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Phycoremediation of Rice Parboiling Industry Wastewater by Micro Algae and Utilization of Treated Water for Crop Production

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Abstract— The screening of freshwater microalgae which is grown in the outdoor paddy fields for phycoremediation of rice parboiling industries wastewater is investigated in the current project. Diluted sample of wastewater with distilled water in 1:1 ratio is denoted as Raw and Distilled Water (RDW) sample and diluted sample with tap water in 1:1 ratio is denoted as Raw and Tap Water (RTW) were allowed for phycoremediation. The highest growth of microalgae biomass has increased and shown great biomass in RDW sample of rice parboiling industries wastewater. At Day 15 approximately 13.72×10^6 cell/mL with the highest removal of nitrate nitrogen, ammonia nitrogen, total nitrogen, phosphate and total organic carbon (BOD and COD) are 82.62%, 98.23%, 87.66%, 76.26, 93.32% and 95.32% respectively. RTW wastewater was able to reach maximum growth by Day 18 where cell concentration was approximately 19.7×10^5 cell/mL. Significantly reduction for some nutrients such as nitrate nitrogen, ammonia nitrogen, total nitrogen, phosphate and total organic carbon (BOD and COD) were observed. These findings provide good indication of microalgae growth and phycoremediation of rice parboiling industries wastewater. The information from these findings could be of potential use for biotechnology industries, for further development of bio-based product from microalgae biomass. The current study is extended to know the impact of raw undiluted rice parboiling industries wastewater and treated i.e., phycoremediation by RDW and RTW on Bengal gram (*Cicer arietinum*) for germination, growth, physiological and biochemical response. Plant growth is measured using growth analysis parameters such as, % germination, germination index, mean germination time, relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), leaf weight ratio (LWR), specific leaf area (SLA), specific leaf weight (SLW), leaf area duration (LAD). The physiological response of crop irrigated with undiluted raw rice parboiling industry wastewater, treated RDW and treated RTW are calculated by measuring % phytotoxicity, % inhibition, tolerance indices, seed vigor index. The biochemical response of seedlings irrigated with RRW, RDW and RTW are calculated by measuring total carbohydrates, total protein, chlorophyll pigment concentrations and peroxidase enzyme activity. The obtained results concluded that phycoremediation by RDW and RTW has shown positive growth and increased biochemical response of *Cicer arietinum.*

Keywords— Phycoremediation, Micro algae, Wastewater treatment, Parboiled rice industry wastewater, Agricultural reuse, Bengal gram, Physiological response, Biochemical response.

I. INTRODUCTION

Economic development of India widely depends on agriculture and rice is the major crop with largest cultivation area. Recently it reaches a record of high production of 104.32mt (2017–18). This high production has needed to cater the need of population and emerged as an important industrial activity in small and medium scale sectors. Water-intensive industrial activity i.e., rice par-boiling [1], the most popular and widely adopted method to produce high quality rice. it is an important aspect of rice processing which determines to large extent. It is a hydrothermal treatment of rice kernels. Parboiled rice sounds like it’s precooked, but it’s not. Instead, it’s processed quite differently from other types of rice. Water is the most important factor involves in the preprocessing of rice. After processing a certain quantity of water will be exerted as wastewater, rather than the absorbed water by paddy. The effluents discharged by these rice processing industries pose an ecological hazard to human beings as well as pollute the water and soil, as it contains high amount of organic matter, high chemical oxygen demand (COD), high biological oxygen demand (BOD) and high amount of phosphates. Therefore, the treatment of such effluents is highly essential to render the effluents suitable for discharge into surface water or on land.

II. RELATED WORK

There are various successful treatment models proven by many researchers such as Adsorption [2] Coagulation / Flocculation [3-4], Bio remediation [5-6], treatment by bio reactor, photo-remediation, chemical treatment and phytoremediation [7]. These models successfully helped in removal of nutrients. But less attention is being paid towards phycoremediation of rice parboiling industry wastewater by using micro algae. However, few above mentioned conventional treatment systems involve high cost and skillful management which ultimately results in increased production costs and yet to be commercialized. Hence, the present study phycoremediation of rice parboiling industry wastewater using micro algae to utilize the treated water as irrigation sources for crop production is carried out.

Aims& Objectives:

The main objective of the present study is the evaluation of phycoremediation capacity of selected microalgae in the removal of various physical and chemical parameters of rice parboiling industry wastewater. A special attempt is carried out by taking Bengal gram (*Cicer arietinum*) seeds. To evaluate the impact of treated and untreated wastewater on germination, growth, physiological and biochemical responses.

In order to achieve this objective, the investigations carried out were as follows:

- Physico-chemical characterization of rice parboiling industrial wastewater, collected from industries to find out chemical complex nature of rice parboiling industrial effluent.
- Collection, screening and identification of various microalgae from the paddy fields.
- Optimization of environmental parameters for the treatment of rice parboiling industrial wastewaters by algae using a photoreactor.
- Physicochemical characterization of rice parboiling industrial wastewater after phycoremediation to evaluate the removal efficiency of microalgae used in treatment.
- Determination of germination, growth, physiological and biochemical response of Bengal gram (*Cicer arietinum*) under treated (RDW & RTW) and untreated rice parboiling industrial (RRW) wastewaters.

