

Microalgal Remediation Of Electroplating Wastewater And Biomass Production -An Ecofriendly Approach

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Abstract: Electroplating industrial(EPI)effluents are the major source of heavy and toxic metals discharged into the waste water lines. Sustainable technology through the greener path using microalgae helps to recycle the EPI effluent. In our research the removal of heavy metals in light and dark cycles using *Westiellopsis* was assessed and recorded the results as follows:Cr-91%,Cu-90% ,Ni-84%,Zn-86% .Response of *Westiellopsis* to recover the heavy metals in EPI effluent was recorded in the descending order:Cr > Cu > Zn>Ni. The cell growth of the *Westiellopsis* was increased upto 95% in raw EPI effluent(T4). Cell recovery was achieved upto 100%.Among all treatments,in T4 maximum yield of biomass and protein was recorded upto 97% and 95% respectively. Thus the current research on cultivating *Westiellopsis* in EPI effluent was an ecofriendly approach with dual advantage of reduction in heavy metal load and high yield of dry biomass .

Keywords: Electroplating effluent, microalgal growth, *Westiellopsis*,biomass yield, protein yield.

INTRODUCTION

Increasing population and industrialization had caused new and increasing menace to the ground water system. By 2050 the availability of surface and ground water would decline to less than 80 cubic kilometers from about 500 cubic kilometers. (Cabanelas et al.,2013; Sun et al.,2016;Acién fernández et al.,2018; Ali et al.,2021). Heavy metals such as nickel, chromium, cadmium, zinc etc are toxic and non degradable discharged through industrial effluents pose a serious threat on potable water to become unfit for consumption(Rangsayatorn et al.,2004; Romera et al.,2007).The dissolved heavy metal constituents primarily affected ground water recharge operations are targeted for the recovery through biosorption technologies.

Recently a large number of small scale industries have been established throughout India.The industries discharge a large amount of waste water into the rivers and lakes mainly through increased pollutants from the industrial wastes and green house emissions at higher rate affected the environment that leads to global warming and climate change.Hence there is a great responsibility for the researchers and industrialists to involve in curtailing pollution through immediate steps to implement the green technologies, life cycle assessment and circular economy approaches (El-Bestawy, 2008;Colica et al.,2010).

Microalgae are also known as cyanobacteria or blue green algae have unique photosynthetic efficiency which offers a greener path to produce biomass from the waste waters. Use of various microalgal biomass for the detoxification of different industrial effluents for environmental protection and recovery of valuable metals provides a potential alternative to the existing expensive treatment technologies(Kocberber and Donmez 2007; Hodaifa et al.,2008; Indu sharma et al.,2023). *Westiellopsis* sp is an oxygenic phoyosynthetic filamentous microalga.It has special structure called heterocyst able to fix atmospheric nitrogen and it is intercalary in nature.The branched thallus consists of vegetative cells with blue green pigments known as trichomes.

Here we mainly concentrate on the conservation of ground water which is affected by the heavy metal pollution from electroplating industrial effluents.Hence in the present study analysis of pollutants present in the various processing units of electro plating industrial effluent and its influence on growth

and yield of selected microalga in terms of dry biomass and protein had been experimented. For heavy metal recovery, biosorption technology was effected on the selected microalga *Westiellopsis*.

MATERIALS AND METHODS

Electroplating industries are characterized based on its infrastructure, production and quality of raw materials used. Source of water in each unit varies that influences the growth and yield of *Westiellopsis sp.*

Physico chemical Profiling of Electroplating industrial Effluent

Various physico chemical parameters were analysed before and after microalgal remediation of EPI effluent. Estimation of physicochemical parameters viz., pH, dissolved oxygen, Total dissolved solids, Total suspended solids, Chemical Oxygen Demand, Biological Oxygen Demand, Sulphate, Chloride, Nickel, Chromium, Zinc and Copper by American Public Health Association (APHA,2017) methods.

