RESEARCH PAPER   OPEN ACCESS

Degrading heavy metals from waste water in the presence of efficient cyanobacteria

S. Abirami[*1], A. Muruganandam[1], M. Gopinathan[1], V. Ambikapathy[2],

A. Panneerselvam[3], P. Prakash[2]

[1]*P.G. & Research Department of Botany, M. R. Government Arts College*

(Affiliated to Bharathidasan University, Trichy- 24), Mannargudi, Tamil Nadu, India

[2]*P.G. & Research Department of Botany, A.V.V.M. Sri Pushpam College (Autonomous)*

(Affiliated to Bharathidasan University, Trichy- 24), Poondi, Thanjavur, Tamil Nadu, India

[3]*Indian Biotrack Research Institute, Thanjavur, Tamil Nadu, India*

Abstract

The current study illustrates the degradation of heavy metals in the presence of cyanobacteria by different waste water. The waste water samples were collected and cyanobacteria also isolated and identified. Totally, there were 22 isolates of cyanobacteria such as *Arthrospira jenneri, Aphanocapsa koordersi, A. platensis, Gloeocapsa crepidium, G. gelatinosa, G. livida, G. punctata, G. samoensis, G. sanguine, Hyella caespitose, Oscillatoria acuminate, O. amoena, O. homogenea, O. laetevirens, O. minimus, O. pseudogeminata, O. schultzii, O. subbrevis, O. trichoides, Spirulina laxissima, S. meneghiniana* and *S. subtilissima*. The cyanobacteria incorporate with waste water to interrupt and degrade the heavy metals in various waste water treatments such as dairy waste water, kitchen waste water, fish pond discharge and municipal waste water. Screening and effect of cyanobacterial treatment of wastewater of BOD, COD, TN and TP were analysed. The maximum percentage of degradation were determined in the kitchen waste water and fish pond discharge, whereas biochemical oxygen demand, chemical oxygen demand, total nitrogen, and total phosphorus by the selected potential cyanobacteria like *Oscillatoria trichoides* and *Spirulina laxissima*. The degradation of heavy metals such as Cu^{2+}, Fe^{2+}, Zn^{2+} and Pb^{2+}. The maximum degradations were recorded at 99.88% and 99.84% for Zn^{2+} *O. trichoides and S. laxissima* in kitchen waste water and fish pond discharge respectively. The significance of variance at a confidence level of $p<0.05$ and $p<0.01$ is recorded. The use of cyanobacteria performs a variety of tasks in the assembly of excess food, the treatment of wastewater, and the production of valuable biomass, all of which have a variety of uses. For the cyanobacteria *O. trichoides and S. laxissima* were suitable candidature for the healthy environment and future enduovurs.

*Corresponding Author: S. Abirami ✉ abi25sekar@gmail.com

Introduction

Wastewater is produced in huge quantities each day in developing nations like India as a result of increasing population and growing industry. In India, a variety of conventional methods are employed for wastewater treatment, however they are very costly and not practical. The problems of traditional methods are being resolved by the introduction of various innovative environmentally friendly technical wastewater treatment methods nowadays (Satpal and Khambete, 2016). Wastewater treatment systems exploiting photosynthetic microorganisms have lately arose as an encouraging substitute to conservative biological processes (Goncalves *et al.*, 2017). However, over the last ten10 years, there has been a significant rise in research interest in the use of cyanobacteria to treat wastewater discharged from various locations, with encouraging results for both organic and inorganic discharge (Subramaniyam *et al.*, 2016; Papadopoulos *et al.*, 2020a). Additionally, cyanobacterial biofilms show significant prospects for use in the treatment of wastewater. The biofilm matrix defends the microbial inhabitants from the stresses of the environment (Papadopoulos *et al.*, 2020b). Microalgae are especially helpful for reducing the contents of inorganic nitrogen and phosphorus in wastewater because they may use both of these wastewater pollutants for growth (Liu *et al.*, 2017; Hena *et al.*, 2015).

Wastewater-derived microalgae cultures can significantly contribute in the management of marine ecosystems by providing an inexpensive and environmentally friendly method for wastewater treatment. It is beneficial to use dairy effluent for micro algal cultures because it uses more freshwater, costs less to add nutrients, removes the remaining nitrogen and phosphorus, and produces micro algal biomass that may be used to make biofuel or even other high-value byproducts (Kothari *et al.*, 2013; Kumar *et al.*, 2015; Kothari *et al.*, 2012). For basic physiological and chemical processes in both plants and animals, heavy metals are essential. However, some heavy metals have the potential to poison living organisms. Arsenic As, cadmium Cd, copper Cu,

chromium Cr, lead Pb, manganese Mn, Mercury Hg, nickel Ni, and zinc Zn are heavy metals that are usually present in wastewater (Lu *et al.*, 2015; Sabbir and Tatsufumi, 2024). Activities that discharge wastewater with significant levels of contaminants, such as heavy metals, must be removed before discharging the effluent to natural water sources (Ahmad *et al.*, 2022; Miao *et al.*, 2021; Bavatharny *et al.*, 2023). Traditional physical techniques are also used to treat industrial wastewater, with adsorption being the most common due to its simplicity of operation (Sun *et al.*, 2020; Shahrokhi-Shahraki *et al.*, 2021; Ram *et al.*, 2011; Shrestha *et al.*, 2021). The conventional biological nutrient removal method needs many reactors, which raises the operational complexity. Microalgae's strong capacity for nitrogen uptake has garnered interest in wastewater treatment in recent years (Sun *et al.*, 2019; Renuka *et al.*, 2015; Ji *et al.*, 2018). When compared to physical and chemical systems, biological treatment systems have attracted global attention and assisted in the development of comparatively effective, affordable, and environmentally safe treatment technologies (Menger-Krug *et al.*, 2012; Choudhary *et al.*, 2007).

Cyanobacteria are among the most promising microorganisms for absorbing heavy metals and carrying out oxygenic photosynthesis. Cyanobacteria provide a number of advantages that make them desirable hosts for biodegradative genes to increase their capacity for biodegradation (Lebkuecher *et al.*, 2015). Aquatic plant biomass, either micro or macro organisms, can be used to accumulate metal ions from water in one of two ways (Kuritz and Wolk, 1995; Zakaria, 2001; Pena-Castro *et al.*, 2004; Keskinkana *et al.*, 2003). It was previously demonstrated that a packed bed column was the most practical method for heavy metal absorption because it had a high absorbent capacity and improved effluent quality (Kulshreshtha *et al.*, 2014; Veenstra *et al.*, 1999). Wastewater discharge is increasing together with the water shortage as a result of increased urban, agricultural, and industrial pollution. Additionally, a number of governmental organisations have now embraced the circular economy concept in an attempt to reduce and

control the pollution which wastewater produces (Ahmad *et al.*, 2019). The generated sludge would mainly be made up of microalgae after the wastewater had completed micro algal bioremediation (Capodaglio, 2020; Mohd Udaiyappan *et al.*, 2017). A further objective of the present study was to screen was to screen for four different waste water treatments and the efficiency of heavy metal removal from cyanobacteria.

Materials and methods

The collection of dairy waste water, kitchen waste water, fish pond discharge and municipal wastewater. The basic parameters of waste water samples were all investigated. The removal efficiency of heavy metals like Cu^{2+}, Fe^{2+}, Zn^{2+} and Pb^{2+} are selected potential cyanobacteria were analysed. From research sample, 22 cyanobacteria were isolated and this work was screened by the 22 cyanobacteria from four different wastewaters. Then the efficiency of heavy metal removal from selected potential cyanobacteria.

Cultivation of cyanobacteria

A conical flask with a non-absorbent cotton plug was loaded with around 20 ml of the collected algae, which was then immersed in four media solutions that were previously prepared. These flasks were placed in an incubator, and for 10 to 30 days, the growth of cyanobacteria in all four media was observed. The incubator was set to a 25°C temperature with such a 2000–2500 lux light intensity. Cyanobacteria cultures received 16 and 8 hours, respectively, of alternate exposure to light and dark. The pH of the solution was kept at 7-8. The BG-11 medium was discovered to be the best of the four for the growth of cyanobacteria. Wastewater was treated using algae and a media solution (Larsdotter, 2006; Becker, 1994; Kawaguchi, 1980; Raven, 1988; Singh and Kumar, 2024; Yolanys *et al.*, 2024).

Screening of cyanobacteria and treatment of wastewater

Cyanobacteria used to treat wastewater in a batch system. Filtered algae from the media solution were mixed with about 1000 ml of wastewater in a 2 lit beaker. Using an air pump, pumped through the solution. For approximately 10 to 12 days, the temperature, colour, and pH of the solution were continuously recorded. The treated solutions BOD, COD, TN and TP were estimated after twelve days (Sirsam and Bhangale, 2017). In accordance with the standards provided by the American Public Health Association (Zhao, 2019), the BOD, COD, TN, and TP were measured.

Cyanobacterial growth rate determination

An ultraviolet spectrophotometer UV-1800, Shimadzu was used to measure the optical density OD of each sample of cyanobacteria culture in order to detect chlorophyll (APHA, 2012). The following formulas were employed to calculate each strain's corresponding first-order growth rate k_1, day^{-1}.

$$OD_{strain} = OD_i - OD_{control}$$
$$LnOD^t_{strain} = k_1 t + Ln\ OD^t_{strain}$$

The optical density of the inoculated ADE sample is represented by OD_i, and the optical density of the control ADE sample is denoted by $OD_{control}$ positive control. Using Excel, related linear regression statistics were calculated.