III. Methodology

The experimental methodology is categorized into 5 different stages as follows:

1. Collection & characterization of rice parboiling industrial wastewater
2. Collection, isolation & Identification of microalgae
3. Optimization of algal growth parameters and phycoremediation of rice parboiling industry wastewater.
4. Characterization of treated industrial wastewater (or) removal efficiency of microalgae used in current investigation.
5. Potential use of treated water (RDW and RTW) for *Cicer arietinum* crop production under controlled condition.

1. Collection & characterization of rice parboiling industry wastewater

The water samples used in the investigation are collected in the plastic bottles from rice parboiling industry. During sampling, temperature and pH were recorded using thermometer and Hanna portable pH meter respectively. The collected samples were transferred to institute laboratory for analysis, using APHA standard methods [8]. Total alkalinity and acidity are determined using titrimetric method. For determining turbidity, Turbidity meter was used and results expressed in NTU. Total hardness determined by EDTA titrimetric method and chemical oxygen demand (COD), biological oxygen demand (BOD) was calculated according to standard testing protocol of APHA 2nd edition. Heavy metals are determined using atomic absorption spectrophotometer (AAS).

2. Collection, isolation and identification of micro algae

(a) Sample collection from paddy field

Water samples with visible micro algal population were collected from the paddy fields that are located in Green Fields Institute of Agriculture, Ibrahimpatnam, Ranga Reddy, Telangana, India. All the samples from the fields are collected in 50 mL tubes and maintained in the refrigerated conditions while transferring to the laboratory.

(b) Isolation

In order to isolate single micro algal species from the field water samples, standard plating methods were used. Multiple media recipe’s were utilized to isolate the colonies. The field samples need to be diluted initially to aid in the isolation process. Sterilized plastic petri dishes (100 × 15 mm) containing approximately 40 mL of agarized medium are used to plate these diluted samples. One milliliter of the diluted sample is transferred to a media plate and evenly spread across the surface. Inoculated plates are now placed in a temperature-controlled incubator (20-25°C, approximately 27 μE/m/s) where the algae are allowed to grow for about 14 days. Grown algae cultures are streaked using sterile technique onto additional sets of nutrient media plates and placed back in the incubator for isolation. This method of streaking should be repeated until isolation into axenic unialgal cultures are achieved. The number of colonies that were transferred from each dilution plate onto other nutrient media plates depends on the amount of contamination, identification of the colonies present based on the colony morphology and the microscopic cellular morphology of each isolate.

1) (c). Morphological identification

Microalgal and cyanobacterial cultures, initially are separated based on morphological examination of the colonies on agar nutrient media. This general classification method is only used to distinguish isolates on the most basic level. Identification of these isolates to the genus level is based on the morphology of the individual cells followed by microscopic examination according to Prescott 1975 [9].

3. Optimization and cultivation of algal biomass for phycoremediation

The isolated pure colonies of micro algae are incubated in BG11 medium under light intensity of 30 µmol photon m^2/s at 30°C for 14 days. BG11 contains 17.6 mM $NaNO_3$, 3.0 mM $MgSO_4.7H_2O$, 0.24 mM $CaCl_2.2H_2O$, 0.188 mM Na_2CO_3, 0.18 mM K_2HPO_4, 27.9 mM sodium EDTA, 3.12 mM citric acid, 46.3 mM H_3BO_3, 4.2 mM $MnCl_2.4H_2O$, 0.77 mM $ZnSO_4.7H_2O$, 1.66 mM $NaMoO_4.2H_2O$, 0.32 mM $CuSO_4.5H_2O$, 0.17 mM Co $(NO_3)_2.6H_2O$ and 22.5 mM $FeNH_4$ citrate [10]. After 14 days by using centrifuge, the biomass is separated from media.

(a). Chlorophyll measurement

100 µL of cell culture mixed with 900 µL of absolute methanol. These are mixed by vortexing and incubated in darkness for one hour before centrifugation. It is then centrifuged at 12000xg for 2 minutes. Chlorophyll concentration of extract is determined by measuring the absorbance at 665 nm for cyanobacteria and both 665 and 650 nm for green algae and calculated using the formula described by Gandhi et al., 2017 [11].

(b). Phycoremediation

The pure colonies of micro algae centrifuged, the biomass was collected and allowed for microscopic identification. Totally 8 pure algal colonies were isolated and identified (Figure-2 & 3). 3 gm wet biomass of each microalgae culture is transferred into a beaker and final biomass measured was 24 gm by using electrical weighing balance. The obtained microalgal culture is mixed well to make composite sample and then inoculated into 20 liters of raw/ undiluted rice parboiling industries wastewater. Which is denoted as raw rice parboiling industry wastewater (RRW), and another set of photoreactor is filled with 10 liters of distilled water and 10 liters of raw rice parboiling industry wastewater and inoculated with 24 gm of mixed microalgae and denoted as raw water diluted with distilled water (RDW), the schematic diagram of overall water treatment procedure shown in figure-1. One more set of photoreactor is filled with 10 liters of raw rice parboiling industry wastewater and 10 liters of tap water (water which is generally used as source of irrigation for the research farms of Green Fields Institute of Agriculture Research & Training) and inoculated with 24 gm of mixed microalgae culture. This set of treatment is denoted as raw rice parboiling wastewater diluted with tap water (RTW). All the experiments were performed in triplets and the mean values are taken for analysis of results.

