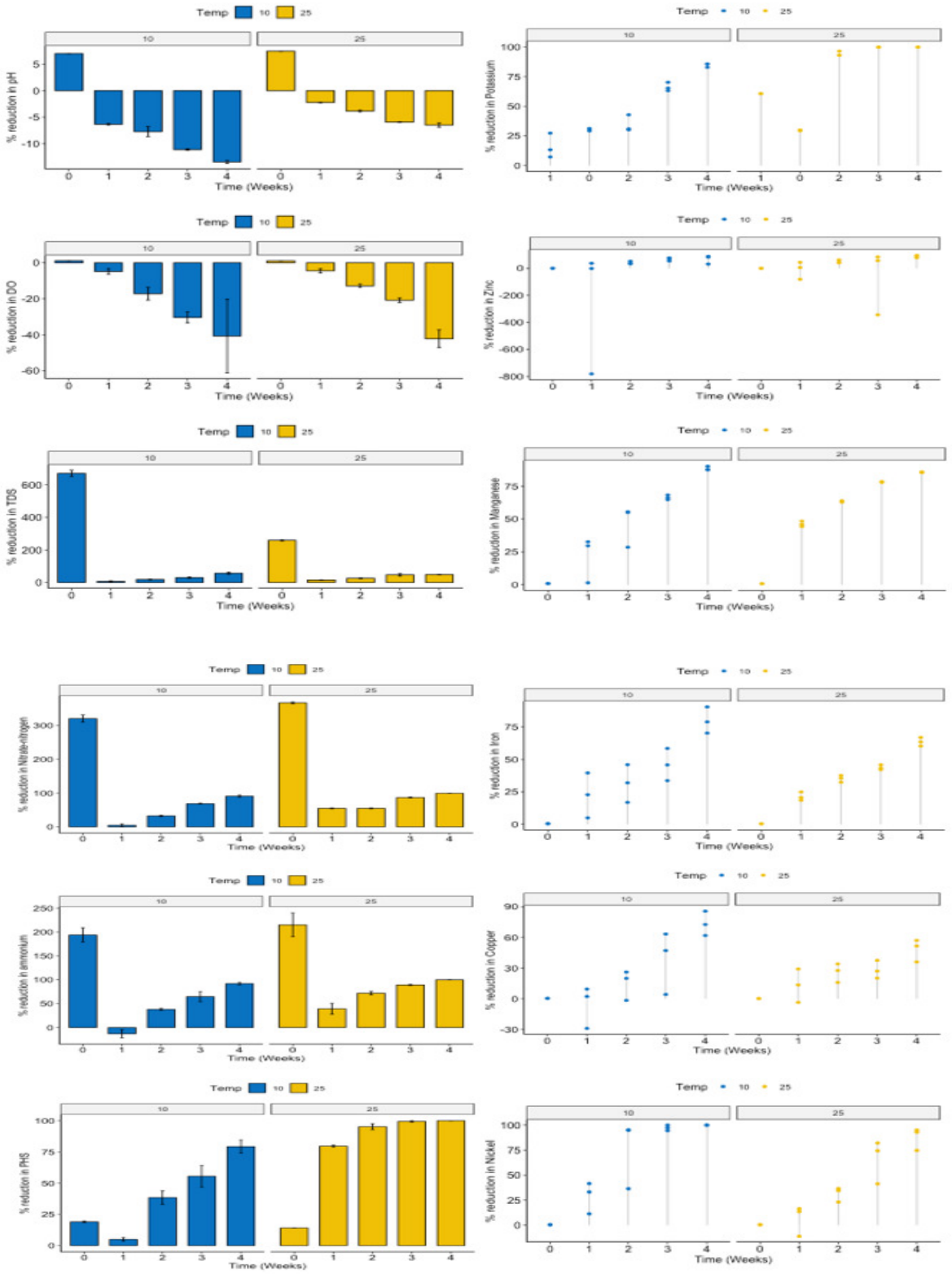

Fig. 6: Shows comparison reduction percentage on Physico-chemical parameters by mixed culture of the all used species under different temperature of 10°C and 25°C

