JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2023/30943

RESEARCH ARTICLE

Microalgae: Nature's Green System to Recycle Waste to Resource

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Abstract

Dairy wastewater management is a major concern for many milk-producing countries as it is a serious nuisance to the surroundings when disposed of untreated, increasing the organic load and foul smell. Microalgae remediation is an easy and cost-effective treatment to treat the effluent and simultaneously enhance a few agronomic traits. In this research, the phytoremediation technique was validated by treating dairy effluent using microalgal consortium and to study its impact on seed germination assay of *Vigna mungo* (Black gram). The results exhibited a significant increase in germination index, vigor index, and germination percentage when undiluted effluent was treated with microalgal consortium followed by other dilutions. Seedling growth was found maximum in 100% microalgal treated (TE100) undiluted effluent followed by 75% dilution (TE75), 50% dilution (TE50), 25% dilution (TE25), (TE0) treated effluent and compared with controls: water, effluent, fertilizer. So, we conclude that dairy effluent treated with suitable microalgae can be used directly for irrigation purposes to produce plants with high yield and significant biomass, indirectly making the industry a place of zero-waste discharge.

Keywords: Dairy Effluent, Phytoremediation, Seed Germination, Sustainable Growth, and Development, Zero-Waste Management

1. Introduction

Food demand and increased pollution rate are the two major issues of the current scenario in our country[1]. Feeding the exploding global population in India is one of the serious threats to the agriculture industry. Due to soil and land reduction, low rainfall, pest, and pathogen, the crop yield is not sufficient to fulfil the food demand of the country. Another big problem in agriculture is the necessity for large volumes of water. It is a non-renewable resource that is in high demand because of rapid utilization and pollution due to anthropogenic activities of industries and domestic exploitation[2]. All these factors deteriorate the quality of water and make it scarce for even human consumption.

The dairy industry is one of the industries that utilize the large volume of underground water in every stage of processing like production, cleaning, packaging, and tanker washing and expel out as dairy effluent with a foul smell and increased organic load that makes the surrounding water bodies unpleasant and unsuitable for survival[3,4]. Dairy effluent released from the above processes contains soluble organic matter, suspended solids, trace metals, oil, and grease[5]. These increase the Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD)[6,7] of the effluent to a higher concentration. Also due to the fermentation of milk sugar to lactic acid, dairy waste becomes rapidly acidic and contributes to immediate high oxygen demand making it unsuitable for the survival of the aquatic ecosystem[8].

The demand for water in urban communities is found to be increasing rapidly and the estimated wastewater expelled from urban centres is found to have reached 61,754 Million Litres every Day (MLD) by 2015. But the treatment facilities available for treatment are limited to 22,963 MLD. This research study mainly aims in bridging the water scarcity problem on one end and bulk volumes of wastewater expelled from industries[9]. The major concern

Article Received on: 10.08.2022 Revised on: 14.11.2022 Accepted on: 11.01.2023

of releasing untreated kitchen and sewage wastewater is the eutrophication of the nearby water sources due to high nitrogen and ammonium[10].

Common physical and chemical methods of effluent treatment plants are very expensive, and laborious, occupying more space and unit operations[11]. Various physiological disorders and imbalanced usage of chemical fertilizers decrease the nutrient availability in soil[12]. Enhancing crop productivity in a cost-effective and friendly way using some alternate irrigation techniques is the suggested alternative to solve this problem. Bioremediation is a cohesive approach where microbes are employed to transmute toxic elements to release CO_2/CH_4 and H_2O[13]. Among them, researchers have gained attention towards modern technology using plants including microalgae and seaweeds (phytoremediation/phytoremediation) to take up excess nutrients, heavy metals, organic matter, dyes, pigments, etc.,[14] from industrial wastewater making it a promising option. The high nutrient content of industrial wastewater acts as an efficient substrate for microalgal cultivation to increase its biomass and bioenergy production. The notifiable merits of microalgae like natural biotransformation/bioaccumulation/bioaugmentation of the toxic chemical pollutants present in the environment, CO_2 sequestration potency[15,16], easy adaptability to any environment, and plant growth promotion by increasing nutrient availability, finally lead to zero waste management[17].