4. Physico chemical analysis of treated rice parboiling industry wastewater

The pollutant/nutrient removal efficiency of microalgae are determined at regular time intervals (2, 5, 8, 10, 12, 15 and 20 days) by collecting water samples used for phycoremediation. For analysis APHA 2^{nd} edition standard methods are used.[8].

5. Potential use of treated water for crop production under controlled condition

Current experiments were conducted for 30 days at Green Fields Institute of Agriculture Research & Training, Ibrahimpatnam, Ranga Reddy, Telangana, India, to evaluate the impact of phycoremediated rice parboiling industry wastewater on Bengal gram (*Cicer arietinum*). For this the soil samples were collected from open fields located at research institute during the winter season. The collected soil samples are initially dried under sunlight for four days and made free from all vegetation, solid & unwanted materials. Then these soil samples are allowed for physicochemical analysis according to standard testing procedures stated in APHA 2^{nd} edition and remaining soil is transferred into plastic tubes for further experiments [12-13]. The results of physicochemical analysis of collected composite soil samples are depicted in table-1.

Into a series of 12 plastic tubs equal quantity of soil (≈ 2 kg/tub) was transferred and three tubs were labeled as C (Control), a set of three tubs labeled as RDW-TW (crop irrigated with phycoremediated RDW treated water), another set of three tubs labeled as RTW-TW (crop irrigated with phycoremediated RTW treated water) and final set of three tubs named as RW (crop irrigated with rice parboiling industry wastewater without any treatment). All the experiments were carried out in triplets and mean values were recorded for analysis of results.

Seed Selection & Seed Treatment

The seeds of Bengal gram (*Cicer arietinum*), chickpea used in current investigation are purchased from the local market, located at Ibrahimpatnam and the seeds collected were certified and pretreated. Hence, there is no further pretreatment performed before sowing the seeds into the experimental tubs.

The set of 30 seeds were presoaked into solution-A (50 ml of RDW phycoremediated water) and denoted as RDW-TW treatment. The second set of 30 seeds were soaked in solution-B (50 ml of RTW phycoremediated water) and denoted as RTW-TW treatment. The third sets of 30 seeds were soaked in solution-C (50 ml of untreated/raw rice parboiling industry wastewater) and denoted as RW treatment. A set of 30 seeds were sown after soaking them in normal bore well water and considered as control (C). The pre soaked Bengal gram (*Cicer arietinum*) seeds in solution – A, B, C and RW were sown into respective tubs, randomly and irrigated with respective treated water immediately. The water used for irrigation for control treatment was allowed for physicochemical analysis [14-16] and results were depicted in table-2. From the time of seed sowing, the experimental tubs are irrigated once a day regularly with respective treated, untreated/raw and bore well water to maintain soil moisture at saturation level. The experimental setup is kept in open area for better sunlight and air.

Growth Analysis

The following data are required to calculate different growth parameters in order to express the instantaneous values and mean values over the time intervals. In the following discussion W, WL, WS and WR are used to represent the dry weights of total plant, dry leaves, stem and roots respectively. Whereas A is the leaf area.

Relative Growth Rate (RGR)
Blackman coined the term RGR, which is defined as the rate of increase in dry matter per unit of dry matter already present. This can also be referred as Efficiency index, since the rate of growth is expressed as the rate of interest on the capital. It provides a valuable overall index of plant growth. RGR can be calculated by using the following formulae [17].

$$Relative\ Growth\ Rate = \frac{loge^{W_2} - loge^{W_1}}{T_2 - T_1}$$

Net Assimilation Rate (NAR)
The NAR is the measure of, the amount of photosynthetic product going into plant material i.e., it is the estimate of net photosynthetic carbon assimilated by photosynthesis - the carbon lost by respiration. The NAR can be determined by measuring plant's dry weight and leaf area, periodically during growth and is commonly reported as grams of dry weight increase/ square centimeter of leaf surface/particular time period. This is also termed as unit leaf rate because the assimilatory area includes only the active leaf area in measuring the rate of dry matter production. The mean NAR over a time interval from T_1 - T_2 is given by

$$NAR = \frac{W_2 - W_1}{T_2 - T_1} X \frac{loge^{A_2} - loge^{A_1}}{A_2 - A_1}$$

Leaf Area Ratio (LAR)
The LAR is a measure of proportion of the plant which is engaged in photosynthetic process. It gives relative size of the assimilatory apparatus. It is also called as capacity factor. It is defined as the ratio between leaf area in square centimeters and total plant dry weight. It represents leafiness character of crop plants on area basis [18].

$$Leaf\ Area\ Ratio = \frac{A}{W}$$

Leaf Weight Ratio (LWR)
It is one of the components of LAR, and it is defined as the ratio between grams of dry matter in leaves and total dry matter in plants. LWR is dimensionless, Since the numerator and denominator are on dry weight basis. It is the index of leafiness of the plant on weight basis.

$$Leaf\ Weight\ Ratio\ (LWR) = \frac{W_L}{W}$$

Specific Leaf Area (SLA)
It is one more component of LAR and it can be defined as the ratio between leaf area in cm^2 and total leaf dry weight in grams. This is used as a measure of leaf density. The mean SLA can be calculated by using the following formulae.