Heavy metal determination

The two living species of the tested cyanobacteria namely *Oscillatoria trichoides* and *Spirulina laxissima* were selected for removal efficiency of heavy metals. Using an atomic absorption spectrophotometer AAS and the standard procedure described in the "Standard Methods for Examination of Water and Wastewater" (Sorakin, 1979), treated samples and raw water were both characterised for the selected dissolved heavy metals after treatment. Membrane filters were used to separate water samples, and each sample was then individually analysed with a specific lamp and wavelength. The proposed system's heavy metal removal efficiencies were calculated.

Statistical analysis

The results of the experiments were carried out in triplicate and are expressed as mean values with a

standard deviation by using Microsoft Excel 2007. The differences between the mean values were calculated using Tukey's test at the 0.05 and 0.01 level and using the Origin Software.

Results and discussion

Screening of cyanobacteria from wastewater and treatments

A total of four cyanobacteria strains were involved in the primary screening method, and their time series growth curves were examined (Sirsam and Bhangale, 2017). In the present study, a total of 22 cyanobacteria were screened, such as *Arthrospira jenneri, Aphanocapsa koordersi, A. platensis, Gloeocapsa crepidium, G. gelatinosa, G. livida, G. punctata, G. samoensis, G. sanguine, Hyella caespitose, Oscillatoria acuminate, O. amoena, O. homogenea, O. laetevirens, O. minimus, O. pseudogeminata, O. schultzii, O. subbrevis, O. trichoides, Spirulina laxissima, S. meneghiniana* and *S. subtilissima* for various waste waters, such as dairy waste water, kitchen waste water, fish pond discharge and municipal waste water.

Dairy wastewater

According to the species, different microalgae have varied growth rates. Different biomass productivity of microalgae in dairy wastewater has been reported by various researchers (Lu *et al.*, 2015).

Large amounts of seriously polluted wastewater were produced by the dairy wastewater. When the algal cells were cultivated in dairy wastewater, cyanobacterial Chlorella usually achieved a high level of nutrient pollutant removal and was effectively used (Chiu *et al.*, 2015; Gupta *et al.*, 2015).

Cyanobacteria are a very appealing option for low-cost and sustainable wastewater treatment due to their ability to grow well in nutrient-rich environments, consume nutrients effectively, and accumulate metals from the wastewater (Devi *et al.*, 2012). Cyanobacteria were used to treat dairy wastewater and produce biomass while reducing the nutritional content of the wastewater (Hena *et al.*, 2015).

On a bench outdoor scale, it was possible to cultivate *Chlorella zofingiensis* in dairy wastewater and obtain a maximum TP and TN removal of 97.5% and 51.7%, respectively (Huo *et al.*, 2012). In an uncertain and changing indoor lab-scale experiment using dairy wastewater (Ding *et al.*, 2014) obtained 0.86 g/L biomass, 89.92-91.97% TP, and 84.18-89.70% COD removal. In this study, the removal efficiency of dairy wastewater, COD and BOD mg/L were presented at highest concentration in the cyanobacteria were *Oscillatoria trichoides* 74.2 mg/L and 79.5 mg/L and *Spirulina laxissima* 65.1 mg/L and 69.3 mg/L. Typical nitrogen TN and phosphorus TP highest concentration in the *O. trichoides* were 28.9% and 10.6% and *S. laxissima* 29.1% and 10.8% respectively. The maximum concentration in the COD and BOD mg/L were *Aphanocapsa koordersi* 55.2mg/L and *O. laetevirens* 58.6mg/L in COD and *G. punctata* 65.1mg/L and *O. laetevirens* 62.1mg/L in BOD, *Arthrospira jenneri* 28.1% and *G. gelatinosa* 27.4 in TN and *Arthrospira jenneri* 9.2%, *Aphanocapsa koordersi* 9.3% and *G. livida* 9.6% in TP. The minimum concentration in the COD and BOD mg/L from dairy wastewater were presented at the cyanobacteria was *Gloeocapsa gelatinosa* 25.1mg/L, *G. crepidium* 25.4mg/L, *Hyella caespitose* 26.5mg/L and *O. subbrevis* 26.9mg/L in COD and *Hyella caespitose* 29.5mg/L, *O. minimus* 28.6mg/L and *O. subbrevis* 27.1mg/L in BOD. The minimum TN and TP were presented at *Aphanocapsa platensis* 18.6%, *G. livida* 18.9%, *G. punctata* 16.5%, *O. Schultzii* 17.1% and *S. subtilissima* 17.6% in TN and *G. samoensis* 5.2%, *O. homogenea* 5.1%, *O. pseudogeminata* 5.7%, *O. schultzii* 5.3% and *S. meneghiniana* 5.8% in TP. The cyanobacteria of not applicable N/A are *G. gelatinosa* from BOD mg/L, *O. homogenea* and *S. meneghiniana* from TN % and *G. crepidium, Hyella caespitosa* and *O. minimus* from TP % are recorded. The four removal efficiency COD mg/L, BOD mg/L, TN % and TP % were maximum and minimum are mostly recorded with the cyanobacteria is *Aphanocapsa koordersi, Arthrospira jenneri, O. schultzii, G. livida* and *O. subbrevis,* respectively (Fig. 1 & 2).
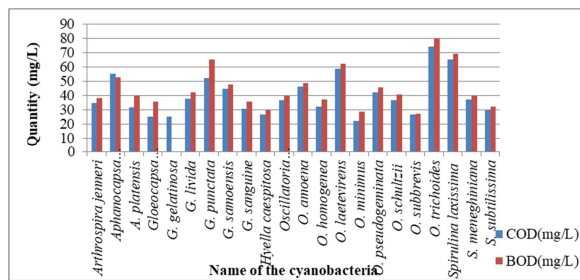
Fig. 1. Screening of COD and BOD from cyanobacteria strains in dairy wastewater
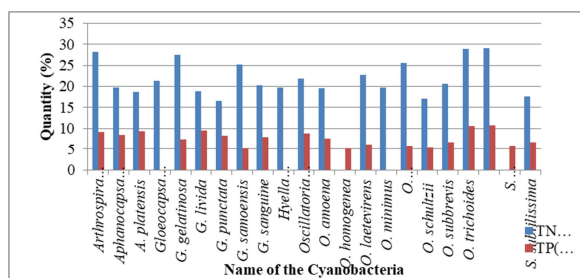

Fig. 2. Screening of TN and TP from cyanobacteria strains in dairy wastewater

Kitchen wastewater
Additionally, 68.4% BOD and 67.2% COD were removed from household wastewater using biological treatment with algae (Aslan and Kapdan, 2006). In 48 hours of interaction with *C. vulgaris* at 30°C, (Rana, 2010) reported a 78% COD reduction. The algal-bacterial system (Hoffman, 1998) used for wastewater treatment was able to remove approximately 70–80% of the COD. By using filamentous green algae in an artificial wetland and a high rate algal pond (Wood *et al.*, 1989) reported a relatively low efficiency for COD removal in the range of 59.2% to 69.4%. The domestic wastewater, the average specific growth rates in the exponential period were 0.412, 0.429, 0.343, and 0.948. It also revealed that the removal rates for phosphorus, and COD were 74–82%, 83–90%, and 50–83.0%, respectively (Wang *et al.*, 2010). In this study, the efficiency of kitchen wastewater removal, the cyanobacteria with the highest COD and BOD mg/L concentrations were *O. trichoides* 121.0mg/L and 135.5mg/L and *S. laxissina* 105.7mg/L and 115.4mg/L. The greatest typical concentrations of nitrogen TN and phosphorus TP were *O. trichoides* 30.5% and 10.9% and *S. laxissina* 32.7% and 11.5%

respectively. The maximum concentration of *G. samoensis* 94.1mg/L and *Aphanocapsa koordersi* 91.1mg/L in COD, *Arthrospira jenneri* 95.7mg/L, *Aphanocapsa koordersi* 96.2 mg/L and *G. samoensis* 96.8 mg/L in BOD, *Arthrospira jenneri* 29.8% and *O. pseudogeminata* 27.5% in TN and *G. livida* 10.2%, *Arthrospira jenneri* 9.5%, *Aphanocapsa platensis* 9.7%, and *O. acuminata* 9.6% in TP. The minimum presented at the cyanobacteria were *G. gelatinosa* 65.7mg/L, *G. sanguine* 67.2mg/L, *O. homogenea* 69.2mg/L and *S. meneghiniana* 65.2mg/L in COD, *G. gelatinosa* 68.2mg/L and *S. meneghiniana* 69.4mg/L, *G. livida* 19.4%, *O. homogenea* 19.6%, *O. schultzii* 19.3% and *S. subtlissima* 19.3% in TN and the *G. samoensis* 5.8%, *O. homogenea* 6.8%, *O. pseudogeminata* 6.7%, *O. schultzii* 6.4% and *S. meneghiniana* 6.6% in TP. The cyanobacteria of not applicable N/A are *G. gelatinosa* and *Hyella caespitose* from TN % and *G. crepidium*, *Hyella caespitose* and *O. minimus* from TP % are recorded. The four removal efficiency COD mg/L, BOD mg/L, TN % and TP % were maximum and minimum are mostly recorded with the cyanobacteria is *S. meneghiniana*, *Arthrospira jenneri*, *Aphanocapsa koordersi* and *O. homogenea,* respectively (Fig. 3 & 4).
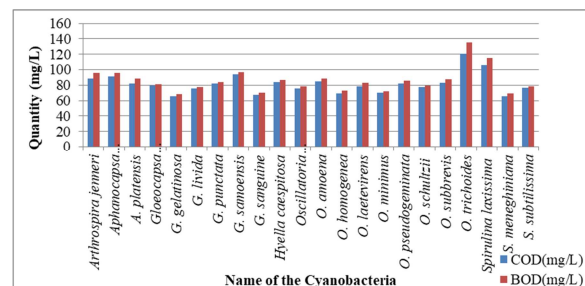