Lab-scale Study on Microalgal Cultivation

A microalgal cultivation studies on the growth efficiency of *Westiellopsis* in light and dark cycle was conducted. *Westiellopsis* sp cultures were grown in BG11 medium at pH 7.2 and 24±1°C at bioprocess laboratory of Madha Engineering College. A controlled environmental chamber illuminated with cool white fluorescent lamps (Philips 40w, cool daylight, 6500k) at an intensity of 2000 LUX in a 12/12 hours light/dark cycle (Parks, 2004). The colonies of *Westiellopsis* were grown on liquid BG11(control), EPI effluent of undiluted (raw effluent-T4) and diluted solutions(T1,T2andT3) for 24 days under standard conditions (Desikachary, 1959; Rippka et al., 1979).

The cell growth of *Westiellopsis* sp was monitored by measuring the wavelength at 436 nm by a spectrophotometer (Priyanka et al.,2021). Treatments of the experiments in triplicates are as follows: Control(BG-11medium) was kept without any dilutions. 25% ,50%,75% (v/v) dilutions were made with EPI effluent and water in T1,T2,T3 respectively. In T4 (100%) raw EPI effluent was taken. For cell recovery membrane filtration technology was used. The dry biomass yield (mg/ml) and protein yield (mg/g) of *Westiellopsis* were assessed at week 1,2 and 3. The amount of microalgal protein was determined according to Lowry's method (1951). Profiling of aminoacids in *Westiellopsis* was performed by HPLC as per methodology given by Rao et al.,2010.

Statistical Analysis

Triplicates were maintained in all experiments and IBM SPSS Version 18 was used for statistical analysis. Comparison of data was performed by one way ANOVA test and t-test with 95% confidential intervals.

RESULTS AND DISCUSSION

Physico Chemical Profiling of EPI Effluent

The physico chemical experiments for the four processing units of EPI effluent resulted with various micro and macro nutrients viz., pH 3.2-4.5; DO 6.0-7.4 (mg/l) ;TDS-1175.5(mg/l) ; SO₄ 131.4-138 (mg/l) ;Cl₂-275.9 - 293(mg/l);Cr 37.5 (mg/l) ; Cu-32.5(mg/l) ; Zn -25 (mg/l); Ni 82.5 (mg/l). Details of the four processing units (Table-1)are discussed below.

Table 1: Characteristics of Electroplating industrial effluent at Processing Units 1,2,3 and 4

S.No	Parameters	Unit 1	Unit 2	Unit 3	Unit 4
1	pH	3.2±0.02	3.6±0.05	4.2±0.05	4.5±0.09
2	dissolved oxygen(mg/l)	7.4±0.09	7.1±0.03	6.12±0.05	6.0±0.07
3	Total dissolved solids (mg/l)	1175±9.02	1206±11.7	1277.6±9.70	1215.1±10.2
4	Total suspended solids (mg/l)	740±5.51	793±12.28	755 ±8.55	801±14.01
5	Chemical Oxygen Demand (mg/l)	218.4±2.62	225.5±1.33	268.4 ±3.35	265.0±1.51
6	Biological Oxygen Demand (mg/l)	390±5.10	395±7.01	490 ±6.11	470±4.02
7	Sulphate(mg/l)	138±2.09	145.2±1.01	149.4±1.02	137.1 ±1.37

8	Chloride (mg/l)	250.9±11.05	262.1±14.02	274.9±10.07	276 ±10.09
9	Nickel (mg/l)	-	-	81.5 ±0.86	83.05±0.75
10	Chromium (mg/l)	-	-	37.5±0.61	38.5±0.42
11	Copper (mg/l)	-	-	35.3±0.55	34.7±0.72
12	Zinc (mg/l)	22±0.02	25 ±0.01	27.5±0.04	26.1±0.11

* Data represented as mean values ± standard derivation, Significance level at p<0.05

Processing unit -1: Highly acidic effluent with pH 3.2 because of cleansing the raw materials was found.TDS 1175.5mg/l and TSS 805mg/l were made the effluent turbid . Chloride level was found moderate. Concentration of Zn(II) was 21 mg/l.BOD,COD ratio was 4.94.

Processing unit -2 (pre plating section):pH of the effluent in this unit was highly acidic with 3.6.Total suspended and dissolved solids.Chlorides, BOD,COD ratio was 7.1. There was no contents of Ni,Cr and Cu.