$$Specific\ Leaf\ Area\ (SLA) = \frac{A}{W_L}$$

Specific Leaf Weight (SLW)
The reciprocal of SLA is SLW. It is the ratio between total dry weight and leaf area. It indicates the relative thickness of the leaf of different genotypes.

$$SpecificLeafWeight(SLW) = \frac{W_L}{A}$$

Leaf Area Duration (LAD)
It is usually expressed as a measure of leaf area integrated over a time period. Some takes into account both the magnitude of leaf area and its persistence in time. It also represents the leafiness of the crop growing period. Thus, the unit of measurement of LAD may be in day or weeks or months [19].

$$LeafAreaDuration(LAD) = \frac{LA_1 + LA_2\ (T_2 - T_1)}{2}$$

Plant Sampling and Analysis
A seed was considered as germinated when root had emerged more than 2 mm. The number of germinated seeds per time is termed as seed germination rate. Germination percentage and tolerance indices determined by using the following formula [20].

$$\%\ of Germination = \frac{NumberofSeedsGerminated}{TotalNumberofSeedsPlanted} X\ 100$$

$$Germination\ Index\ (GI) = \sum_{i=1}^{k} \frac{No.of\ germinated\ seeds}{the\ count\ day}$$

Where i=1 day one, k is the last day of observation.

$$Mean\ Germination\ Time\ (MGT) = \frac{\sum_{i=1}^{k} n_i\ t_i}{\sum_{i=1}^{k} n_i}$$

Where t_i is the time from day one of the observation to the last day of observation, n_i is an observed number of germinated seeds every day and k is the last germination day of observation.

$$Mean\ Germination\ Rate\ (MGR) = \frac{1}{Mean\ Germination\ Time}$$

$$Co-efficient\ of\ variation\ of\ the\ time = \frac{S_t}{Mean\ Germination\ Time} X\ 100$$

Where S_t is standard deviation of germination time

$$Toleranceindices = \frac{Meanrootlengthoftreatedseed}{Meanrootlengthofcontrol}$$

The inhibition of seedling growth was expressed according to the formula [21]

$$\%inhibition = \frac{Lengthofcontrol - Lengthoftreatedseed}{Lenghtofcontrol} X\ 100$$

Seedling Vigor Index
Seedling vigor index are that properties of the seed, which determine the levels of activity and performance of seed during germination and seedling emergence. It is a single measurable property like germination describing several characteristics associated with various aspects of the performance of seed. Seedling vigor index is calculated by using formula: [22, 23]
SVI = Germination percentage × Seedling length.

Percentage Phyto-toxicity
Percentage phytotoxicity of heavy metals on root and shoot growth of Bengal gram are calculated the regular time

intervals (5 to 30 days of seedling growth). The following formula is used for calculating the percentage phytotoxicity [24].

$$\%Phytotoxicity = \frac{\frac{S}{R} length of control - \frac{S}{R} length of treated seed}{\frac{S}{R} length of control} X\ 100$$

Estimation of Biochemical Attributes

Biochemical attributes were studied in term of photosynthetic pigments. The chlorophyll-a, chlorophyll-b and total chlorophyll (a + b) can be determined spectrophotometrically. Initially, Leaves were cut into small pieces, mixed thoroughly and 0.25 g of leaves are taken into a mortar to grind them finely by using pestle with 25 ml of 80% acetone for 5 minutes. The homogenate is then filtered through filter paper (Whatman No.42), and then it was made up to a volume of 25 ml with 80% acetone. By applying Anthrone method, the total Carbohydrates were determined, total proteins are determined through Biuret method and peroxidase activity using O-dianisidine method enzymatically [25-27].

Extract Monitoring by Spectrophotometer

After the extraction, chlorophyll contents are monitored by UV-Vis's spectrophotometer [25]. The optical density/absorbance of each solution are measured at 663 and 645 nm against 80% acetone blank in 1 cm quartz cuvette at room temperature. The Arnon's equation was used to calculate the amount of chlorophyll-a, chlorophyll-b and total chlorophyll (a + b) [26, 27]:

Chl a ($mg \cdot g^{-1}$) = [(12.7 × A663) − (2.69 × A645)] × ml acetone/mg leaf tissue,

Chl b ($mg \cdot g^{-1}$) = [(22.9 × A645) − (4.68 × A663)] × ml acetone/mg leaf tissue,

Total Chl = Chl a + Chl b.

Statistical Analysis

Data is statistically analyzed using one-way ANOVA on GraphPad Prism 6.01 software [28]. The results presented are mean ± S.D. (standard deviation) and data from different treatments and control are compared by using Duncan's multiple-range test at $p < 0.05$.

IV. RESULTS AND DISCUSSION

1. Isolation & Morphological Identification of Microalgae

The pure colonies of microalgae were screened, which were collected from the paddy field water samples. Then allowed for morphological identification under microscope. From the identification of cultures that were produces the study 4 and 4 genus were identified belonging to Cyanophyta and Chlorophyta respectively.