Deploying microalgae for remediation is the most cost-effective technology to remove nutrients and increase biomass productivity for by-product development[2,9,18]. Moreover, the treated water can be utilized for irrigation purposes having enriched nutrient content that can promote plant growth and development. Microalgae are found very attractive to benefit the agrochemical industries and agriculturists due to their activity as plant growth biostimulants and biofertilizers[19]. They have multiple positive correlations with plant growth like shoot length, rooting, crop yield, fruit quality, and tolerance against drought and salinity. Microalgae with high-stress tolerance can be formulated as a soil conditioning agent, fertilizer, foliar spray, granules, and pesticides that can be used to boost the economy of the industry when incorporated in the effluent treatment plant[20]. Using the microalgae (post-treatment) is an added advantage when utilized for by-product production apart from the remediation process. This makes the industry a zero-waste discharge site, alongside producing various value-added products as a revenue generator[21].

The agronomist and scientific community are exploring new technologies to restore the environment in an eco-friendly method increasing the economic value of crops by nutrient supplementation. So our team indulged in studying the impact of treated and untreated wastewater released at the outlet discharge point in a dairy production unit located in Tamil Nadu on black gram germination, growth, and productivity.

2. Materials and Methods

2.1 Characterization of Dairy Wastewater

The diary effluent samples were collected directly from the collection point of a milk production unit located in the Vellore district, India. A sampling of untreated raw dairy wastewater was done at the collection point which connects all the water expelled from the industry including production, cleaning, packaging, and pantry etc., 20-litre empty plastic water cans and stored at 4°C[3]. The physicochemical characterization of the effluent was done based on the American Public Health Association (APHA 1992) and tabulated below in Table 1[22]. For the experiment, 8 types of treatment – tap water, raw untreated effluent, and urea (fertilizer) as control, whereas TE100 (raw 100% undiluted effluent + microalgal consortium), TE75 (75% effluent + 25% water + microalgal consortium), TE50 (50% effluent + 50% water + microalgal consortium), TE25 (25% effluent + 75% water + microalgal consortium), TE0 (Pure algal consortium) as treatment samples were taken.

2.2 Biological Treatment with Algal Consortium

Based on the literature, algal cultures with significant degradation potential were selected and purchased from the National Repository for Microalgae and Cyanobacteria (NRMC-F), Bharathidasan University, Tiruchirapalli and Phycospectrum Environmental Research Centre, Anna Nagar, Chennai. The cultures selected for the consortium development were *Scenedesmus dimorphus* (NRMCF0157), *Chlorella vulgaris* (NRMCF0128), *Chlorococcum* sp. (NRMCF0103) and *Desmococcus olivaceous* in equal ratios.

2.3 Growth of Seedling

Black gram, a pulse crop with high protein content is considered in this study to supplement our nutrition diet with a significant increase in yield by adapting to an alternate green system to utilize waste as a resource[23,24]. Black gram (*Vigna mungo*) purchased from a certified agronomy shop was surface sterilized with 0.1% mercuric chloride for 2 min and washed thrice with distilled water to remove traces of mercuric chloride. In blotting paper, different dilutions of effluent treated with microalgal consortium and controls were added to the sterilized seeds[25] placed inside the blotting paper. 10 seeds were placed in a row for each treatment, labelled and kept undisturbed in a dry place for 7 days. Any 3 randomly selected seeds were used for data analysis and documentation. Various parameters were analysed using the following formulae given below:

2.3.1 Germination Percentage

Germination percentage is the calculation of no. of seeds germinated to the total no. of seeds plated was done using the formulae[25]:

GP = (No. of seeds germinated / Total no. of seeds tested) * 100

2.3.2 Germination Energy

Germination energy can be calculated by considering the no. of seeds germinated highest on a particular day to the total no. of seeds plated using the formulae:

GE = (No. of seeds germinated on day 4 / Total no. of seeds tested) * 100

2.3.3 Germination Rate Index

The germination rate index can be calculated using the formulae:

GRI = (G1/1) + (G2/2) + (G3/3) +………. (Gi/i)

Where G1, G2….Gi – No. of seeds germinated on Day 1, 2........i

2.3.4 Mean Germination Time

Mean Germination Time, MGT can be calculated on the summation of the multiplication value of the no. of seeds germinated each day and the respective day considered by the formulae:

MGT = ∑Fx / ∑(F1*X1)+(F2*X2)+…………………… (Fi*Xi)

Where F, F1, F2….Fi is the No. of seeds germinated and X, X1, X2,…….Xi are the days in which the seeds germinated.

2.3.5 Vigour Index

The length vigour index was calculated using the formulae:

VI = (Average root length + Average hypocotyl length) * GP

Where, Average root length and shoot length was calculated using a thread that exactly resemble the curve and folding of the stem and root, later measured with a ruler.

2.3.6 Biomass Estimation

The fresh and dry weight of the seeds was calculated by weighing the seeds immediately after 7 days (fresh weight) and after drying them in the oven at 40°C for 2 days (dry weight).

3. Results and Discussion

3.1 Characterization of Dairy Effluent

The physicochemical parameters of raw undiluted effluent were analyzed immediately after collection from industry and the results are compared with different dilutions algal consortium treated effluent after 7 days in Table 1. The raw effluent was turbid, milky white with oil and grease having a stringent foul smell. The treated effluent of all dilution falls within the permissible limit specified by Government regulations and is used for seed germination assay[3].

Table 1. Physicochemical characteristic parameters of raw and treated dairy effluent

Parameters	Untreated Raw effluent	TE100	TE75	TE50	TE25	TE0
Color	Turbid-Milky white	Dark Green	Dark Green	Dark Green	Dark Green	Pale Green
Odor	Foul smell	No smell	No smell	No smell	No smell	No smell
pH	5.68±0.12	8.47±1.24	8.64±3.23	8.84±1.54	8.43±0.67	8.35±1.76
COD	1060±1.50	115±1.05	63.7±0.52	31.9±2.53	15.9±2.23	23.9±1.23
BOD	263±1.21	10.9±1.75	16.2±2.3	6.2±1.24	12.0±3.65	6±3.87

(*Continued*)

Table 1. (Continued)

Parameters	Untreated Raw effluent	TE100	TE75	TE50	TE25	TE0
Nitrite	0.68±0.04	0.16±0.02	0.15±0.12	0.16±0.21	0.14±1.21	0.15±1.22
Nitrate	32.6±1.90	13.2±0.83	11.25±1.0	12.32±0.98	15.63±2.13	16.45±0,34
Phosphorous	28.4±2.54	5.1±0.20	5.3±2.31	4.8±2.54	7.4±3.21	6.5±1.09
Ammoniacal Nitrogen	55.25±1.89	6.19±0.19	5.51±3.23	7.23±5.43	6.83±1.24	5.59±2.45

All data are represented as mean±SD values, where n = 3 (TE - Treated effluent with the microalgal consortium in various dilutions)

3.2 Biological Treatment with Algal Consortium

In a bioreactor, algal strains get adapted to the effluent with continuous aeration for its growth and multiplication. The 50% and 25% diluted effluents (TE50, TE25) improved the water quality parameters equal to the pure microalgal consortium (TE0) followed by TE75 and TE100 with better degradation potential reducing the BOD and COD of dairy effluent after treatment with the microalgal consortium.