Processing unit -3 (plating):The nature of EPI effluent due to plating process resulted as follows:pH is 4.2 ;DO 6.12 (ppm) ; SO₄ 149.4 (mg/l) ;Cl₂-274.9 (mg/l);Cr 37.1 (mg/l) ; Cu 35.3(mg/l) ; Zn 27.8 (mg/l); Ni 81.5(mg/l). Heavy metal contents of rinsing unit is higher compared to the other 3 units.

Processing unit-4(rinsing): Rinsing process effected the EPI effluent with the following results:The pH was slightly lesser than other units.Sulphates contents were lesser .But the chlorides were more.TDS & TSS were found in high quantity.The BOD,COD values were higher in this unit compared to other units.

The electroplating industrial (EPI) effluents are the major source of heavy and toxic metals discharged into the waste water lines. Units 3 and 4 of the EP industries use distilled water as source for chrome plating and zinc plating.The other two units of EP industries use tap water or ground water based on their availability.Because of this nature of EPI effluent , the fresh water microalga *Westiellopsis*, was able to enhance its metabolic activity very faster in light and dark cycles(Table-2).

Table 2: Light and dark uptake of heavy metals of electroplating industrial effluent by *Westiellopsis* sp *

Heavy Metals	Uptake Rate by <i>Westiellopsis</i> sp (mg / 10 min) in Raw EPI effluent under Light and dark cycles	
	Light Uptake	Dark Uptake
Cr	0.633±0.02	0.525±0.02
Zn	0.811±0.04	0.609 ±0.01
Cu	0.753±0.03	0.566 ±0.02
Ni	0.540 ±0.02	0.371±0.01

* Data represented as mean values ± standard derivation, Significance level at p<0.05

In T1 biomass yield was insignificant due to the maximum dilution.The heavy metal removal efficiency of *Westiellopsis* sp was found to be 12.-15% in a retention time of 10 minutes. In T2 and T3 increasing the retention time increased the removal percentage for Cu and Zn.There was no significant removal Cr and Ni after 20 minutes.This might be due to the growth reached to stationery phase which affected the uptake of heavy metals.In T4 the removal percentage for Ni and Zn was ranging from 84 % to 86% within 15 minutes of retention time.Removal percentage upto 90%-91% of Cu and Cr respectively by *Westiellopsis* was recorded in raw effluent(Table-3).

Table 3: Percentage of Removal of heavy metals by *Westiellopsis* sp in electroplating industrial effluent

% of Removal of heavy metals in Raw Effluent by <i>Westiellopsis</i>	Retention Time (minutes)			
	5	10	15	20

Cr	46.5 ±0.12	90.5±1.09	93.5±1.04	92.8±1.04
Zn	35.2±0.12	86.4±0.14	87.3±1.00	80.1±0.42
Cu	42.7±0.09	90.3±1.12	90.1±0.75	86.4±1.06
Ni	38.1±0.15	84±1.08	87.1±1.01	87±0.33

*Data represented as mean values ± standard derivation, Significance level at p<0.05

The nutrients in the EPI effluent influenced the growth of *Westiellopsis sp* in a positive trend in all the treatments(Figure-1) except T1 which was insignificant compared to other treatments .The enhancement of dry biomass was ranging from 26.4% to 49.9% in T2 and T3 respectively and 39 to 97% in T4 for *Westiellopsis* . Furthermore the dual advantage of cultivating *Westiellopsis* in EPI effluent was potentially reduced the heavy metal load after biosorption experiments and high yield of dry biomass ranging from 82-95%.The effect of pH, agitation time, adsorbant dosage and eluting agents were the parameters (standardized by various trials in our previous study) demonstrated the efficiency of microalga *Westiellopsis* for the recovery of heavy metals (Table-3). Microalgal heavy metal removal from various waste waters was experimented by Whitton et al.,2015; Prabha Tripathi and Judia harriet sumathy,2018; Leong and chang,2020; Yan et al.,2021.