Cyanophyta: *Nostoc* (Figure-2a) was observed under microscope with cells arranged in beadlike chains that are grouped together in a gelatinous mass (Prescott 1975). These belongs to genus of blue green algae containing two pigments, blue phycocyanin and red phycoerythrin, and chlorophyll which has the ability to fix nitrogen in specialized cells called heterocysts. *Anabaena macrospora* (Figure-2b) was observed during microscopic identification having barrel like cells and interspersed enlarged spores. Trichomes are planktonic, straight and somewhat ellipsoid (Prescott 1975). *Spirulina* sp. (Figure-2c) was observed under microscope during examination having disk shaped soft cells and was thick bluish green in colour. *Spirulina* is a genus belongs to gram negative bacteria with soft cell walls that consist of complex sugars and proteins (Prescott 1975). An irregular shaped dark green-coloured colonies containing 2-8 spherical cells enclosed by a transparent hyaline was also observed under microscope and identified as *Chroccoccus* sp. (Figure-2d) with help of images and description reported by Prescott 1975.

Chlorophyta: *Scendesinus* sp. (Figure-3a) colonies were found during microscopic study. it consists of four naviculoid cells arranged in single series, spines on terminal cells 2 at each pole, which are long and curved. The spines on apices of inner cells are observed short and straight (Prescott 1975). A green coloured unicellular, spherical, individual colonies in the range of 10 µM – 14 µM are observed under microscope and were identified as *Chlorella vulgaris* (Figure-3b) with help of description and images reported by Prescott 1975 (page no. 237). Green coloured unicellular, flagellated dense colonies were observed under microscope and were identified as *Chlamydomonas* sp. (Figure-3c) according to Prescott 1975. The cells of most *Chlamydomonas* species consist of non-cellulosic membrane (Theca) a stigma (eyespot) and cup shaped chloroplast. colonies of elliptical, flattened and compressed cells with four flagella of equal length were found under microscope and are identified as *Tetraselmis* sp. (Figure-3d) with help of Prescott 1975 description reported in algal isolation from lake water.

2. Experimental Conditions

The average temperature of wastewater samples recorded during the period of sampling for undiluted rice parboiling industry wastewater is 26–33°C. while it is 25–30°C for RDW, RTW diluted rice parboiling industry wastewater inoculated for algal biomass treatment system. The mean minimum, maximum ambient temperatures and relative humidity during the study period recorded are 26°C and 35°C and 41%-58% respectively. Comparatively lower temperatures observed in algal biomass treatment system with diluted water i.e., RDW and RTW is due to the presence of covered dense biomass [29].

3. Physicochemical Analysis of Rice Parboiling Industry Wastewater

The results of physicochemical analysis report of rice parboiling industry wastewater study are summarized in table-3. The undiluted raw wastewater had an average COD value 1645 mg/L and BOD_5 value 1062 mg/L, COD and BOD_5 ratio being (1.45:1) that often considered for suitability of biodegradability of organics present in the system [30]. The BOD_5: TN (total nitrogen): TP (total phosphorus) ratio of raw wastewater being 35:1.15:1 and further supports for aerobic degradation of the rice parboiled wastewater. The pH of raw wastewater is highly acidic (pH 5.7 to 6.2) and the DO (dissolved oxygen) content is practically absent. The tap water, used to dilute wastewater is found to have neutral pH and had a moderate range of BOD_5 and COD values with less concentration of total nitrogen and total phosphorus. Both the soil used for crop production and water used as source of irrigation were allowed for physicochemical analysis. Obtained results are mentioned in table- 1 (soil) and table-2 (water) respectively.

4. Visual Observation

The algal culture inoculated in the treatment, with undiluted raw wastewater became more turbid and browner by the third day of experiment, gradually turns necrotic in nature and algal cells finally produced unpleasant smell within the fifth day of experiment. Therefore, study using raw wastewater treatment cannot be monitored for rest of the study. Sooknah and Wilkie, 2004 [29] also reported similar results when they studied the nutrient removal in dairy manure wastewater by floating macrophytes. The algal biomasses were observed growing rapidly in both the diluted (RDW & RTW) approaches covering nearly the entire surface area within a week (7 days). The biomass (initial weight 24 gm at time of inoculation) has been increased robustly in the RDW & RTW phycoremediation systems (final weights ∼350 g in RDW and ∼372 gm in RTW) atmost in two weeks. Similar results have also been reported by Fonkou *et al.* 2002 where aquatic plant water lettuce and algal biomass has been used to treat domestic sewage [31].

5. Removal Efficiency of Nutrients

The results of nutrients removal (%) are depicted in Figure-4 (RDW) and Figure-5 (RTW). The removal percentage of ammonia nitrogen in RTW is higher when compared to RDW dilution phycoremediation system. Additional removal of total nitrogen, nitrate nitrogen, total phosphorus and soluble phosphorus were also found higher in RTW compared to RDW. Similar removal percentages are reported by Li et al., 2011 [32], when they treated highly concentrated municipal wastewater for nutrient removal and biodiesel production by *chlorella* sp. Zhou et al., 2017 [33] has also reported similar results when they studied nutrients removal and recovery from saline wastewater by *Spirulina platensis*. Singh et al., 2011 [34], also reported similar type of nitrogen removal when they treated poultry industry wastewater. From the statistical analysis of results, it is observed that both dilutions do not differ significantly. Reduction in total nitrogen content in both treatments due to the uptake of ammonia nitrogen and nitrate nitrogen for the algal growth, volatilization of ammonia and settling. In current study, the reduction rates of total phosphorus show higher values than those of soluble phosphorus in both dilution treatments. This reduction of total phosphorus is mainly due to algal uptake for their growth. Similar results were reported by Cai et al., 2013 [35] when they conducted experiments for nutrients recovery from wastewater streams by microalgae. Lin et al., 2017 [36] has also reported similar type of phosphorus removal when they studied textile wastewater treatment for biogas and microalgae biomass production.