3.3 Growth of Seedlings

3.3.1 Germination Bioassay

After 7 days of effluent addition to seeds, the blotting paper was opened and the following parameters were calculated: germination percentage, germination index, average shoot length, average root length, vigor index, fresh weight, and dry weight of biomass. Maximum seed germination and dry biomass were exhibited on 100% effluent treatment with consortium supporting the use of dairy effluent in plant growth promotion. In previous studies when raw untreated effluent was used for irrigation in black gram and green gram reported growth inhibition in a higher concentration above 50% dilution[26,27]. But in our study, phycoremediated effluent with algal consortium gives maximum growth potential suggesting this method is the better alternative to treat dairy effluent and also use the treated wastewater for growth enhancement and yield promotion. TE100 and TE75 outperformed when compared to other treatments. Raw effluent also comparatively did not affect the seed growth. But in a few papers direct effluent irrigation of various industries and also 100% treated effluent has been reported to have inhibited the growth of certain plants[28,29]. But while treating with microalgae, the sequential growth of seeds is found from high to low as the dilution of effluent decreases, confirming the positive correlation of treated effluent and seed growth pattern. Average root length, average shoot length, fresh weight, dry weight, and seed germination rate significantly differ with the effluent concentration promoting seed germination assay. Figure 1, Illustrates the seed germination of *Vigna mungo* seeds in two different generations (a) zeroth generation (raw truly labelled seeds) (b) First generation seeds (Harvested seeds of zeroth generation treated with different treatments placed in the following order: (1) Water, (2) Effluent, (3) Fertilizer, (4) TE100, (5) TE75, (6) TE50, (7) TE25, (8) TE0.

Figure 1. Generation-wise seed germination study of V*igna mungo* seeds.

Maximum seed germination on day 4 was found to be maximum in TE100 and TE50 compared to other treatments but almost equal to water and fertilizer control seeds. The lowest seed germination was found in TE0 because of the lack of effluent addition. In comparison between both generations, the seeds of the first generation gave higher yield than zeroth generation treated seeds that exhibit significant growth in initial seedling growth rate confirming the increase in and germination time in the next generation, confirming the positive correlation of our treatment with the seed quality. In Figure 2, the seed germination rate of zeroth and first-generation seeds of *Vigna mungo* is compared individually for each treatment, as follows: (a) Water (b) Effluent (c) Fertilizer (d) TE100 (e) TE75 (f) TE50 (g) TE25 and (h) TE0.

Germination percentage, Germination energy and GRI clearly illustrated the hike in seedling growth in first-generation seeds in TE100, TE75, TE50 and TE25 than the zeroth-generation seeds (Figure 3). Similarly, in measuring the average shoot length it is very clear that the overall growth was maximum in the first generation of TE25 than TE 75 and TE100. But for root length, the result was high in first-generation TE100 followed by TE25, and TE75. In the same way, the fresh weight was more in TE100 followed by TE75, TE25, TE50 and TE0 of first-generation plants (Figure 4).

From our study, it is very clear that first-generation seeds are more potent than raw seeds purchased from the shop. So, it implies that treated plants are not only giving better yield but also their productivity increases in the next offspring too. So, when our consortium is used,