Furthermore the uptake/removal efficiency of heavy metals within 5 to 15 minutes as follows:Cr 47% to 94%; Ni 38-92%; Cu 48-90%; Zn 35-87% (Table-4). Microalgal treatment leads to the improvement in the quality of the recycled waste water(Table-4) by decreasing COD, phosphates and specifically heavy metals (Michalak and Chojnacka ,2010; He et al.,2020;Al-jabri et al.,2021).

Hence from our research it had been prominently proved that the culture of microalgal biomass in EPI effluent facilitated triple advantages as its role in conserving natural resources especially the ground water , recycling of waste water and dense growth of microalgal biomass in the effluent.

Study on Growth , Biomass and Protein Yield of *Westiellopsis*

Microalgal cultivation study and heavy metal removal efficiency of *Westiellopsis* in light and dark cycle and the growth profile had shown the potentiality of *Westiellopsis* to utilize the nutrients in electroplating effluent(Table-3 and 4). The pollutants were metabolized for the growth and decontaminated the EPI effluent to reuse in the industry. This effect has been recorded as a part of lifecycle assessment and bioeconomy approach in the electroplating industries. Similar trend of research was observed in the earlier work of Lim et al., 2010 ; Udom et al.,2013; Palaniswamy and Veluchamy 2017;Silambarasan et al.,2021).

Biomass yield represents the efficacy of *Westiellopsis* in raw EPI effluent effected 48% which was comparable with the control in which 49% yield obtained. Dilutions of 25% -75% influenced only 19-43%.Yield with reference to the dry biomass in the EPI effluent was ranging from 38.7% to 97% for *Westiellopsis*. The growth of microalga *Westiellopsis sp* in electroplating industrial effluent with reference to the vegetative cells , heterocysts and its frequency (Figure-1& 2) brings about oxygenation and mineralization which accounts for the increase in biomass and protein yield .

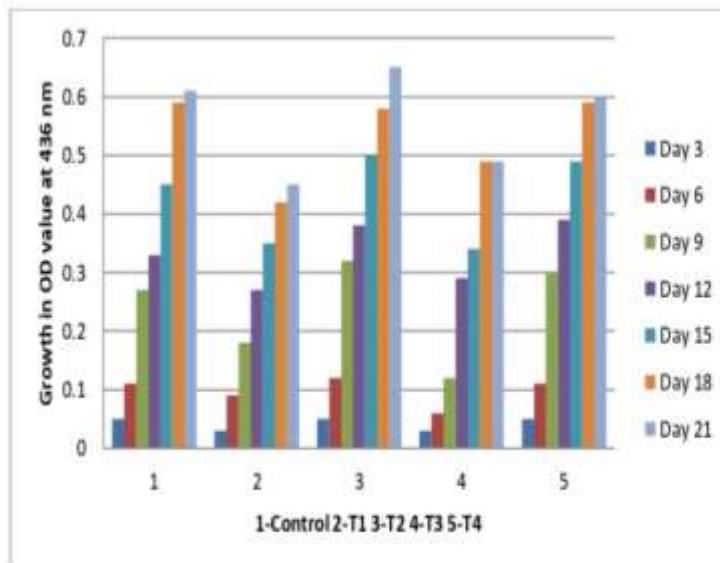


Fig. 1- Growth Profile of *Westiellopsis* in electroplating industrial effluent (Mean of triplicates)