6. Removal Efficiency of Organic Carbon (COD & BOD)

The removal percentage of BOD_5, COD are higher in both RDW and RTW. The results of BOD and COD percentage removal are shown in figure-6 for RDW and RTW treatments respectively. From statistical analysis of results, it is observed that p values of BOD_5, 0.043 (for RDW treatment), 0.052 (for RTW) and COD 0.008 (for RDW), 0.024 (for RTW) which is less than 0.05, supporting the conclusion that removal of BOD and COD differs significantly for different treatments in both the dilution approaches. Suvidha et al., 2016 [37] also reported similar type of results when they treated food industry wastewater under mixotrophic conditions.

7. Removal Efficiency of Turbidity & Colour

The colour of the treated effluent with algae changed from ash grey to green on the 5^{th} day. The change in colour may be due to the growth of algae utilizing organic matter in the effluent that has made the water clear. After centrifugation at 2000 rpm for 2 minutes the samples were allowed for turbidity analysis using nephelometer and observed decreased turbidity which shown in figure-7. Similar types of results are reported by Gulab Singh and Patidar 2020 [38].

8. Removal Efficiency of Solids (TSS and TDS)

The removal percentage of solids increased with increased biomass of algae in both RDW and RTW treatment systems (Figure-8 & Figure-9). The percentage removal of solids (dissolved and suspended) was uptake by the algal biomass during growth and sediments settled. The similar type of results were reported by Kim et al., 2013 [39] in their experiments on growth rate, organic carbon and nutrient removal rates of *Chlorella sorokiniana* in autotrophic, heterotrophic and mixotrophic conditions.

9. Removal Efficiency of Hardness

There was a significant reduction in calcium and magnesium, hardness observed with increased experimental duration (Figure-10 & Figure-11). The obtained results, concluding that the mixed algae used for current study have a vital role in up taking calcium and magnesium from rice parboiling industry wastewater in both dilutions. Similar type of results reported by Mennaa et al., 2015 [40] while working on urban wastewater treatment by seven species of microalgae and an algal bloom for biomass production.

10. Removal Efficiency of Acidity & Alkalinity

The high concentrations of acid and alkali salts create a great non-sense to living biota of fresh or marine eco system. The percentage removal of acidity and alkalinity were increased with increased algal biomass (Figure-10 & Figure-11) in both diluted algal biomass treatment system. Similar type of results are reported by Zhan et al., 2016 [41], when studies conducted on eight fresh water green algae strains for synchronous water purification and lipid production.

11. Seed germination and growth analysis under RRW, RDW and RTW irrigation

The global population increasing day by day and reaching food scarcity line. Hence, there is a need to double the food production by optimizing the environmental parameters to improve crop yield, with less amount of water at water scarcity areas. The aim of present experiment was to study the effect of seed germination and seedling growth under different irrigation water conditions i.e., untreated raw rice parboiling industry wastewater (RRW), phycoremediated RDW water and phycoremediated RTW water. Scientific literature and experimental results obtained by Sinha & Dipak 2013 [42], explained role of sewage water. Marthandan et al., 2018 explained role of various toxic and metal pollutants on seed germination and seedling growth of *Cicer arientinum* L and *Pisum sativum* L. The results obtained by their experiments concluded that water quality of sewage and other different pollutants has shown adverse impact on germination and growth of *Cicer arientinum* L and *Pisum sativum* L and decreased seed vigor index, shoot length, root length, fresh and dry biomass of crop and final commercial edible part of *Cicer arientinum* L and *Pisum sativum* L was reported. There is very less attention paid towards reuse of treated wastewater for crop production, especially phycoremediated water and their impact on crop growth and yield of the crop in tropical and sub-tropical areas of India. Taking this factor into consideration the present experiment was carried with RRW, phycoremediated RDW and phycoremediated RTW as source of irrigation to investigate role of treated water's reuse efficiency in plant growth against to surface water/borewell water as positive control and RRW as the negative control.

Bengal gram (*Cicer arietinum*), is an important commercial tropical crop in various dry locations of north and south India. It is commonly called as chickpea, which is a major pulse and plays a vital role in ensuring economic stability and food security. From the past few years due to the increase in global pollution leaded decrease in rate of rain fall and water in ground table. The imbalance in both the ecological, environmental components lead to the unstable yields and low-quality seed production. To overcome the above-mentioned problem the current studies were conducted to study and identify the impact of RRW, RDW and RTW on Bengal gram (chickpea). Growth experiments carried as described in materials, methods and results (Table-4) showed that all the treatments significantly shown difference in the germination percent, germination rate, germination index and vigor index in comparison with the both positive (Bore well water) and the negative controls (RRW). The maximum values of germination percent (90.06%) and rate (96.06 n/d) were recorded in the control treatment. There is decrease in germination percentage and germination growth with RDW and RTW treatments. Less amount of germination found with RRW treatment (38.21%) and the maximum average of mean germination time of seeds with control treatment (5 days) are recorded and minimum values were recorded in seeds irrigated with RRW. The pollutants contain in rice parboiled industry wastewater shown adverse impact on seed viability and vigour resulting less germination and growth. Farhan et al., 2021 [43] also reported the same type of results in his studies conducted on effect of industrial wastewater on wheat germination, growth, yield, nutrients and bioaccumulation of lead. The seedlings irrigated with RRW turned into yellow because of necrosis and started browning from 9^{th} day onwards and has become scaly and totally dried on the 16^{th} day of experiment. Hence, further plant sampling and growth analysis were not performed with respect to RRW treatment.