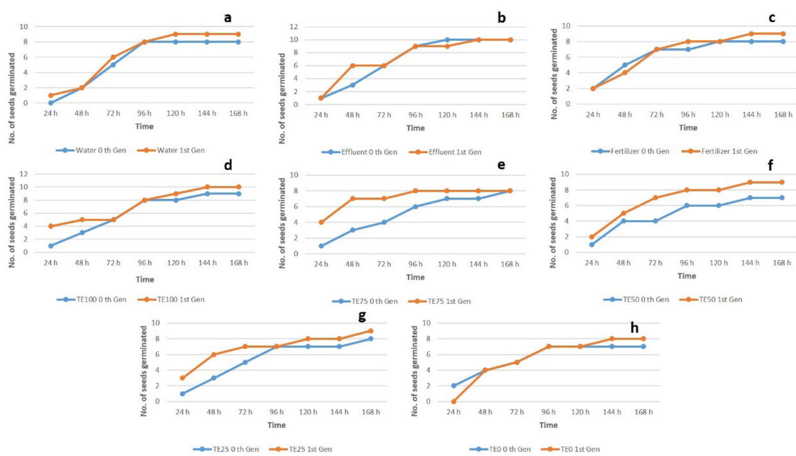

Figure 2. Comparative analysis of seed germination in zeroth and first-generation for the eight different treatments: **(a)** Water **(b)** Effluent **(c)** Fertilizer, **(d)** TE100 **(e)** TE75, **(f)** TE50 **(g)** TE25 and **(h)** TE0.

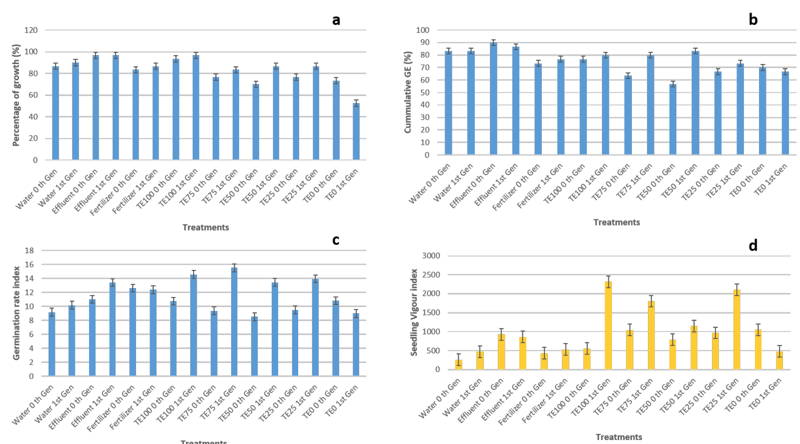

Figure 3. Germination percentage, Germination energy, Germination rate index and Vigour index of *Vigna mungo* seeds of zeroth and first-generation treated with the microalgal consortium.

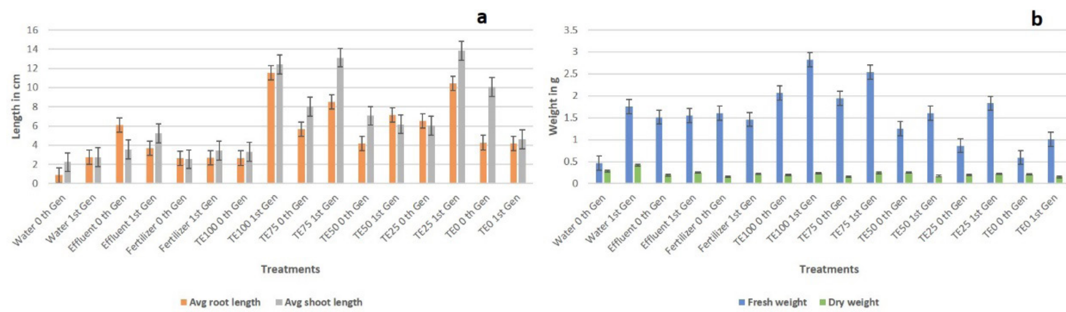

Figure 4. Average root length vs. shoot length and Fresh weight vs. dry weight of zeroth and first generation of seeds treated with microalgal strain.

we are sure that the yield will be good for the next-next generations also. Raw effluent without any dilution had an inhibitory effect on the seed growth as compared to other treatments in literature[30]. In our study with the untreated raw effluent, the plants got infected with some disease, causing poor yield in our field study.

Hence the positive effect of treated dairy effluent can be utilized for agricultural irrigation for a better impact on the plants and surrounding ecosystem. The excess amount of nitrogen will cause better vegetative yield. But if beyond the limit, sometimes may lead to delayed maturity and growth bringing economic loss to the farmers. And sometimes the excess humus developed from the solid sludge may alter the soil to an anaerobic condition that can retard the growth of plants[31].