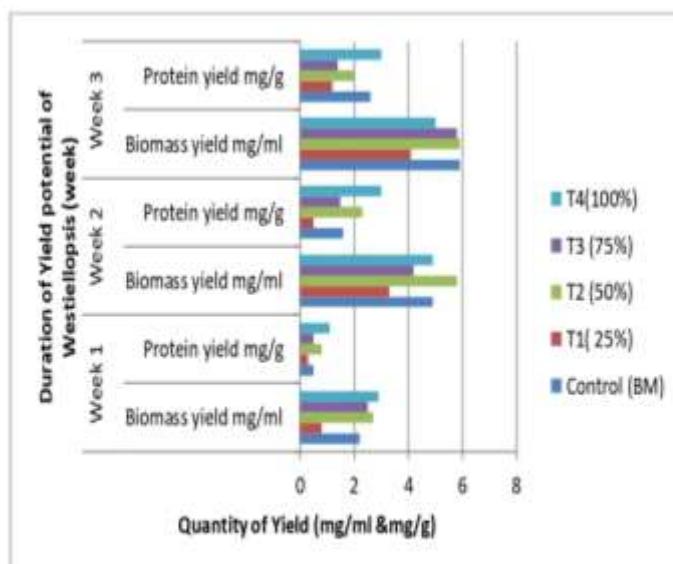
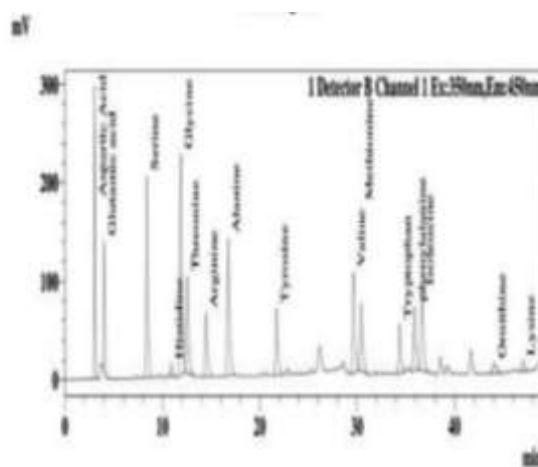


Fig.2 Dry Biomass and Protein Yield of *Westiellopsis* in Electroplating industrial Effluent (Mean of triplicates)

Protein yield of *Westiellopsis* in all the treatments were ranging from 25% to 37% and in basal medium it was 46%. The raw EPI effluent was effectively influencing the microalga *Westiellopsis* to yield protein upto 43% which was confirmed by the aminoacid profile performed by HPLC(Figure-3).



Peak Table					
Peak#	Name	Ret. Time	Area	Conc.	Unit
1	Aspartic Acid	3.87	151043	0.00	ng/L
2	Glutamic acid	4.09	93242	0.00	ng/L
3	Serine	5.45	261154	0.00	ng/L
4	Histidine	10.92	150091	0.00	ng/L
5	Glycine	11.82	319529	0.00	ng/L
6	Threonine	12.58	140715	0.00	ng/L
7	Arginine	14.48	97232	0.00	ng/L
8	Alanine	16.79	223455	0.00	ng/L
9	Tyrosine	21.71	1009635	0.00	ng/L
10	Valine	24.20	1864710	0.00	ng/L
11	Methionine	30.41	1212910	0.00	ng/L
12	Tryptophan	34.30	65545	0.00	ng/L
13	phenylalanine	35.86	188570	0.00	ng/L
14	Isoleucine	36.64	140962	0.00	ng/L
15	Ornithine	44.03	252325	0.00	ng/L
16	Lysine	47.03	193131	0.00	ng/L

Fig.3. Aminoacid profile of *Westiellopsis* Performed by HPLC

Microalgal proteins contain all the essential amino acids and possess balanced total amino acid profiles Lucakova *et al.* 2022. Because of these specific essential aminoacids *Westiellopsis* thalli were divide faster in light and dark cycles. Light uptake of 0.592- 0.723 mg /10 min and dark uptake of 0.470-0.542 for copper by the cyanobacterial biomass were recorded . For zinc the light uptake of 0.604- 0.781 mg /10 min and dark uptake of 0.515- 0.535 mg /10 min by the cyanobacterial biomass were recorded which showed the potentiality of *Westiellopsis* sp to uptake the nutrients and heavy metals. Light: Dark cycle exposure of *Westiellopsis* to illumination for 12:12 hrs influenced protein yield in all the treatments from 25% upto 37% during the exponential phase of growth (Wong,2016; Sui *et al.* 2019).

Exposure to EPI effluent at various dilutions by the trichomes of *Westiellopsis* proved that the microalgal protein yield in which specific essential aminoacids are the backbone to withstand harsh environmental condition. Ursu *et al.*,2014 discussed the functional role and the properties of proteins with the

aminoacid contents of *Chlorella vulgaris*. Few research on various microalgal aminoacids are in accordance with the current study on aminoacid profile by HPLC (Lourenço *et al.* 2004; Kolmakova and Kolmakov, 2019). Amino acid contents of microalga used to indicate the direct impact on growth status and it is directly proportional to the dry biomass yield. These results are in accordance with the earlier experiments of Seyfabadi *et al.* 2011; Biancarosa *et al.* 2017; Gorissen *et al.* 2018.