Fig. 3. Screening of COD and BOD from cyanobacteria strains in Kitchen wastewater
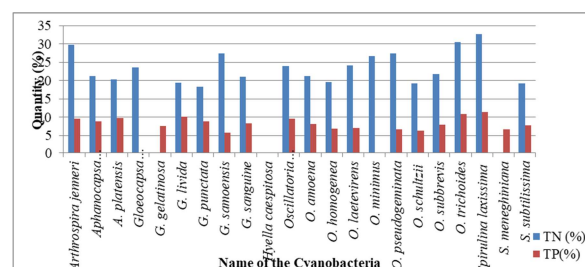

Fig. 4. Screening of TN and TP from cyanobacteria strains in Kitchen wastewater

Fish pond discharge

The effluent for the intensive aquaculture system usually has a high concentration of dissolved nitrogen compounds, generally in the form of ammonia, which are produced by the undigested feed and the wastes (Milhazes-Cunha and Otero, 2017). Cyanobacterial absorption of these nitrogenous compounds could be a sustainable alternative to bacterial nitrification of these compounds to gaseous nitrogen, as the produced biomass could be used as superior feed ingredients (Gao *et al.*, 2016; Tossavainen *et al.*, 2019; Halfhide *et al.*, 2014).

In microalgal-bacterial floc was used in a sequencing batch reactor to treat aquaculture wastewater successfully (Omojevwe and Ezekiel, 2016); the simple separation of microalgal-bacterial floc by gravity sedimentation could reduce the total cost of aquaculture wastewater treatment. In China, investigate the removal of nutrients by the combined use of high-rate algal ponds and macrophyte systems. COD removals as a percentage were 54.5% in "winter" 8 days and 44.5% in "summer" 4 days. Only around 50% of COD was removed on an annual average.

However, the amount of dissolved COD that was removed as a percentage of the influent's overall COD was almost 73%. Although the effluent from the had a low dissolved COD concentration about 60 mg/l, the total COD could be significant because of the algal biomass. The average removal rates for COD and phosphorus were around 50%, 75% and 90%. In particular, this system was effective at removing ammonia from wastewater (Chen *et al.*, 2003). In this study, the removal efficiency of fish pond discharge, COD and BOD mg/L were presented at highest concentration in the cyanobacteria were *Oscillatoria trichoides* 88.3 mg/L and 92.5 mg/L and *Spirulina laxissima* 79.5 mg/L and 86.4 mg/L. Typical nitrogen TN and phosphorus TP highest concentration in the *O. trichoides* were 29.4% and 10.7% and *S. laxissima* 30.7% and 10.9% respectively. The maximum concentration in the COD and BOD mg/L were *Aphanocapsa koordersi*

78.5mg/L and *G. samoensis* 75.3mg/L in COD, *Aphanocapsa koordersi* 81.2mg/L, *G. samoensis* 78.6mg/L and *Hyella caespitose* 72.5mg/L in BOD, *Arthrospira jenneri* 28.5% and *G. gelatinosa* 28.2% in TN and *Arthrospira jenneri* 9.4%, *Aphanocapsa platensis* 9.5%, *G. livida* 9.8% and *O. acuminata* 9.2% in TP. The minimum concentration were noted at the cyanobacteria of *G. gelatinosa* 43.0mg/L, *G. livida* 48.7mg/L, *O. acuminata* 46.1mg/L, *O. homogenea* 39.8mg/L and *S. subtilissima* 36.7mg/L in COD, *G. gelatinosa* 45.7mg/L, *O. acuminata* 48.1mg/L, *O. homogenea* 41.1mg/L, *S. meneghiniana* 41.5mg/L and *S. subtilissima* 38.1mg/L in BOD, *O. schultzii* 18.4% and *S. subtilissima* 18.5% in TN and *G. samoensis* 5.4%, *O. homogenea* 5.6%, *O. schultzii* 5.6% and *O. pseudogeminata* 5.9% in TP respectively. The cyanobacteria of not applicable N/A are recorded with the *G. punctata* and *S. meneghiniana* from TN and *G. crepidium*, *Hyella caespitose* and *O. minimus* from TP. The four removal efficiency COD mg/L, BOD mg/L, TN % and TP % were maximum and minimum are mostly recorded with the cyanobacteria is *G. samoenis, Arhtrospira jenneri, O. homogenea and S. subtlissima,* respectively (Fig. 5 & 6).
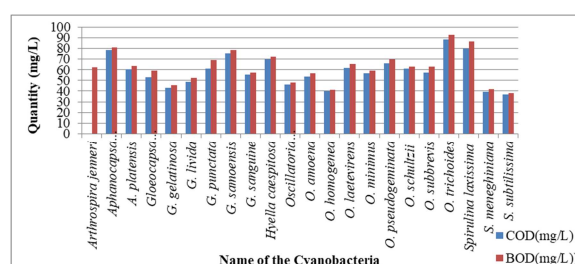

Fig. 5. Screening of COD and BOD from cyanobacteria strains in Fish pond discharge
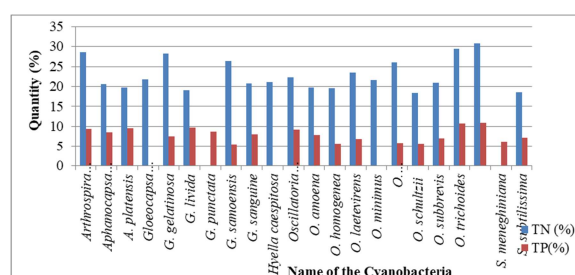

Fig. 6. Screening of TN and TP from cyanobacteria strains in Fish pond discharge

Municipal wastewater

Municipal sewage wastewater MSWW usually has nitrogen and phosphorus values of 21.9–28.8 and 8.2-10.4 mg/L, respectively (Schwinn and Dickson, 1972). For the bacteria to completely consume the nitrogen and phosphorus in the MSWW, the concentration of dissolved organics is typically low (Yu *et al.*, 2019). Consequently, an advanced treatment procedure is used to remove too many nutrients after the activated sludge process ASP N, P. The residual nitrogen and phosphorus from the ASP effluent could be removed by microalgae (Wang *et al.*, 2010; Milhazes-Cunha and Otero, 2017; Gao *et al.*, 2016; Tossavainen *et al.*, 2019; Halfhide *et al.*, 2014; Omojevwe and Ezekiel, 2016; Chen *et al.*, 2003; Schwinn and Dickson, 1972; Yu *et al.*, 2019; Abdel-Raouf *et al.*, 2012; Yang *et al.*, 2020). However, the MSWW may effectively remove nitrogen, phosphorus, BOD, and heavy metals through the production of microalgae. Pathogens from the MSWW may also be removed by microalgae (Chiu *et al.*, 2015). A local municipal wastewater treatment plant's treatment process flow was sampled at four different places, and carried out a study to assess the development of green algae Chlorella sp. and how effectively the algal growth removed metal ions, nitrogen, and phosphorus from the wastewaters (Wang *et al.*, 2010). The primary issue with most wastewaters is the high nutrient concentrations, particularly the TN and TP concentrations, which must be removed during wastewater treatment using expensive chemical-based treatments. In municipal wastewater, agricultural effluent, and farm wastewater, the TN and TP concentrations range from 10 to 60 mg/L, more than 1,000 mg/L, and 500 to 600 mg/L, respectively (Alcántara *et al.*, 2015). In this study, the removal efficiency of fish pond discharge, COD and BOD mg/L were presented at highest concentration in the cyanobacteria were *Oscillatoria trichoides* 77.2 mg/L and 86.0 mg/L and *Spirulina laxissima* 71.0 mg/L and 82.6 mg/L. Typical nitrogen TN and phosphorus TP highest concentration in the *O. trichoides* were 26.7% and 9.6% and *S. laxissima* 25.9% and 9.9% respectively. The maximum concentration of cyanobacteria were *G. punctata*