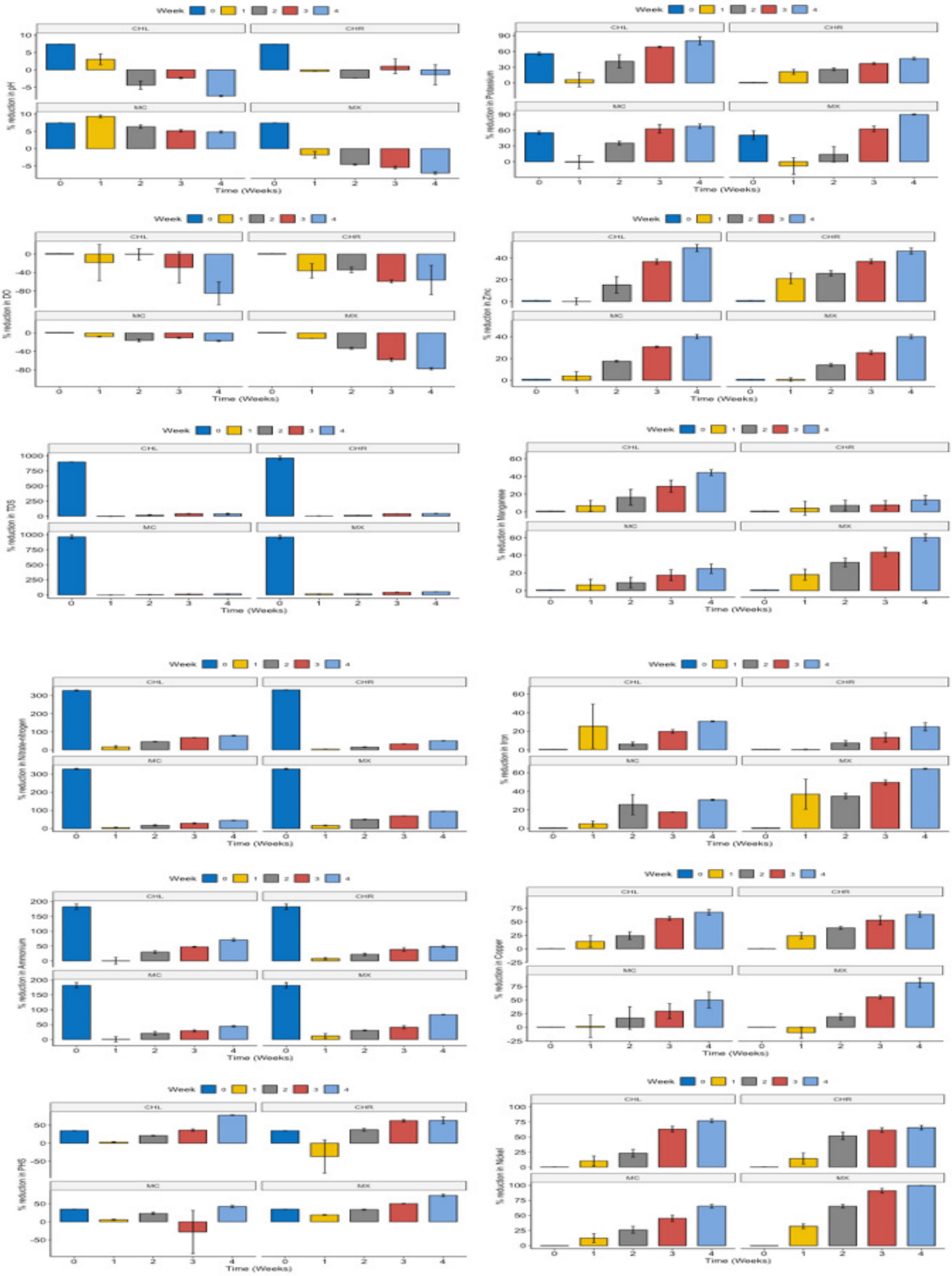

Fig. 7: Shows species and mixed culture comparison reduction percentage on Physico-chemical parameters under 12/12 light/dark photoperiod