Microalgal proteins have technofunctional properties provide uniqueness for its physiology that can withstand diverse and harsh challenging environments. Thus it is suggested that the EPI effluent could be used as an ambient medium to cultivate the microalga *Westiellopsis* with a potential of yielding protein as a valuable product.

From these findings it is recommended that the raw EPI effluent could be treated effectively in a faster manner as the heavy metal load was reduced by biosorption technology. Future research scope pertaining to extraction of valuable products from *Westiellopsis* and its role as multipurpose raw material to the industries will be experimented.

CONCLUSION

From the lab scale experiments it is demonstrated that *Westiellopsis* sp utilised EPI effluent as an ambient medium for the cell growth in all the treatments (T1- T4). Furthermore the 50% dilution is highly effective in enhancing the growth of *Westiellopsis* sp which could be compared with its growth pattern in the basal medium. The raw EPI effluent was effectively influencing the microalga *Westiellopsis* to yield protein upto 43% which was confirmed by the aminoacid profile performed by HPLC .

The scope of future research enlightened towards the effects of heavy metals on the microalgal physiology and gene expression involved in decontamination of various pollutants. Furthermore, it is essential to assess the consequences of heavy metal pollution and the biotechnological exploitation of the resistant strains for the treatment of industrial effluents. The microalgal (*Westiellopsis*) biomass could be used as a potential resource for the production of biofuel and other valuable products. Thus it would be an added advantage to account for bioeconomy and sustainable technology for the electro plating industries.

CONFLICT OF INTEREST

Author declares that there is no conflict of interest.

REFERENCES

1. Acién Fernández FG, Gómez-Serrano C and Fernández-Sevilla JM (2018) Recovery of nutrients from wastewaters using microalgae. *Frontiers in Sustainable Food Systems* 2:59. <https://doi.org/10.3389/fsufs.2018.00059>
2. Ali S, Peter AP, Chew KW, Munawaroh HSH and Show PL (2021) Resource recovery from industrial effluents through the cultivation of microalgae: a review. *Biores Technol* 337:125461. <https://doi.org/10.1016/j.biortech.2021.125461>
3. Al-Jabri, H., Das, P., Khan, S., Thaher, M and AbdulQuadir, M. (2021). Treatment of Wastewaters by Microalgae and the Potential Applications of the Produced Biomass—A Review. *Water*, 13(1), 27. <https://doi.org/10.3390/w13010027>
4. APHA.(2017).Standard Methods for the Examination of Water and Wastewater, 23rd edition,American Public Health Association,Washington,D.C.
5. Biancarosa, I, Espe, M, Bruckner, C.G, Heesch, S, Liland N,Waagbø, R, Torstensen, B and Lock, E.J(2017) Amino Acid Composition,Protein Content, and Nitrogen-to-Protein Conversion Factors of 21 Seaweed Species from Norwegian Waters. *J. Appl. Phycol*, 29, 1001-1009
6. Cabanelas ITD, Ruiz J, Arribi Z, Chinalia FA, Garrido-Pérez C, Rogalla F, Nascimento IA and Perales JA (2013) Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. *Biores Technol* 131:429-436.<https://doi.org/10.1016/j.biortech.2012.12.152>.