60.4mg/L, *Hyella caespitose* 53.4mg/L and *G. crepidium* 52.7mg/L in COD, *G. punctata* 65.1mg/L and *G. sanguine* 67.1mg/L in BOD, *Arthrospira jenneri* 28.6%, *Aphanocapsa koordersi* 21.1%, *G. crepidium* 20.6%, *G. gelatinosa* 21.4%and *O. pseudogeminata* 20.8% in TN and *Arthrospira jenneri* 8.7% and *Aphanocapsa platensis* 8.1% in TP were noted. The minimum concentration of cyanobacteria were recorded with *Aphanocapsa koordersi* 21.0mg/L, *G. gelatinosa* 20.8mg/L, *O. amoena* 21.8mg/L, *O. pseudogeminata* 19.8mg/L and *S. subtilissima* 23.8mg/L in COD, *Aphanocapsa koordersi* 26.7mg/L, *G.gelatinosa* 27.4mg/L, *O.amoena* 24.3mg/L, *O. laetevirens* 23.7mg/L, *O. pseudogeminata* 21.6mg/L and *S. subtilissima* 26.4mg/L in BOD, *G. punctata* 15.0%, *O. laetevirens* 12.7%, *O. schultzii* 15.5% and *S. subtilissima* 13.7% in TN and *G. samoensis* 4.9%, *O. homogenea* 4.7%, *O. pseudogeminata* 4.5% and *O. schultzii* 4.9% in TP. The cyanobacteria of not applicable N/A are recorded with the *A. platensis* and *O. schultzii* from COD, *G. livida* from BOD, *S. meneghiniana* from TN and *G. gelatinosa*, *Hyella caespitose* and *O. minimus* from TP were noted. The four removal efficiency COD mg/L, BOD mg/L, TN % and TP % were maximum and minimum are mostly recorded with the cyanobacteria is *Aphanocapsa koordersi, S. subtilissima* and *O. schultzii,* respectively (Fig. 7 & 8).
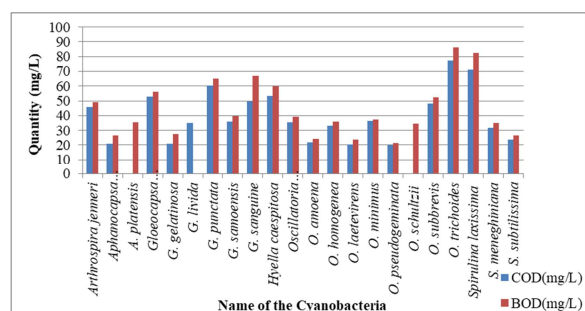

Fig. 7. Screening of COD and BOD from cyanobacteria strains in Municipal wastewater

In the current study, the dairy wastewater, kitchen wastewater, fish pond discharge and municipal wastewater in all parameters COD mg/L, BOD mg/L, TN % and TP % the cyanobacteria were *Arthrospira jenneri* and *Aphanocapsa koordersi* are maximum

and minimum screened were *O. pseudogeminata, O. schultzii* and *O. homogenea.* The mostly screened by the cyanobacteria were *Oscillatoria trichoides* and *Spirulina laxissima* in all parameters. So, the next study is efficiency of removal heavy metals are the tested for selected cyanobacteria.
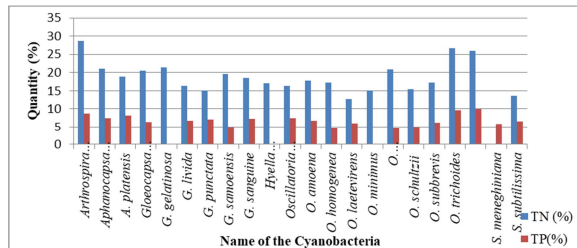

Fig. 8. Screening of TN and TP from cyanobacteria strains in Municipal wastewater

Removal efficiency of heavy metals

Four heavy metals, copper, iron, zinc, and lead, were found to be present in this effluent at high amounts. Because of this, the immobile cultures of *Anabaena variabilis* and *Tolypthrix ceytonica* were chosen to evaluate their ability to remove Cu^{2+}, Fe^{3+}, Zn^{2+}, and Pb^{2+} ions from the contaminated industrial wastewater (El Bestawy, 2019). The appearance of four heavy metals, namely copper, iron, zinc, and lead, was found after an analysis of the four different types of waste water used in this work, including dairy wastewater, kitchen wastewater, fish pond discharge, and municipal wastewater. As a result, *Oscillatoria trichoides* and *Spirulina laxissima* were chosen to test whether they were able to remove the Cu^{2+}, Fe^{3+}, Zn^{2+} and Pb^{2+} ions from the four different wastewaters. RE% is a function of the type of heavy metal, exposure time, and microbial species, according to the results of the residual concentrations and removal efficiencies of the four metals and four different wastewater by the individual or mixed free cultures of potential cyanobacteria *Oscillatoria trichoides* and *Spirulina laxissima* Table 1-4. Regardless of metal type, microbial species, or metal concentration, RE% of all the investigated metals are proportionally increased with exposure time. As a result, the experiment's highest removal % was observed 5h.

Efficiency of heavy metals removal in dairy wastewater

Anabaena variabilis and *Tolypthrix ceytonica,* two species of highly resistant cyanobacteria, were used to effectively treat the raw effluent of the Varta Company, which was contaminated with copper, iron, zinc, and lead. They showed a very rapid 6 h increase in REs % for all the contaminating metals, which can be slowed down by using serial units successively. *T. ceytonica* and the mixed culture removed 88.00%, 79.09%, 92.35%, and 37.11% of the copper, iron, zinc, and lead from the wastewater, respectively, whereas the free *Anabaena variabilis* culture removed 89.33%, 93.44%, 98.23%, and 86.67%. The results indicated that REs % varies on the kind of heavy metal, the length of incubation, and the type of microbial species (Ansari *et al.,* 2016). This was confirmed by, who observed that the competition between metal ions for binding sites on algal surfaces varied depending on the species of algae and the metal ions. The quantity and concentration of heavy metals, the type of biosorbent used, the physiological state of the cells, and the chemical makeup of the wastewater all influence the effectiveness of metal biosorption (Tien, 2002). In this study, the residual concentration levels are reported at 0.16, 12.74, 6.21 and 4.52 mg/L for Cu^{2+}, $Fe^{3+,}$ Zn^{2+} and Pb^{2+} respectively. The highest RE% ranges achieved by the tested *O. trichoides, S. laxissima* and mixed *O. trichoides* and *S. laxissima* after five exposure hours were 85.09%, 75.09% and 74.19% in copper, 88.19%, 86.35% and 82.49% in iron, 99.62%, 94.32% and 95.41% in zinc and 82.52%, 76.12% and 34.86% in lead. The lowest RE% ranges in tested cultures were 21.52%, 52.30% and 33.25% in copper, 65.56%, 71.28% and 65.47% in iron, 82.33%, 78.25% and 84.26% in zinc, 6.59%, 5.27% and 6.23% in lead. The significance of variance at confidence level of cyanobacteria cultures presented were 0.003, 0.013 and 0.023 in copper, 0.001, 0.002 and 0.002 in iron, 0.001, 0.001 and 0.028 in zinc and 0.001, 0.003 and 0.002 in lead are noted.

Table 1. Using separate and combined free-living cultures of at various exposure times, measurements of certain heavy metals in dairy wastewater

Heavy metal	Time h	*Oscillatoria trichoides*		*Spirulina laxissima*		*Oscillatoria trichoides* and *Spirulina laxissima*		p
		RC	RE%	RC	RE%	RC	RE%	
Cu	0	0.162±0.002	-	-	-	-	-	-
	1	0.125±0.006	21.52[l]	0.084±0.001	52.30[l]	0.088±0.003	33.25[l]	0.023
	2	0.105±0.008	27.31	0.061±0.003	63.07	0.055±0.001	66.74	0.013
	3	0.044±0.001	75.06	0.052±0.005	67.48	0.052±0.003	69.15	>0.05
	4	0.036±0.003	77.11	0.042±0.001	71.44	0.047±0.008	71.05	>0.05
	5	0.017±0.004	85.09[h]	0.036±0.002	75.09[h]	0.013±0.006	74.19[h]	>0.05
r, p		0.91, 0.003*	-	0.77, 0.013*	-	0.69, 0.023*	-	-
Fe	0	12.74±0.542	-	-	-	-	-	-
	1	4.231±0.112	65.56[l]	4.192±0.122	71.28[l]	4.655±0.152	65.47[l]	>0.05
	2	4.220±0.105	65.91	4.025±0.134	75.23	4.405±0.143	68.23	>0.05
	3	3.564±0.137	73.26	3.265±0.108	76.10	4.223±0.141	72.19	>0.05
	4	3.025±0.107	78.15	2.843±0.086	78.06	4.008±0.132	78.14	>0.05
	5	2.184±0.068	88.19[h]	1.653±0.051	86.35[h]	3.114±0.103	82.49[h]	0.016
r, p		0.86, 0.001*	-	0.89, 0.002*	-	0.72, 0.002*	-	-
Zn	0	6.215±0.021	-	-	-	-	-	-
	1	0.520±0.011	82.33[l]	1.055±0.012	78.25[l]	0.685±0.021	84.26[l]	>0.05
	2	0.261±0.007	88.52	0.744±0.035	85.12	0.610±0.005	88.74	>0.05
	3	0.227±0.004	96.21	0.557±0.018	88.23	0.445±0.008	91.52	>0.05
	4	0.116±0.002	97.28	0.487±0.016	91.18	0.421±0.003	92.57	>0.05
	5	0.091±0.007	99.62[h]	0.361±0.011	94.32[h]	0.320±0.016	95.41[h]	>0.05
r, p		0.93, 0.001*	-	0.85, 0.001*	-	0.63, 0.028*	-	-
Pb	0	4.523±0.004	-	-	-	-	-	-
	1	4.571±0.003	6.59	4.224±0.152	5.27	4.450±0.153	6.23	0.041
	2	4.380±0.005	21.42	3.274±0.108	29.21	4.328±0.147	4.56	0.023
	3	3.513±0.008	57.10	2.744±0.096	41.74	4.105±0.132	8.75	0.001
	4	2.062±0.062	66.09	2.220±0.036	52.08	4.045±0.125	10.47	0.001
	5	1.563±0.050	82.52	1.056±0.012	76.12	2.847±0.110	34.86	0.012
r, p		0.83, 0.001*	-	0.74, 0.003*	-	0.62, 0.002*	-	-

[l] The lowest recorded RE%, [h] The highest recorded RE%, * Level of Significant variance at 0.05 and 0.01 2-tailed

The maximum ranges are analysed by the heavy metal is zinc and very lowest ranges are presented at lead metal. The results indicated that free individual and mixed cultures of the two selected species were able to remove the tested metals at a very high rate and with very high RE% in the following order is $Zn^{2+} > Fe^{3+} > Cu^{2+} > Pb^{2+}$. The tested cultures also showed high selectivity Table 1.