So the usage of microalgal treated (phycoremediated) wastewater is recommended for application as a foliar spray, biostimulant or phytoprotectant is an opportunity wide open for the algologists and environmentalists to improve an eco-friendly and sustainable agricultural practice to the upcoming generation[32]. Effective technology transfer to industries will protect the surrounding environment and also aid in the production of some value-added products to benefit society and create economic revenue for the industry for converting waste into resources.

4. Conclusion

Wastewater emitted from the dairy industry if left untreated will contaminate the environment and increase rapid algal blooms completely reducing the quality of aquatic life by reducing colour, pH, oxygen and suspended solids of the water source. Nature's Green magic (phytoremediation/ phycoremediation) to reduce pollution and increase nutrient availability to plants improves the water quality and also increases plant growth by supplementing the excess nutrients taken from the dairy effluent.

The treated effluent increases crop productivity and yield, in an eco-friendly and sustainable approach for better livelihood. This research will bridge the gap between ETP facilities at industries and agronomists to increase yield by utilizing wastewater as a resource.

Many papers on using raw effluent like a distillery, brewery, oil mill, and plasma-activated water to irrigate crops have been published in the last few decades[30,32-35]. But in all the studies, low concentrations only influenced growth, and higher concentrations were reported to inhibit plant growth promotion. Also, the accumulation of those untreated toxic materials is having chances contained in their plant parts indirectly causes some health issues during consumption. So treating them with a natural system and then using them for irrigation is better by all means for the society and environment. We conclude that undiluted dairy effluent (TE100) grown with single or consortia of algae are very suitable for use as biofertilizer or spray for increased productivity and making the industry a better place with zero discharge of waste to the ecosystem.

5. Acknowledgement

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sector. The authors are thankful to the management of Vellore Institute of Technology for providing the required facilities, infrastructure, support and encouragement. A special acknowledgement to Bharathidasan University, Tiruchirappalli and Phycospectrum Environmental Research Centre, Anna Nagar, Chennai for providing us with the microalgal strains selected for the study and helping us with technical guidance when required. The authors also acknowledge Mr. Arunram, for his extended support in formatting and alignment of the manuscript.