Shoot length, root length, fresh seedling weight and dry seedling weight of RRW, RDW and RTW were measured at 15 and 30 days' time intervals (Table-5 & 6), and compared with control treatment. The results concluded that control treatment has shown significantly higher seedling vigor, fresh and dry weight compare to RRW, RTW and RDW. There is not much significant change observed seedling irrigated with RTW and RDW. These two treatments shown statistically very much difference with seedlings irrigated with RRW. The obtained results concluded that both the phycoremediated RTW and RDW doesn't show much difference on seed vigor, fresh and dry mass of Bengal gram seedling. The similar sort of results obtained with Rajendra et al., 2010 [44] in the studies he conducted on dairy industry wastewater for seed germination and early seedling growth of soyabeans.

All the biochemical parameters visually total carbohydrates, total proteins, photosynthetic pigments and POD enzyme activities were determined with respect to all treatments and then the results obtained are shown in table-7 and table-8. From the results it is observed that the biochemical content increased when compared to control treatment. The present obtained results concludes that the phycoremediated RTW and RDW are safer to reuse for crop production of Bengal gram in water scarce and low rainfall areas of India.

The plant stress parameters i.e., % phytotoxicity (Figure-12), % inhibition (Figure-13) and tolerance indices (Figure-14), are calculated according to formula which is described in the above-mentioned materials and methods section. From the obtained results it was clear phycoremediated RTW and RDW which are used in current study doesn't show any negative impact or toxicity with respect to growth parameters. Whereas, seedlings irrigated with RRW has shown greater phytotoxicity and inhibition of growth when compare to control treatment.

The seedling growth analysis parameters such as relative growth rate, net assimilation rate, leaf area ratio, leaf weight ratio, specific leaf area, specific leaf weight, leaf area duration of Bengal gram (*Cicer arietinum* L.)

seedlings are estimated and RRW treatment only resulted in negative and decreased growth and there is no significant change were observed with RTW and RDW treatments when they are compared to control.

Discussion

The characteristics of effluents generally has varied considerably within industries depending on the processes involved. In rice parboiling industry, various operations like cleaning, washing soaking, boiling etc., produce wastewater which is generally rich in the organic matter thus leading to the creation of odorous and high BOD and COD contents in water. The discharge of that wastewater into the environment without giving any kind of treatment poses significantly high risk for public health in one hand and causing environmental pollution on other hand. Hence, there is a serious need to treat those effluents before they are flown into the environment. Physicochemical analysis which is generally performed as per WHO guideline in rice parboiling effluent includes pH, DO, TDS, TSS, BOD, COD and hardness. Algae are known to grow more rapidly in nutrient-rich water and are able to remove those unwanted and toxic nutrients from wastewater successfully. In the current study, the effect of the micro algal consortium on physicochemical properties of rice parboiling industry wastewater has been carried out. The analysis of physicochemical characteristics of rice parboiling industry wastewater by using traditional method has been carried out with an algal consortium based parboiled wastewater treatment and their efficiency was checked. Various parameters like BOD, COD, TDS, TSS, calcium, magnesium hardness and other nutrients including heavy metals were measured which showed differences before and after treatment. The morphological identification of micro algae screened from paddy fields was made by microscope and the algae were identified based on their morphological characteristics and found to be inclusive *Nostoc*, *Anabaena macrospora*, *Spirulina* sp., *Chlorella vulgaris*, *Chroccoccus* sp., *Scendesinus* sp., *Chlamydomonas* sp., *Tetraselmis* sp., A large number of pollutants impart colour and odour to the water, making them unaesthetic. The removal of those colours from wastewater is more important as they also contribute to major BOD load. In the current study colour of the effluents treated with mixed algal consortium changed from ash grey on the day of collection to green after 5 days of algal treatment. The change is colour may be considered due to the use of the nutrients present in the effluent by the algal consortium. These findings are in concordance with Kotteswari et al., 2012 [45]. COD is used to measure pollution load in terms of quantity of oxygen required for oxidation of inorganic matter. The resulted reduction of COD indicated that the microalgae could utilize inorganic carbon in the wastewater as a source of energy and as a substrate for its cell growth. In this study, the reduction of COD is higher to the study reported by Kotteswari et al., 2012 who reported a reduction of COD levels to 24.69% when the dairy effluent was treated with *Spirulina platensis* and Gani et al., 2014 [46] who reported a reduction of COD in dairy effluent after treatment with microalgae *Botryococcus* sp. to be 4.8%. Presence of high BOD indicates the high quality of biological oxidizable organic matter present in the effluent. High contents of BOD cause depletion in oxygen. In the current study, the BOD levels were reduced from 1062 mg/L to 72 mg/ L (93.32%) in RDW system and 1062 mg/L to 70 mg/L (93.40%) in RTW treatment. The reduction in BOD of the present experiment is much higher than the reduction level of 40.25% reported by Kotteswari et al., 2012 using Nostoc sp [45]. The total suspended solids affect the light intensity of water along with it it's influencing the turbidity and transparency. Total suspended solids of the effluent decreased upon treatment with microalgae from initial 176 µg/L to 36.92 µg/L which may be due to the utilization of various nutrients by microalgae and there could have been a conversion of the total suspended solids in the effluent into dissolved materials favourable for algal uptake and assimilation. Shivsharan et al., 2013 reported a reduction in TDS to 9% in treated sewage [47]. The total dissolved solids are a measure of total inorganic salts and other substances that are dissolved in water. The total dissolved solids remained the same after treatment. Alkalinity is a measure of buffering capacity of the water. It is considered as important parameter which indicates the ability of water to neutralize acids from wastewater. In the present study, the alkalinity was reduced by 80% (RDW) and 83% (RTW) after treatment. This is significantly high when compared to Kotteswari et al., 2012 who reported an 18% reduction in alkalinity of dairy effluent using Nostoc sp [45]. The initial phosphate concentration was 1.82 mg/L was reduced to 0.47 mg/L (RDW) and 0.42 mg/L (RTW), the overall phosphate reduction percentage was 74% (RDW) and 77% (RTW). In the present experiment, the calcium & magnesium hardness was reduced to 82% & 86% for RDW and 84% & 93% for RTW can be noticed. The hardness of water mainly depends on the calcium and magnesium salts. Along with this in the current investigation, the performance of microalgal consortium in treatment of rice par boiling industry wastewater was evaluated and the results proved that algal consortium can be used to remove pollutants load and nutrients. The germination, physiological and biochemical behavior of Bengal gram irrigated with RTW and RDW shown positive response. Whereas, growth rate was recorded less compare to control treatment. The results of growth index, seed vigor and biomass are found much higher than results obtained by Farhan et al., 2021 [43]; Rajendra et al., 2010 [44] and Marthandan et al., 2018 [48] in their study when they used industrial wastewater for crop production of wheat, soybean and other crops respectively. Hence, the current studies conclude that the pycoremediated RTW and RDW can be used for crop production in water scarce areas of India.