Efficiency of heavy metals removal in kitchen wastewater
The ranges of REs% obtained for Cu2+ by *A. variabilis* and *T. ceytonica*, and the mixed culture of *A. variabilis* and *T. ceytonica* are 20.67-89.33, 51.33-85.33, and 34.67-88.00, respectively. These ranges showed *A. variabilis* superior ability to remove Cu2+ compared to the other two cultures, followed by the mixed culture and *T. ceytonica.* The three cultures respective Fe3+ REs % ranges were 68.59–93.44, 70.02–97.22, and 66.89–79.09, with *T. ceytonica*

achieving the highest ranges, followed by *A. variabilis*, and then the mixed culture. The three cultures showed REs % for Zn^{2+} of 86.47–98.23, 79.21–93.33, and 85.88–92.35, respectively, with *A. variabilis* removing more Zn^{2+} than the other two cultures, followed by the mixed culture, especially at exposures up to 5 h, then *T. Ceytonica* (El Bestawy, 2019). In this study, Cu^{2+}, Fe^{3+}, Zn^{2+} and Pb^{2+}, respectively, the residual concentration levels are reported to be 0.16, 12.74, 6.21, and 4.52 mg/L. The tested *O. trichoides, S. laxissima* and mixed *O. trichoides* and *S. laxissima* had the highest RE% ranges in copper 92.17%, 80.19%, and 85.05%, iron 90.28%, 91.52% and 86.13%, zinc 99.88%, 97.13% and 98.08%, and lead 87.44%, 78.06% and 39.48% after five exposure hours. The tested cultures lowest RE% ranges were mostly for copper 42.13%, 59.28% and 48.11%, iron 67.23%, 75.15% and 68.09%, zinc 85.21%, 86.32% and 86.51%, and lead 8.19%, 8.56% and 8.44%. There were 0.004, 0.014 and 0.034 in

copper, 0.001, 0.003and 0.001 in iron, 0.003, 0.002 and 0.004 in zinc, and 0.002, 0.002 and 0.001 for lead in the cyanobacteria cultures that were significant levels are presented. Zinc has the maximum ranges when analysed as a heavy metal, and lead metal has the lowest very highest ranges.

The results showed that free individual and mixed cultures of the two selected species were able to remove the tested metals in the following order: Zn^{2+} > Cu^{2+} > Fe^{3+} > Pb^{2+} at a very high rate and with very high RE%. The tested cultures showed a high level of selectivity Table 2.

Table 2. Using separate and combined free-living cultures at various exposure times, measurements of certain heavy metals in kitchen wastewater

Heavy metal	Time h	*Oscillatoria trichoides*		*Spirulina laxissima*		*Oscillatoria trichoides* and *Spirulina laxissima*		p
		RC	RE%	RC	RE%	RC	RE%	
Cu	0	0.196±0.003	-	-	-	-	-	-
	1	0.142±0.004	42.13	0.141±0.002	59.28	0.159±0.002	48.11	0.012
	2	0.129±0.005	45.08	0.120±0.001	64.10	0.137±0.003	68.74	>0.05
	3	0.074±0.002	88.16	0.096±0.005	69.08	0.115±0.001	74.28	>0.05
	4	0.045±0.001	89.05	0.088±0.002	74.66	0.093±0.004	77.23	0.024
	5	0.021±0.003	92.17	0.053±0.001	80.19	0.044±0.002	85.05	>0.05
r, p		0.96, 0.004	-	0.79, 0.014	-	0.71, 0.034	-	-
Fe	0	13.67±0.416	-	-	-	-	-	-
	1	4.458±0.124	67.23	4.316±0.158	75.15	4.812±0.168	68.09	>0.05
	2	4.423±0.108	68.52	4.302±0.145	79.00	4.745±0.127	70.28	>0.05
	3	4.216±0.091	74.11	3.864±0.123	83.15	4.675±0.103	75.62	0.011
	4	4.116±0.074	81.04	3.571±0.101	87.64	4.319±0.081	80.07	>0.05
	5	4.005±0.052	90.28	3.170±0.053	91.52	4.211±0.052	86.13	0.012
r, p		0.92, 0.001	-	0.90, 0.003	-	0.77, 0.001	-	-
Zn	0	6.758±0.062	-	-	-	-	-	-
	1	0.729±0.032	85.21	1.418±0.016	86.32	0.845±0.023	86.51	>0.05
	2	0.674±0.015	89.12	1.257±0.074	88.16	0.816±0.014	89.01	>0.05
	3	0.530±0.009	97.05	1.108±0.019	89.29	0.770±0.010	93.64	>0.05
	4	0.456±0.006	99.46	1.089±0.024	92.46	0.618±0.008	96.33	0.015
	5	0.320±0.002	99.88	1.032±0.010	97.13	0.553±0002	98.08	0.026
r, p		0.97, 0.003	-	0.89, 0.002	-	0.71, 0.004	-	-
Pb	0	4.805±0.002	-	-	-	-	-	-
	1	4.612±0.001	8.19	4.513±0.127	8.56	4.716±0.129	8.44	0.023
	2	4.571±0.002	25.09	4.356±0.115	34.18	4.673±0.106	5.21	0.016
	3	3.720±0.003	59.06	4.328±0.102	46.29	4.611±0.052	8.82	0.003
	4	2.642±0.001	78.11	4.119±0.084	54.71	4.570±0.036	11.12	0.001
	5	1.925±0.003	87.44	3.817±0.017	78.06	4.336±0.012	39.48	0.001
r, p		0.85, 0.002	-	0.76, 0.002	-	0.66, 0.001	-	-

[l] The lowest recorded RE%, [h] The highest recorded RE%, * Level of Significant variance at 0.05 and 0.01 2-tailed

Efficiency of heavy metals removal in fish pond discharge

Treatment methods for heavy metal pollution in wastewater includes chemical, physical, and biological processes. Biosorption, bioremoval, bio-separation, and sometimes phytoremediation are terms used to describe a promising method that employs living aquatic plants to sorb metals from water as an alternative to physiological and chemical methods (Clesceri *et al.*, 1999). When it comes to waste water, the electroplating, electronics, and metal cleaning sectors commonly heavy metals and causes significant issues with water pollution. It has been

demonstrated that microalgae are very efficient at removing as a result of their large surface area and strong binding affinity, heavy metals from wastewater. In addition, microalgae have a negative surface charge and a strong affinity for heavy metal ions, they are especially useful for the analysis of effective at purifying wastewater (Grandova *et al.*, 2001). In this study, the residual concentration levels are reported at 0.17, 13.08, 6.51 and 4.58 mg/L for Cu^{2+}, Fe^{3+}, Zn^{2+} and Pb^{2+} respectively. The highest RE% ranges achieved by the tested *O. trichoides, S. laxissima* and mixed *O. trichoides* and *S. laxissima* after five exposure hours were 88.09%, 76.20% and

80.52% in copper, 90.18%, 88.13% and 83.45% in iron, 99.84%, 96.22% and 96.05% in zinc and 85.62%, 77.19% and 36.17% in lead. The lowest RE% ranges in tested cultures were 30.02%, 55.27% and 37.41% in copper, 67.02%, 72.35% and 67.18% in iron, 84.26%, 80.25% and 84.87% in zinc, 7.25%, 7.54% and 7.41% in lead. The significance of variance at confidence level of cyanobacteria cultures presented were 0.003, 0.001and 0.003 in copper, 0.001, 0.003 and 0.004 in iron, 0.002, 0.001 and

0.002 in zinc and 0.003, 0.002 and 0.001 in lead are recorded. The maximum ranges are analysed by the heavy metal is zinc and very lowest ranges are presented at iron metal. The results indicated that free individual and mixed cultures of the two selected species were able to remove the tested metals at a very high rate and with very high RE% in the following order is $Zn^{2+} > Fe^{3+} > Cu^{2+} > Pb^{2+}$. The tested cultures also showed high selectivity Table 3.