6. References

1. World Health Organization. The state of food security and nutrition in the world 2020: transforming food systems for affordable healthy diets. Food and Agriculture Org. 2020.
2. Do JM, Jo SW, Kim IS, Na H, Lee JH, Kim HS, *et al*. A feasibility study of wastewater treatment using domestic microalgae and analysis of biomass for potential applications. Water. 2019; 11(11):2294. https://doi.org/10.3390/w11112294
3. Khaleel Rana I, Ismail N, Ibrahim Mahamad H. The impact of wastewater treatments on seed germination and biochemical parameter of *Abelmoschus esculentus* L. Procedia Soc Behav Sci. 2013; 91:453-60. https://doi.org/10.1016/j.sbspro.2013.08.443
4. Mostafa SS. Microalgal biotechnology: prospects and applications. Plant Sci. 2012; 12:276-314.
5. Chokshi K, Pancha I, Ghosh A, Mishra S. Microalgal biomass generation by phycoremediation of dairy industry wastewater: An integrated approach towards sustainable biofuel production. Bioresour Technol. 2016; 221:455-60. https://doi.org/10.1016/j.biortech.2016.09.070
6. Kothari R, Pathak VV, Kumar V, Singh DP. Experimental study for growth potential of unicellular alga Chlorella pyrenoidosa on dairy waste water: An integrated approach for treatment and biofuel production. Bioresour Technol. 2012; 116:466-70. https://doi.org/10.1016/j.biortech.2012.03.121
7. Kamrul HM, Sohidul Islam M, Rafiqul Islam M, Nuur Ismaan H, Sabagh A EL. Germination and early seedling growth of mungbean (*Vigna radiata* L.) as influenced by salinity. Azarian J Agric. 2018; 5(2):49-59.
8. Liu C, Subashchandrabose S, Ming H, Xiao B, Naidu R, Megharaj M. Phycoremediation of dairy and winery wastewater using Diplosphaera sp. MM1. J Appl Phycol. 2016; 28(6):3331-41. https://doi.org/10.1007/s10811-016-0894-4
9. Kumar PK, Krishna SV, Naidu SS, Verma K, Bhagawan D, Himabindu V. Biomass production from microalgae Chlorella grown in sewage, kitchen wastewater using industrial CO2 emissions: Comparative study. Carbon Resour Convers. 2019; 2(2):126–33. https://doi.org/10.1016/j.crcon.2019.06.002
10. Wang L, Li Y, Chen P, Min M, Chen Y, Zhu J, *et al*. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. Bioresour Technol. 2010; 101(8):2623-8. https://doi.org/10.1016/j.biortech.2009.10.062
11. Shekhawat DS, Bhatnagar A, Bhatnagar M, Panwar J. Potential of treated dairy waste water for the cultivation of algae and waste water treatment by algae. **Univers J Environ Res Technol**. 2012; 2(1):101-4.
12. Sajib SA, Billah M, Mahmud S, Miah M, Hossain F, Omar FB, *et al*. Plasma activated water: the next generation eco-friendly stimulant for enhancing plant seed germination, vigor and increased enzyme activity, a study on black gram (*Vigna mungo* L.). Plasma Chem Plasma Process. 2020; 40(1):119-43. https://doi.org/10.1007/s11090-019-10028-3
13. Choi YK, Jang HM, Kan E. Microalgal biomass and lipid production on dairy effluent using a novel microalga, chlorella sp. isolated from dairy wastewater. BBE. 2018; 23(3):333-40. https://doi.org/10.1007/s12257-018-0094-y
14. Lalge A, Terzin F, Djordevic B, Winkler J, Vaverkova MD, Adamcova D, *et al*. effects of wastewater on seed germination and phytotoxicity of hemp cultivars (*Cannabis sativa* L.). Proceedings of 24th International PhD Students Conference 2017. 2017; 652-7.
15. Enamala MK, Enamala S, Chavali M, Donepudi J, Yadavalli R, Kolapalli B, *et al*. Production of biofuels from microalgae - A review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. Renew. Sust. Energ. Rev. 2018; 94:49-68. https://doi.org/10.1016/j.rser.2018.05.012
16. Swetha C, Sirisha K, Swaminathan D, Sivasubramanian V. Study on the treatment of dairy effluent using chlorella vulgaris and production of biofuel (Algal treatment of dairy effluent). Biotechnol Ind J. 2016; 12(1):12-7.
17. Malik S, Shahid A, Betenbaugh MJ, Liu CG, Mehmood MA. A novel wastewater-derived cascading algal biorefinery route for complete valorization of the biomass to biodiesel and value-added bioproducts. Energy Convers Manag. 2022; 256:115360. https://doi.org/10.1016/j.enconman.2022.115360
18. Ummalyma SB, Sukumaran RK. Cultivation of microalgae in dairy effluent for oil production and removal of organic pollution load. Bioresour Technol. 2014; 165:295-301. https://doi.org/10.1016/j.biortech.2014.03.028