7. Colica G, Mecarozzi P. C., and De Philippis, R(2010). "Treatment of Cr(VI)-containing wastewaters with exopolysaccharide producing cyanobacteria in pilot flow through and batch systems," *Applied Microbiology and Biotechnology*, vol. 87, no. 5, pp. 1953–1961.
8. Desikachary T.V. *Cyanophyta*, Indian council of agricultural research, New Delhi p 686.1959
9. El-Bestawy, E(2008). "Treatment of mixed domestic-industrial wastewater using cyanobacteria," *Journal of Industrial Microbiology and Biotechnology*, vol. 35, no. 11, pp. 1503–1516.
10. Gorissen, S.H.M.; Crombag, J.J.R.; Senden, J.M.G.; Waterval, W.A.H.; Bierau, J.; Verdijk, L.B.; van Loon, L.J.C (2018) Protein Content and Amino Acid Composition of Commercially Available Plant-Based Protein Isolates. *Amino Acids*, 50, 1685–1695.
11. He X., Yao B., Xia Y., Huang H., Gan Y and Zhang W.(2020). Coal fly ash derived zeolite for highly efficient removal of Ni²⁺ in waste water. *Powder Technol.*, 367, 40–46. DOI: 10.1016/j.powtec.2019.11.037.
12. Hodaifa, G., Martinez, M.A and Sanchez, S. (2008) Use of industrial wastewater from oliveoil extraction for biomass production of *Scenedesmus obliquus*. *Bioresource Technology*, 99: 1111-1117
13. Indu Sharma, Sandeep, Raj Bala, Nakul Kundra, Tejinder Kaur and Ashutosh Sharma (2023). Microalgae-Mediated Wastewater Treatment for Biofertilizer Production, *Water Wastewater Resource Recovery and Biological Methods*, p. 231-247 https://doi.org/10.1007/978-3-031-40198-5_11
14. Ishida Y, Fujita T and Arai K (1981) New detection and separation methods for amino acid by high performance liquid chromatography. *Journal of Chromatography* 204: 143-148.
15. Kolmakova, A.A.; Kolmakov, V.I. (2019). Amino Acid Composition of Green Microalgae and Diatoms, Cyanobacteria, and Zooplankton (Review). *Ecol. Physiol. Biochem. Hydrobiol.* 12, 452–461.
16. Kocberber N and Donmez G (2007). Chromium (VI) bioaccumulation capacities of adapted mixed cultures isolated from industrial saline wastewaters. *Bioresource Technology*. 98:2178-2183
17. Leong Y. K. and Chang J. S(2020). Bioremediation of heavy metals using microalgae: Recent advances and mechanisms," *Bioreesour. Technol.*, vol. 303, p. 122886.
18. Lim S. L, Chu W. L and Phang S. M(2010). Use of Chlorella vulgaris for bioremediation of textile wastewater. *Bioreesour. Technol.*, vol. 101, no. 19, pp. 7314-7322.
19. Lourenço, S.O, Barbarino, E, Lavín, P.L, Lanfer Marquez, U.M and Aidar, E(2004) Distribution of Intracellular Nitrogen in Marine Microalgae: Calculation of New Nitrogen-to-Protein Conversion Factors. *Eur. J. Phycol.* 39, 17–32
20. Lowry, O.H and Rosebrough, N.J. Farr, A.L. Randall, R.J. Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275, [https://doi.org/10.1016/s0021-9258\(19\)52451-6](https://doi.org/10.1016/s0021-9258(19)52451-6)
21. Lucakova, S., Branyikova, I., and Hayes, M. (2022). Microalgal proteins and bioactives for food, feed, and other applications. *Appl. Sci.* 12, 4402. doi:10.3390/app12094402
22. Martin-Jézéquel, V.; Sournia, A and Birrien, J.-L. (1992). A Daily Study of the Diatom Spring Bloom at Roscoff (France) in 1985. III. FreeAmino Acids Composition Studied by HPLC Analysis. *J. Plankton Res.* 14, 409–421.
23. Michalak I and Chojnacka K (2010) Interactions of metal cations with anionic groups on the cell wall of the macroalga *Vaucheria* sp. *Engineering in Life Sciences*. 10:209-217.
24. Palaniswamy R and Veluchamy C (2017). Biosorption of Heavy Metals by *Spirulina platensis* from Electroplating Industrial Effluent," *Env. Sci Ind J*, vol. 13, no. 4, p. 139.
25. Parks, L. C. (2004). *Handbook of Microbiological Media* by Ronald Atlas
26. Prabha Tripathi P and Judia Harriet Sumathy V. (2018). A Comparative Study on the Application of Algae for Bioremediation of Cooum Water. *Int. J. Eng. Tech.*, vol. 4, no. 1, pp. 426-442. Rao B.M., Bhandare P., Madhavan P and Someswar Rao N. 2010. Determination of amino acid without derivatization by using HPLC - HILIC column *J. Chem. Pharm. Res.*, 2010, 2(2): 372-380.
27. Priyanka G, Vivekanand V and Nidhi P. (2022). Chapter 17 - Cyanobacterial and microalgal bioremediation: an efficient and eco-friendly approach toward industrial wastewater treatment and value-addition, Editor(s): Surajit Das, Hirak Ranjan Dash, *Microbial Biodegradation and Bioremediation* (Second edition), Elsevier, Pages 343-362, ISBN 9780323854559, <https://doi.org/10.1016/B978-0-323-85455-9.00002-3>.
28. Rangsayatorn N, Pokethitiyook P, Upatham E S and Lanza G R (2004) "Cadmium biosorption by cells of *Spirulina platensis* TISTR 8217 immobilized in alginate and silica gel", *Environment International*, Vol. 30, No. 1, pp. 57-63.
29. Rehman, P (2011). Heavy metals uptake by *Euglena proxima* isolated from tannery effluents and its potential use in wastewater treatment," *Russ. J. Ecol.*, vol. 42, no. 1, pp. 44-49.

30. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology*, 111(1), 1-61.
31. Romera E, Gonzalez F, Ballester A, Blazquez M L and Munoz J A (2007) "Comparative study of biosorption of heavy metals using different types of algae", *Bioresource Technology*, Vol. 98, No. 17, pp.3344-3353
32. Ross, I.S., (1995). Reduced uptake of nickel by a nickel resistance strain of *Candida utilis*. *Microbios*, 83: 261-270.
33. Seyfabadi, J.; Ramezanpour, Z.; Khoeyi, Z.A(2011) Protein, Fatty Acid, and Pigment Content of Chlorella Vulgaris under Different Light Regimes. *J. Appl. Phycol.*, 23, 721-726.
34. Silambarasan S, Logeswari P, Sivaramakrishnan R, Incharoensakdi A, Cornejo P, Kamaraj B and Chi N T L (2021). Removal of nutrients from domestic wastewater by microalgae coupled to lipid augmentation for biodiesel production and influence of deoiled algal biomass as biofertilizer for *Solanum lycopersicum* cultivation. *Chemosphere* 268:129323 <https://doi.org/10.1016/j.chemosphere.2020.129323>
35. Sui, Y, Muys, M, Vermeir, P, D'Adamo, S and Vlaeminck, S.E 2019 Light Regime and Growth Phase Affect the Microalgal Production of Protein Quantity and Quality with *Dunaliella salina*. *Bioresour. Technol.* 275, 145-152.
36. Sun Y, Chen, Z, Wu, G, Wu, Q, Zhang, F, Niu, Z and Hu, H.Y 2016 Characteristics of water quality of municipal wastewater treatment plants in China: Implications for resources utilization and management. *J. Clean. Prod.*, 131, 1-9.
37. Udom I, Zaribaf BH, Halfhide T, Gillie B, Dalrymple O, Zhang Q and Ergas SJ 2013 Harvesting microalgae grown on wastewater. *Biores Technol* 139:101-106 <https://doi.org/10.1016/j.biortech.2013.04.002>
38. Ursu A.V., Marcati A., Sayd T., Sante-Lhoutellier V., Djelveh G and Michaud P. 2014 Extraction, fractionation and functional properties of proteins from the microalgae *Chlorella vulgaris*, *Bioresour. Technol.* 157 134-139, <https://doi.org/10.1016/j.biortech.2014.01.071>.
39. Whitton R, Ometto F, Pidou M, Jarvis P, Villa R, and Jefferson B 2015 Microalgae for municipal wastewater nutrient remediation: mechanisms, reactors and outlook for tertiary treatment *Environ. Technol. Rev.*, vol. 4 no. 1, pp. 133-148.
40. Wong, Y 2016 Effects of Light Intensity, Illumination Cycles on Microalgae *Haematococcus pluvialis* for Production of Astaxanthin. *J. Mar. Biol. Aquac.* 2, 1-6