Table 3. Using separate and combined free-living cultures at various exposure times, measurements of certain heavy metals in fish pond discharge

Heavy metal	Time h	*Oscillatoria trichoides*		*Spirulina laxissima*		*Oscillatoria trichoides* and *Spirulina laxissima*		p
		RC	RE%	RC	RE%	RC	RE%	
Cu	0	0.174±0.001	-	-	-	-	-	-
	1	0.133±0.002	30.02	0.125±0.003	55.27	0.124±0.005	37.41	0.015
	2	0.129±0.001	35.28	0.113±0.001	63.58	0.110±0.001	66.85	>0.05
	3	0.117±0.003	77.14	0.086±0.004	68.17	0.085±0.002	70.15	>0.05
	4	0.096±0.002	80.18	0.075±0.001	73.39	0.066±0.003	75.22	0.005
	5	0.068±0.001	88.09	0.066±0.003	76.20	0.042±0.001	80.52	0.013
r, p		0.92, 0.003	-	0.78, 0.001	-	0.70,0.003	-	-
Fe	0	13.08±4.69	-	-	-	-	-	-
	1	4.338±0.120	67.02	4.251±0.005	72.35	4.715±0.152	67.18	>0.05
	2	4.328±0.112	67.85	4.156±0.003	77.18	4.683±0.140	69.08	>0.05
	3	4.316±0.087	74.26	4.128±0.005	81.09	4.669±0.126	73.11	0.013
	4	4.285±0.065	79.07	4.113±0.007	85.61	4.612±0.108	78.66	0.017
	5	4.215±0.047	90.18	4.095±0.001	88.13	4.502±0.085	83.45	0.026
r, p		0.88, 0.001	-	0.90,0.003	-	0.75,0.004	-	-
Zn	0	6.512±0.033	-	-	-	-	-	-
	1	0.590±0.026	84.26	1.259±0.027	80.25	0.735±0.038	84.87	>0.05
	2	0.442±0.017	89.51	1.247±0.019	86.48	0.645±0.025	88.79	>0.05
	3	0.413±0.009	96.58	1.236±0.011	88.74	0.623±0.014	91.76	>0.05
	4	0.356±0.005	97.89	1.205±0.009	91.82	0.527±0.008	94.13	0.025
	5	0.225±0.001	99.84	1.196±0.004	96.22	0.475±0.002	96.05	0.011
r, p		0.94, 0.002	-	0.87, 0.001	-	0.65, 0.002	-	-
Pb	0	4.584±0.002	-	-	-	-	-	-
	1	4.425±0.001	7.25	4.328±0.132	7.54	4.628±0.143	7.41	0.014
	2	4.381±0.005	26.18	4.310±0.116	31.25	4.607±0.125	4.68	0.016
	3	4.319±0.001	57.64	4.251±0.108	44.27	4.526±0.103	8.80	0.005
	4	4.271±0.004	75.18	4.186±0.047	53.28	4.476±0.086	10.85	0.001
	5	3.841±0.003	85.62	4.058±0.018	77.19	4.265±0.016	36.17	0.002
r, p		0.88, 0.003	-	0.75, 0.002	-	0.65, 0.001	-	-

[l]The lowest recorded RE%, [h] The highest recorded RE%, * Level of Significant variance at 0.05 and 0.01 2-tailed

Efficiency of heavy metals removal in municipal wastewater
Because different cyanobacterial species in mixed cultures fight with one another for resources, individual cultures are almost as effective at removing heavy metals as mixed cultures are. However, it is crucial to use the right immobilisation approach when applying microbial biomass to sorb metal ions during a continuous industrial process. Immobilized

biomass has a number of benefits, including higher biomass loading, less clogging in continuous flow systems, and increased reusability (El-Sheekh *et al.*, 2005; Das and Adholeya, 2015). For the goal of algal tertiary wastewater treatment, immobilisation appears to be one of the finest methods for physically separating micro-algal cells from their growing medium. Additionally, nitrogen removal from wastewater using immobilisation on screens was

higher than with traditional biological tertiary wastewater treatments (Ahmad *et al.*, 2017; Ahmad *et al.*, 2018a; Ahmad *et al.*, 2018b; Martins *et al.*, 2013). In this study, Cu^{2+}, Fe^{3+}, Zn^{2+}, and Pb^{2+}, respectively, the residual concentration levels are reported to be 0.15, 11.59, 6.01 and 4.02 mg/L. The tested *O. trichoides, S. laxissima* and mixed *O. trichoides* and *S. laxissima* had the highest RE% ranges in copper 82.01%, 71.45%, and 72.48%, iron 86.32%, 82.17%, and 80.56%, zinc 97.23%, 91.44%, and 92.56%, and lead 97.23%, 91.44%, and 92.56% after five exposure hours. The tested cultures lowest RE% ranges were mostly for copper 20.18%, 50.26% and 30.11, iron 63.10%, 65.18% and 64.29%, zinc 80.23%, 76.29%,

and 81.06%, and lead 5.14%, 4.25%, and 5.16%. There were 0.004, 0.012, and 0.016 in copper, 0.002, 0.001and 0.001 in iron, 0.002, 0.004 and 0.025 in zinc, and 0.002, 0.002 and 0.003 for lead in the cyanobacteria cultures that were significant levels are presented. Zinc has the maximum ranges when analysed as a heavy metal, and lead metal has the lowest very highest ranges. The results showed that free individual and mixed cultures of the two selected species were able to remove the tested metals in the following order: $Zn^{2+} > Fe^{3+} > Cu^{2+} > Pb^{2+}$ at a very high rate and with very high RE%. The tested cultures showed a high level of selectivity Table 4.

Table 4. Using separate and combined free-living cultures at various exposure times, measurements of certain heavy metals in municipal wastewater

Heavy metal	Time h	*Oscillatoria trichoides*		*Spirulina laxissima*		*Oscillatoria trichoides* and *Spirulina laxissima*		p
		RC	RE%	RC	RE%	RC	RE%	
Cu	0	0.158±0.003	-	-	-	-	-	-
	1	0.101±0.001	20.18	0.079±0.002	50.26	0.074±0.002	30.11	0.015
	2	0.098±0.006	22.56	0.066±0.003	61.74	0.062±0.006	63.51	0.018
	3	0.040±0.005	74.28	0.053±0.004	65.28	0.058±0.002	65.47	>0.05
	4	0.021±0.008	75.11	0.029±0.003	69.18	0.041±0.005	69.23	>0.05
	5	0.009±0.001	82.01	0.011±0.001	71.45	0.021±0.005	72.48	>0.05
r, p		0.87, 0.004	-	0.84, 0.012	-	0.65, 0.016	-	-
Fe	0	11.59±0.427	-	-	-	-	-	-
	1	4.115±0.106	63.10	4.058±0.115	65.18	4.118±0.164	64.29	>0.05
	2	4.108±0.096	63.59	4.021±0.105	73.59	4.012±0.152	65.18	>0.05
	3	4.086±0.074	70.55	3.517±0.074	74.11	3.816±0.122	70.52	>0.05
	4	3.841±0.015	75.29	2.116±0.041	77.08	3.618±0.108	75.19	>0.05
	5	3.547±0.017	86.32	1.528±0.016	82.17	3.418±0.074	80.56	>0.05
r, p		0.85, 0.002	-	0.86, 0.001	-	0.70, 0.001	-	-
Zn	0	6.012±0.054	-	-	-	-	-	-
	1	0.408±0.015	80.23	0.984±0.016	76.29	0.574±0.023	81.06	>0.05
	2	0.378±0.014	84.79	0.819±0.024	83.11	0.489±0.015	85.18	0.014
	3	0.321±0.009	92.16	0.715±0.019	87.19	0.411±0.026	89.14	0.009
	4	0.306±0.005	95.11	0.529±0.025	89.46	0.376±0.015	90.74	>0.05
	5	0.285±0.001	97.23	0.331±0.014	91.44	0.310±0.018	92.56	>0.05
r, p		0.90, 0.002	-	0.83, 0.004	-	0.61, 0.025	-	-
Pb	0	4.028±0.002	-	-	-	-	-	-
	1	4.117±0.009	5.14	3.857±0.129	4.25	4.117±0.124	5.16	0.016
	2	4.103±0.008	20.58	3.518±0.116	22.87	4.057±0.110	3.95	0.024
	3	3.814±0.006	55.13	3.214±0.086	36.54	3.829±0.086	7.48	0.003
	4	3.410±0.006	62.07	3.159±0.024	50.66	3.538±0.019	9.74	0.012
	5	3.226±0.001	80.15	3.085±0.013	71.48	3.110±0.026	30.12	0.005
r, p		0.80, 0.002	-	0.71, 0.002	-	0.60, 0.003	-	-

Also demonstrated that the immobilised *T. ceytonica* was more effective in removing lead from wastewater than *A. variabilis*, and that the bioremoval of lead by the mixed culture was higher than in the individual cultures. It was noted that using sewage effluent that contained heavy metals such Fe^{3+}, Zn^{2+}, Cu^{2+} and

Pb^{2+}encouraged the growth of algae and cyanobacteria as early as 1982 (Wan Maznah and Al-Fawwaz, 2012). Because of their easily accessible low cost, relatively large surface area, and strong binding affinity, algae have been discovered to be potential appropriate biosorbents. In this current study, the

cyanobacteria *O. trichoides* and *S. laxissima* and mixed *O. trichoides* and *S. laxissima* from kitchen wastewater and fish pond discharge are maximum presented in removal efficiency RE% of heavy metals and in the same order for $Zn^{2+} > Fe^{3+} > Cu^{2+} > Pb^{2+}$ when compared with dairy wastewater and municipal wastewater. In four different wastewaters the highest removal efficiency for zinc heavy metal in cyanobacteria *O. trichoides* and *S. laxissima* and mixed *O. trichoides* and *S. laxissima*. The level of significance variance at 0.05 and 0.01 2-tailed are calculated and significant for all heavy metals zinc, copper, iron and lead in four different wastewaters, respectively.