19. Marks EAN, Minon J, Pascual A, Montero O, Navas LM, Rad C. Application of a microalgal slurry to soil stimulates heterotrophic activity and promotes bacterial growth. Sci Total Environ. 2017; 605-606:610-7. https://doi.org/10.1016/j.scitotenv.2017.06.169
20. Zaouri N, Cheng H, Khairunnisa F, Alahmed A, Blilou I, Hong PY. A type dependent effect of treated wastewater matrix on seed germination and food production. Sci Total Environ. 2021; 769:144573. https://doi.org/10.1016/j.scitotenv.2020.144573
21. Fabris M, Abbriano RM, Pernice M, Sutherland DL, Commault AS, Hall CC, *et al*. Emerging technologies in algal biotechnology: toward the establishment of a sustainable, algae-based bioeconomy. Front Plant Sci. 2020; 11:279. https://doi.org/10.3389/fpls.2020.00279
22. S Dhanam. Effect of dairy effluent on seed germination, seedling growth and biochemical parameter in paddy. Bot Res Int. 2009; 2(2):61-3.
23. Naveed S, Huma Z, Rashid A, Ullah A, Khattak I. Effects of domestic and industrial waste water on germination and seedling growth of some plants. Curr Opin Agric. 2012; 1(1):27-30.
24. Dash AK. Impact of domestic waste water on seed germination and physiological parameters of rice and wheat. IJRRAS. 2012; 12(2):280-6.
25. Mosse KPM, Patti AF, Christen EW, Cavagnaro TR. Winery wastewater inhibits seed germination and vegetative growth of common crop species. J Hazard Mater. 2010; 180(1-3):63-70. https://doi.org/10.1016/j.jhazmat.2010.02.069
26. Banupriya G, Uma, Gowrie. A study on microbial diversity of dairy effluent and its impact on growth of different plant species. Int J Curr Sci. 2012; 2012:71-7.
27. Sasikala T, Poongodi N. Effect of dairy effluent on the morphological and biochemical parameters of Black Gram (*Vigna mungo* L. Hepper). IUP Journal of Biotechnology. 2010; 4(2).
28. Kapil J, Mathur N. The Impact of Dairy Effluent on Germination Parameters of Seeds of Mung bean (*Vigna radiata*) and Mustard (*Brassica nigra*). Int J Economic Plants. 2020; 7(4):170-5. https://doi.org/10.23910/2/2020.0386
29. Singh PP, Mall M, Singh J. Impact of fertilizer factory effluent on seed germination, seedling growth and chlorophyll content of gram (*Cicer aeritenum*). J Environ Biol. 2006; 27(1):153-6.
30. O S, A B, N A, Mansour HB. Characterization of Industrial Dairy Wastewater and Contribution to Reuse in Cereals Culture: Study of Phytotoxic Effect. Austin J Environ Toxicol. 2016; 2(2):1013.
31. Mehta A, Bhardwaj N. Phytotoxic effect of industrial effluents on seed germination and seedling growth of *Vigna radiata* and *Cicer arietinum*. Glob J Bio-sci Biotechnol. 2012; 1(1):1-5.
32. Umamaheswari J, Shanthakumar S. Efficacy of microalgae for industrial wastewater treatment: a review on operating conditions, treatment efficiency and biomass productivity. Rev Environ Sci Biotechnol. 2016; 15(2):265-84. https://doi.org/10.1007/s11157-016-9397-7
33. Barrera Bernal C, Vazquez G, Barcelo Quintal I, Laure Bussy A. Microalgal dynamics in batch reactors for municipal wastewater treatment containing dairy sewage water. Water Air Soil Pollut. 2008; 190(1-4):259-70. https://doi.org/10.1007/s11270-007-9598-3
34. Komilis DP, Karatzas E, Halvadakis CP. The effect of olive mill wastewater on seed germination after various pretreatment techniques. J Environ Manage. 2005; 74(4):339-48. https://doi.org/10.1016/j.jenvman.2004.09.009
35. Enaime G, Bacaoui A, Yaacoubi A, Belaqziz M, Wichern M, Lubken M. Phytotoxicity assessment of olive mill wastewater treated by different technologies: effect on seed germination of maize and tomato. Environ Sci Pollut Res. 2020; 27(8):8034-45. https://doi.org/10.1007/s11356-019-06672-z