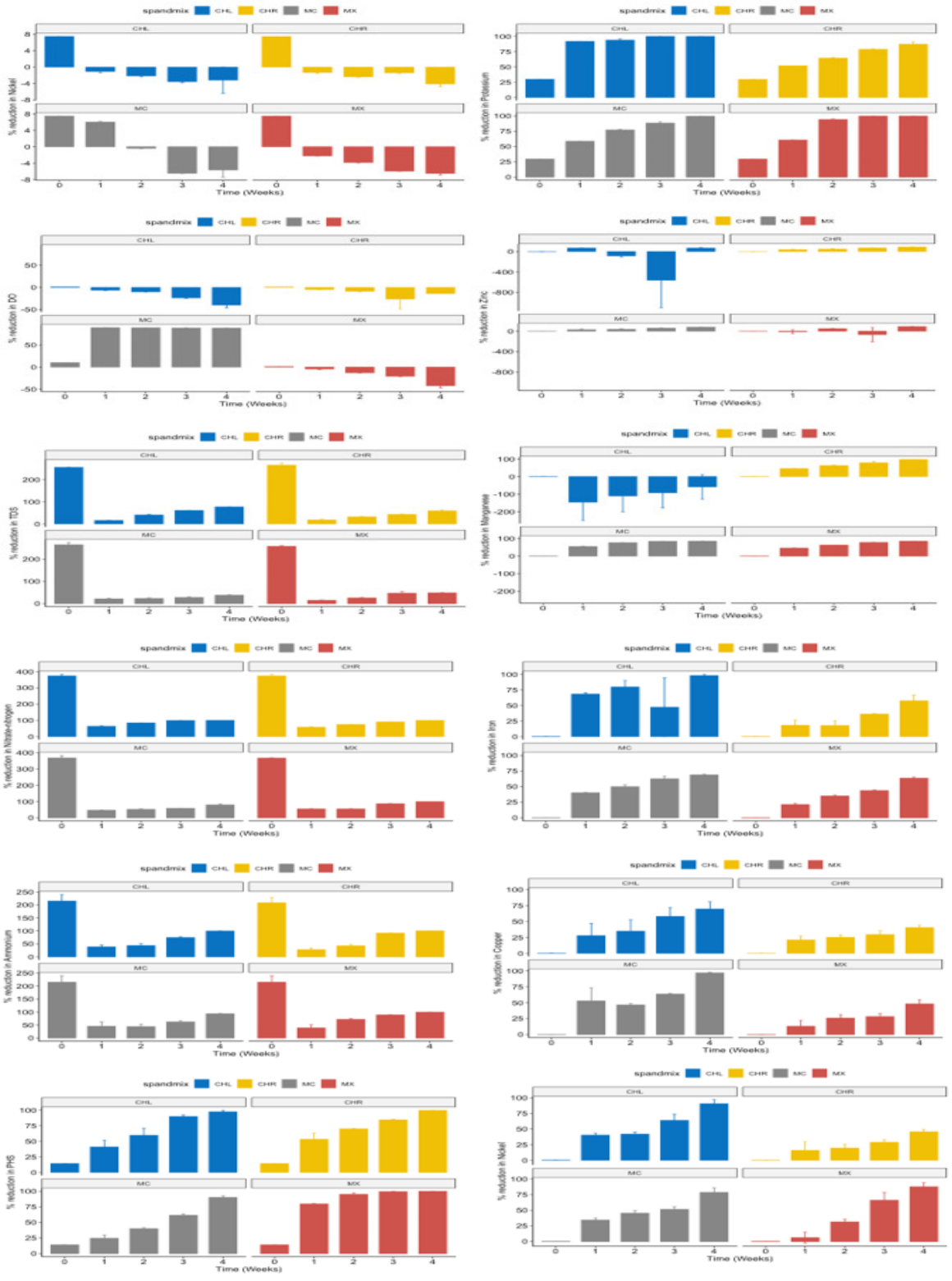

Fig. 8. Shows species and mixed culture comparison reduction percentage on Physico-chemical parameters under temperature of 25°C

Discussion

In this research finding effects of temperature and photoperiod were determined in bioremediation of effluents using three different species of phytoplankton (*Chlorella sorokiniana, Chlorogonium* sp and *Microcystis aeruginosa*) as single culture each, as well as mixed culture by combining both of the species.

Both of the species and the mixed culture were tested under different photoperiod, provided with 12/12h light/dark photoperiods for a period of four (4) weeks. The second were set at 6/18h light/dark photoperiods for a period of four (4) weeks. Both of the species shows significant difference with increase in number of weeks for remediation of effluents under both of the different photoperiod, but both of the species shows more significant potentiality under 12/12h light/dark photoperiods. Light is an essential key for growth of microalgae. Microalgae uses light to process the photosynthetic, but the light energy cannot be stored by microalgae, so the light should be supplied sustainably.