Conclusion

In this study, to screen for four different waste water treatments and the efficiency of heavy metal removal from cyanobacteria. It is informed that before discharging the treated wastewater in the stream, it is necessary to remove cyanobacteria from the treated effluent to conform with general standards for wastewater discharge. This is based on the various studies conducted to treat the wastewater using microalgae show that the cyanobacteria reactor has a significant reduction in nutrients, BOD and COD and other toxic chemicals but increase in total solids due to the growth of cyanobacteria. The efficiency of the cyanobacteria-based wastewater treatment system in removing nutrients is very high. The method is 5-32% effective at removing nitrogen and phosphorus. Additionally, the treatment method is successful in removing 15-135% of COD, BOD and other pollutants from wastewater. The reduction of various wastewater parameters BOD, COD, TN and TP by microalgae indicates that the use of microalgae for industrial wastewater treatment may be an alternative to more involved chemical treatments. After treatment, the cyanobacteria biomass can be utilised to produce biofuels, fertilizer, hydrogen gas, and pharmaceuticals. This method allows for the environmentally and economically favourable treatment of large volumes of wastewater. It is clear that the selected cyanobacterial species, either free-living or fixed, singular or in mixtures, have excellent

bioremoval capacities toward metal contaminants found in water or wastewater, even at extremely high concentrations, with some exhibiting selection preferences over others. This benefit could be effectively employed to decontaminate both natural aquatic ecosystems and wastewater effluents. Additionally, it offers a cost-effective and excellent instrument for the recovery and reuse of treated, suitable wastewater for any purpose, such as irrigation of agricultural non-edible crops. It also provides protection for the received environments. The application of cyanobacteria executes a variety of tasks in the assembly of surplus food, the treatment of wastewater, and the production of useful biomass, all of which have a wide range of applications. In addition to this, as they are photosynthetic autotrophs, they enrich and improve the quality of the water. As a result, wastewater treatment for cyanobacteria cultures may be useful and as an advanced environmental, friendly treatment method. This combined biotechnology plan for wastewater treatment and nutrient recovery will be improved by additional experiments.

References

Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM. 2012. Microalgae and wastewater treatment. Saudi J Biol Sci **19**, 257–275.

Ahmad A, Bhat AH, Buang A, Shah SMU, Afzal M. 2019. Biotechnological application of microalgae for integrated palm oil mill effluent (POME) remediation: A review. Int J Environ Sci Technol **16**, 1763–1788.

Ahmad A, Bhat AH, Buang A. 2017. Immobilized *Chlorella vulgaris* for efficient palm oil mill effluent treatment and heavy metals removal. Desal Wat Treat **81**, 105–117.

Ahmad A, Bhat AH, Buang A. 2018. Biosorption of transition metals by freely suspended and Ca-alginate immobilized *Chlorella vulgaris*: kinetic and equilibrium modeling. J Cleaner Prod **171**, 1361–1375.

Ahmad A, Bhat AH, Buang A. 2018. Enhanced biosorption of transition metals by living *Chlorella vulgaris* immobilized in Ca-alginate beads. J Environ Technol **1**, 1–17.

Ahmad SF, Mofijur M, Parisa TA, Islam N, Kusumo F, Inayat A. 2022. Progress and challenges of contaminant removal from wastewater using microalgae biomass. Chemosphere **286**, 131656.

Alcántara C, Posadas E, Guieysse B, Muñoz R. 2015. Microalgae-based wastewater treatment. In: Handbook of Marine Microalgae: Biotechnology Advances. Kim SK (ed.). Academic Press: Amsterdam, The Netherlands, 439–455.

Ansari AA, Gill SSGR, Newman GRLL. 2016. Phytoremediation management of environmental contaminants. Springer International Publishing, Switzerland, **3**, 29–208.

APHA. 2012. Standard methods for the examination of water and wastewater. 22nd ed. Washington D.C.: American Public Health Association.

Aslan S, Kapdan IK. 2006. Batch kinetic of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecol Eng **28**, 64–70.

Bavatharny T, Gannoru KSHN, Malith P, Wanni AJW, Nimarshana PHV, Malik A, Thilini UA. 2023. Cyanobacterial pigment production in wastewaters treated for heavy metal removal: Current status and perspectives. J Environ Chem Eng **11**, 108999.
https://doi.org/10.1016/j.jece.2022.108999

Becker EW. 1994. Microalgae, Biotechnology and Microbiology. Cambridge: Cambridge University Press.

Capodaglio AG. 2020. Fit-for-purpose urban wastewater reuse: Analysis of issues and available technologies for sustainable multiple barrier approaches. Crit Rev Environ Sci Technol, 1–48.

Chen P, Zhou Q, Paing J, Le H, Picot B. 2003. Nutrient removal by the integrated use of high rate algal ponds and macrophyte systems in China. Water Sci Technol **48(2)**, 251–257.

Chiu SY, Kao CY, Chen TY, Chang YB, Kuo CM, Lin CS. 2015. Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource. Bioresour Technol **184**, 179–189.

Choudhary M, Jetley UK, Khan MA, Zutshi S, Fatma T. 2007. Effect of heavy metal stress on proline, malondialdehyde, and superoxide dismutase activity in the cyanobacterium *Spirulina platensis*-S5. Ecotoxicol Environ Saf **66**, 204–209.

Clesceri L, Greenberg A, Eaton A. 1999. Standard methods for the examination of water and wastewater. 20th ed. APHA, AWWA, WEF, Washington, D.C.

Das M, Adholeya A. 2015. Potential uses of immobilized bacteria, fungi, algae, and their aggregates for treatment of organic and inorganic pollutants in wastewater. In: Water Challenges and Solutions on a Global Scale, ACS Symposium Series **1206**, Chapter 15, 319–337.

Das P, AbdulQuadir M, Thaher MI, Alghasal GSHS, Aljabri HMSJ. 2018. Microalgal nutrients recycling from the primary effluent of municipal wastewater and use of the produced biomass as bio-fertilizer. Int J Environ Sci Technol **16**, 3355–3364.

Devi MP, Subhash GV, Mohan SV. 2012. Heterotrophic cultivation of mixed microalgae for lipid accumulation and wastewater treatment during sequential growth and starvation phases: Effect of nutrient supplementation. Renew Energy **43**, 276–283.

Ding JF, Zhao FM, Cao YF. 2014. Cultivation of microalgae in dairy wastewater without sterilization. Int J Phytoremediation **17**, 222–227.

El Bestawy E. 2019. Efficiency of immobilized cyanobacteria in heavy metals removal from industrial effluents. Desalination and Water Treatment **159**, 66–78.

El-Sheekh MM, El-Shouny WA, Osman MEH, El-Gammal EWE. 2005. Growth and heavy metals removal efficiency of *Nostoc muscorum* and *Anabaena subcylindrica* in sewage and industrial wastewater effluents. Environ Toxicol Pharmacol **19**, 357–365.

Gao F, Li C, Yang ZH, Zeng GM, Feng LJ, Liu JZ, Liu M, Cai HW. 2016. Continuous microalgae cultivation in aquaculture wastewater by a membrane photobioreactor for biomass production and nutrients removal. Ecol Eng **92**, 55–61.

Goncalves AL, Pires JCM, Simoes M. 2017. A review on the use of microalgal consortia for wastewater treatment. Algal Res **24**, 403–415.

Grandova VI, Gromdev SN, Doycheva AS. 2001. Bioremediation of waters contaminated with crude oil and toxic heavy metals. Int J Miner Process **62**, 293–299.

Gupta PL, Lee SM, Choi HJ. 2015. A mini review: Photobioreactor for large scale algal cultivation. World J Microbiol Biotechnol **31**, 1409–1417.

Halfhide T, Åkerstrøm A, Lekang OI, Gislerød HR, Ergas SJ. 2014. Production of algal biomass, chlorophyll, starch and lipids using aquaculture wastewater under axenic and non-axenic conditions. Algal Res **6**, 152–159.

Hena S, Fatimah S, Tabassum S. 2015. Cultivation of algae consortium in a dairy farm wastewater for biodiesel production. Water Resour Industry **10**, 1–14.

Hoffman JP. 1998. Wastewater treatment with suspended and non-suspended algae. J Phycol **34**, 757–763.

Huo SH, Wang ZM, Zhu SN, Zhou WZ, Dong RJ, Yuan ZH. 2012. Cultivation of *Chlorella zofingiensis* in bench-scale outdoor ponds by regulation of pH using dairy wastewater in winter, South China. Bioresour Technol **121**, 76–82.

Ji X, Jiang M, Zhang J. 2018. The interactions of algae-bacteria symbiotic system and its effects on nutrients removal from synthetic wastewater. Bioresour Technol **247**, 44–50.

Kanchana S, Jeyanthi J, Kathiravan R, Suganya K. 2014. Biosorption of heavy metals using algae: a review. Int J Pharm Med Biol Sci **3**, 9.

Kawaguchi K. 1980. Microalgae production systems in Asia. In: Shelef G, Soeder CJ, editors. *Algae Biomass*. Elsevier: Amsterdam, 25–33.

Keskinkana O, Goksub MZL, Yuceera A, Basibuyuka M. 2003. Heavy metal adsorption characteristics of a submerged aquatic plant *Myriophyllum spicatum*. Process Biochem **39**, 179–183.

Kothari R, Pathak VV, Kumar V, Kumar V, Singh DP. 2012. Experimental study for growth potential of unicellular alga *Chlorella pyrenoidosa* on dairy wastewater; an integrated approach for treatment and biofuel production. Bioresour Technol **116**, 466–470.

Kothari R, Prasad R, Kumar V, Singh DP. 2013. Production of biodiesel from microalgae *Chlamydomonas polypyrenoideum* grown on dairy industry wastewater. Bioresour Technol **144**, 499–503.

Kulshreshtha A, Agrawal R, Barar M, Saxena S. 2014. Review on bioremediation of heavy metals in contaminated water. IOSR J Environ Sci Toxicol Food Technol **8**, 44–50.

Kumar S, Gupta N, Pakshirajan K. 2015. Simultaneous lipid production and dairy wastewater treatment using *Rhodococcus opacus* in a batch bioreactor for potential biodiesel application. J Environ Chem Eng **3**, 1630–1636.

Kuritz T, Wolk CP. 1995. Use of cyanobacterial for biodegradation of aromatic pollutants. Appl Environ Microbiol **61**, 238–243.

Larsdotter K. 2006. Wastewater treatment with microalgae – A literature review. VATTEN **62**, 31–38.

Lebkuecher JG, Tuttle EN, Johnson JL, Willis NKS. 2015. Use of algae to assess the trophic state of a stream in Middle Tennessee. J Freshwater Ecol **30**, 349–376.

Liu J, Wu Y, Wu C, Muylaert K, Vyverman W, Yu HQ, Munoz R, Rittmann B. 2017. Advanced nutrient removal from surface water by a consortium of attached microalgae and bacteria: A review. Bioresour Technol **241**, 1127–1137.

Lu W, Wang Z, Wang X, Yuan Z. 2015. Cultivation of *Chlorella* sp. using raw dairy wastewater for nutrient removal and biodiesel production: Characteristics comparison of indoor bench-scale and outdoor pilot-scale cultures. Bioresour Technol **192**, 382–388.

Martins CM, Fiúza LMCG, Sandra ST, Santaella T. 2013. Immobilization of microbial cells: a promising tool for treatment of toxic pollutants in industrial wastewater. Afr J Biotechnol **12**, 4412–4418.

Menger-Krug E, Niederste-Hollenberg J, Hillenbrand T. 2012. Integration of microalgae systems at municipal wastewater treatment plants: Implications for energy and emission balances. Environ Sci Technol **46**, 11505–11514.

Miao X, Hao Y, Liu H, Xie Z, Miao D, He X. 2021. Effects of heavy metals speciations in sediments on their bioaccumulation in wild fish in rivers in Liuzhou—a typical karst catchment in Southwest China. Ecotoxicol Environ Saf **214**, 112099.

Milhazes-Cunha H, Otero A. 2017. Valorisation of aquaculture effluents with microalgae: The Integrated Multi-Trophic Aquaculture concept. Algal Res **24**, 416–424.

Mohd Udaiyappan A, Abu Hasan AF, Takriff MS, Sheikh Abdullah SR. 2017. A review of the potentials, challenges and current status of microalgae biomass applications in industrial wastewater treatment. J Water Process Eng **20**, 8–21.

Omojevwe GO, Ezekiel FO. 2016. Microalgal-bacterial consortium in polyaromatic hydrocarbon degradation of petroleum based effluent. J Bioremed Biodegrad **7**, 359.

Papadopoulos KP, Economou CN, Dailianis S, Charalampous N, Stefanidou N, Moustaka-Gouni M, Tekerlekopoulou AG, Vayenas DV. 2020. Brewery wastewater treatment using cyanobacterial-bacterial settleable aggregates. Algal Res **49**, 101957.

Papadopoulos KP, Economou CN, Tekerlekopoulou AG, Vayenas DV. 2020. Two-step treatment of brewery wastewater using electrocoagulation and cyanobacteria-based cultivation. J Environ Manag **265**, 110543.

Pena-Castro JM, Martinez-Jeronimo F, Sparza-Garcia FE, Canizares-Villanveva KO. 2004. Heavy metals removal by the microalgae *Scenedesmus incrassatulus* in continuous cultures. Bioresour Technol **94**, 219–222.

Ram L, Pravin U, Singare D, Pimple S. 2011. Toxicity study of heavy metals pollutants in wastewater effluent samples collected from Taloja industrial estate of Mumbai, India. Resour Environ **11**, 13–19.

Rana AA. 2010. A study on the effect of temperature on the treatment of industrial wastewater using *Chlorella vulgaris* alga. J Eng Technol **28**, 785–791.

Raven JA. 1988. Limits to growth. In: Micro-algal Biotechnology. Cambridge University Press: Cambridge, 331–356.

Renuka N, Sood A, Prasanna R. 2015. Phycoremediation of wastewaters: A synergistic approach using microalgae for bioremediation and biomass generation. Int J Environ Sci Technol **12**, 1443–1460.

Sabbir H, Tatsufumi O. 2024. Cyanoremediation of heavy metals (As(V), Cd(II), Cr(VI), Pb(II)) by live cyanobacteria (*Anabaena variabilis*, and *Synechocystis* sp.): an eco-sustainable technology. RSC Adv **14**, 10452–10463. https://doi.org/10.1039/D4RA00409D

Sallal AKJ. 1982. Growth of algae and cyanobacteria on sewage effluents. Microb Lett **20**, 7–13.

Satpal, Khambete AK. 2016. Waste water treatment using micro-algae – A review paper. International Journal of Engineering Technology, Management and Applied Sciences **42**, 2349–4476.

Schwinn DE, Dickson BH. 1972. Nitrogen and phosphorus variations in domestic wastewater. J Water Pollut Control Fed **44**, 2059–2065.

Shahrokhi-Shahraki R, Benally C, Gamal El-Din M, Park J. 2021. High efficiency removal of heavy metals using tire-derived activated carbon vs commercial activated carbon: Insights into the adsorption mechanisms. Chemosphere **264**, 128455.

Shrestha R, Ban S, Devkota S, Sharma R, Joshi R, Tiwari A, Kim H, Joshi M. 2021. Technological trends in heavy metals removal from industrial wastewater: A review. J Ind Eng Chem **9**, 105688.

Singh RR, Kumar A. 2024. Waste water treatment through microalgae and cyanobacteria. Nat Resour Conserv Res **7**(1), 4925. https://doi.org/10.24294/nrcr.v7i1.4925

Sirsam R, Bhangale H. 2017. Industrial wastewater treatment by microalgae. Int J Creative Res Thoughts.

Sorakin C. 1979. Growth measurements, division rate. In: Stein RS, editor. Handbook of Physiological Methods, Culture Methods and Growth Measurement. Cambridge Univ. Press, 202, 321–343.

Subramaniyam V, Subashchandrabose SR, Ganeshkumar V, Thavamani P, Chen Z, Naidu R, Megharaj M. 2016. Cultivation of *Chlorella* on brewery wastewater and nanoparticle biosynthesis by its biomass. Bioresour Technol **211**, 698–703.

Sun L, Zuo W, Tian Y. 2019. Performance and microbial community analysis of an algal activated sludge symbiotic system: Effect of activated sludge concentration. J Environ Sci China **76**, 121–132.

Sun Y, Zhou S, Sun W, Zhu S, Zheng H. 2020. Flocculation activity and evaluation of chitosan-based flocculant CMCTS-g-PAM-CA for heavy metal removal. Sep Purif Technol **241**, 116737.

Tien CJ. 2002. Biosorption of metal ions by freshwater algae with different surface characteristics. Process Biochem **38**, 605–613.

Tossavainen M, Lahti K, Edelmann M, Eskola R, Lampi AM, Piironen V, Korvonen P, Ojala A, Romantschuk M. 2019. Integrated utilization of microalgae cultured in aquaculture wastewater: Wastewater treatment and production of valuable fatty acids and tocopherols. J Appl Phycol **31**, 1753–1763.

Veenstra JN, Sanders D, Ahn S. 1999. Impact of chromium and copper on fixed film biological systems. J Environ Eng **125**, 522–531.

Wan Maznah AT, Al-Fawwaz M, Surif M. 2012. Biosorption of copper and zinc by immobilized and free algal biomass, and the effects of metal biosorption on the growth and cellular structure of *Chlorella sp.* and *Clamydomonas* sp. J Environ Sci **248**, 1386–1393.

Wang L, Min M, Li Y, Chen P, Chen Y, Liu Y, Wang Y, Ruan R. 2010. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. Appl Biochem Biotechnol **162**, 1174–1186.

Wood A, Scheepers J, Hills M. 1989. Combined artificial wetland and high rate algal pond for wastewater treatment and protein production. Water Sci Technol **21**, 659–668.

Yang L, Zhang H, Cheng S, Zhang W, Zhang X. 2020. Enhanced microalgal harvesting using microalgae-derived extracellular polymeric substance as flocculation aid. ACS Sustain Chem Eng **8**, 4069–4075.

Yolanys AV, Pankaj B, Jen YH, Paul B, Aparajita B, Hussain AS, Halis S. 2024. Biodegradability and bioavailability of dissolved substances in aquaculture effluent: Performance of indigenous bacteria, cyanobacteria, and green microalgae. Environ Pollut **345**, 123468. https://doi.org/10.1016/j.envpol.2024.123468

Yu G, Peng H, Fu Y, Yan X, Du C, Chen H. 2019. Enhanced nitrogen removal of low C/N wastewater in constructed wetlands with co-immobilizing solid carbon source and denitrifying bacteria. Bioresour Technol **280**, 337–344.

Zakaria AM. 2001. Removal of cadmium and manganese by a nontoxic strain of the freshwater cyanobacterium *Gloeothece magna*. Water Res **35**, 4405–4409.

Zhao Y. 2019. Screening of algal and cyanobacterial strains for optimal biomass production and nutrient recovery from dairy manure-based anaerobic digestion effluent. Baltimore and Maryland, 7–8.