Chlolorella sorokiniana and *Chlorogonium* sp. have shown much significant under both of treatments than *Microcystis aeruginosa* and mixed culture. This finding is in line with the finding of (Molina *et al.,* 2000) who stated that, the applications of PBRs for producing large amount of microalgae biomass are successful, compare to open ponds, PBRs is more expensive but produces more biomass and single-species culture of microalgae is allowed by PBRs for prolonged durations. Also (Chisti, 2007) stated that under certain good control of PBRs, the production of long chain fatty acids and high value such as DHA and EPA is highly recommended. This research agreed with the finding of (Carvalho *et al.,* 2011) who stated that, the microalgae cannot use all the supplied light because microalgae cannot absorb all the photons, and too much light will cause light inhibition for the surface layer of microalgae. The inner portion microalgae cannot reach the light and lack of photons for autotrophic microalgae to convert carbon dioxide in the air into organic compounds, visible light is the main source of energy.

Temperature

Both of the species and the mixed culture were tested under different photoperiod, provided with temperature 25°C for a period of four (4) weeks. The second were set with temperature for 10°C for a period of four (4) weeks. Both of the species shows significant different in remediation of effluents under both of the different temperature, but both of the species shows more and rapid potentiality under temperature of 25°C.

This is in line with the finding of (Huang *et al.,* 2008), who stated that, with the light intensity changing, temperature is an environmental factor which indirectly affects growth of microalgae. Temperature determines the activity and reaction rates of intracellular enzyme, which will have an influence on algal photosynthesis, respiration intensity, affect the growth of microalgae and to limit its distribution (Tan *et al.,* 2009).

pH

The pH value of bioremediation of wastewater using single cultured species and mixed cultured species was maintained at neutral for both single and mixed culture. These are similar result reported by Aarti *et al.* (2008), Makareviciene *et al.* (2011), Mostafa *et al.*

(2015) have found that *Chlorella vulgaris* sustained the maximum growth rate at the range of pH between 6 and 9.

TDS

The removal of TDS for single and mixed cultured differed significantly. These are similar observations made by Mostafa *et al.* (2015) who reported that the TDS of water samples were significantly decreased with algal treatment, this reduction in TDS might be as the result of utilization of various nutrients by algae

DO

The values of DO increases for both single and mixed cultured. The increase in DO is due to photosynthesis. This finding is similar with finding of Oswald *et al., (*2003) who reported that using light as an energy source, microalgae uptake CO_2 from the environment as a vital carbon source to synthesize sugar for their biomass growth and produce O_2 as a by-product

Nitrate Phosphorus Ammonium and Potassium

The Nitrate Phosphorus Ammonium and Potassium reduced significantly for both single and mixed culture under difference temperature and photoperiod. The finding of this research corresponds with the findings of Aslan and Kapdan (2006) that used *C. vulgaris* for nitrogen and phosphorus removal from wastewater. They reported average removal efficiency of 72% for nitrogen and 28% for phosphorus. Shi *et al.* (2007) also conducted experiments with *Chlorella* to remove nitrate from municipal wastewater and reduce levels of phosphate, ammonium and nitrate in synthetic secondary wastewater. In their experiment ammonium was removed less rapidly by the algae than phosphate which is similar to this finding. Azab (2002) who stated that the application of algae for wastewater treatment shows variable percentages of decrease in minerals.

Heavy Metals

The concentration of heavy metals for the both single cultured and mix cultured shows reduction with increase in the number of days. The finding of this research is in line with finding of Chan *et al.* (2014) who also reported that microalgae removed up to 81.7% Cu reaching lowest final concentration of 7.8ppb after 10 days. Zn reduced up to 94.1% reaching 0.6ppb after 10 days. The finding of is research also is in line with finding of Ajayan *et al.* (2011) who observed the highest Cu, Zn and Co removal of 60, 42.9 and 29.6 %, respectively, with microalgae while highest Pb removal of 34.6 % was observed with *Scenedesmus bijuga* in sewage wastewater. In another report, *Chlorella* sp. removed 65.4, 95.4, 98.3, 80, 98.2 and 56.5 % of Al, Ca, Fe, Mg, Mn and Zn, respectively, from municipal wastewater (Wang *et al.,* 2010). El-Sheekh *et al.,* (2005) also studied the removal of heavy metals from Verta Company.

Conclusion

The present result showed that both the algal species had very good potentials to remediate the toxic level of all physico-chemical parameters. This research confirmed that single and mixed culture of microalgae showed high reduction capacity, TDS, phosphate, ammonium, nitrate, potassium and heavy metals. These finding confirmed that temperature and photoperiod play a vital in phycoremediation.

Acknowledgement

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