V. CONCLUSION AND FUTURE SCOPE

Phycoremediation by microalgae is recently gaining a lot of attention for its potentiality in treating wastewater. The current study reveals that, micro algae used in experiments can be successfully used to treat rice parboiling industry wastewater in removing nutrients and also in reduction of organic matter (subjected to use in diluted condition). During batch studies, two dilution approaches has been made herewith (RDW and RTW) in order to effectively use the phycoremediation technology. The results of two different dilution approaches (RDW and RTW) remain practically identical and shown good removal efficiency. The statistical analysis helps to understand that the removal% of BOD, COD and soluble P differ significantly in different treatments (planted/unplanted) however, the scenario of ammonia nitrogen and nitrate nitrogen are different. The study further recommends that harvesting of micro algae after 20 days is an important issue and also need skillful management to score best and successful results. In the present case harvesting at an interval of 20 days is found to be judicious. The study further recommends a pilot-scale as well as full scale field study to finally conclude the success and feasibility of present laboratory-scale research findings. In addition, year-round experiments should be conducted to evaluate the variability of performances due to seasonal changes and other environmental factors. The current study also concludes that the phycoremediated RDW and RTW shown positive growth promontory effect on growth of Bengal gram (*Cicer arietinum* L.) seedlings. The treated wastewater almost enhanced germination and growth compared to the control treatment. These results concluding and solving water crisis problems of various locations of dry land. Further studies are required to know the impact of RDW and RTW at actual field operations.

Figures and Tables

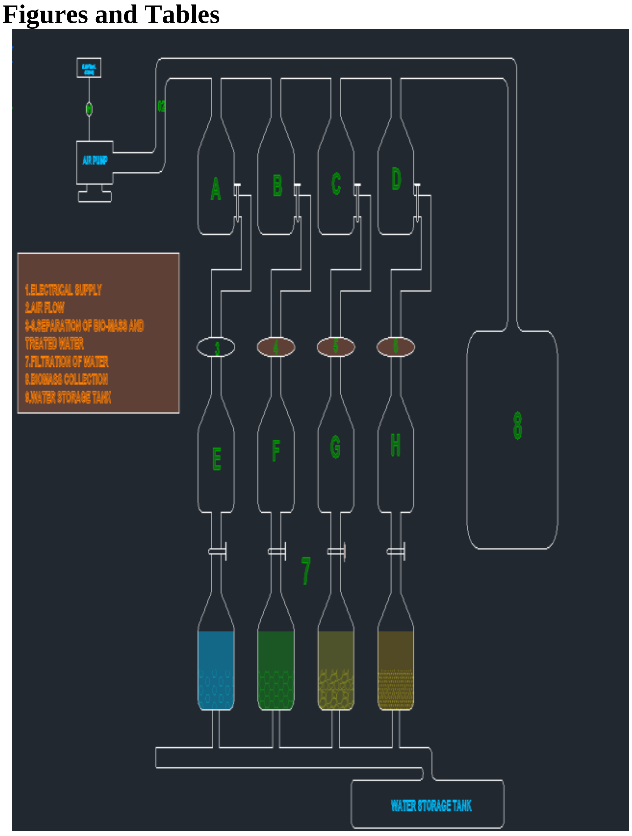

Figure-1: Schematic diagram of experimental procedure

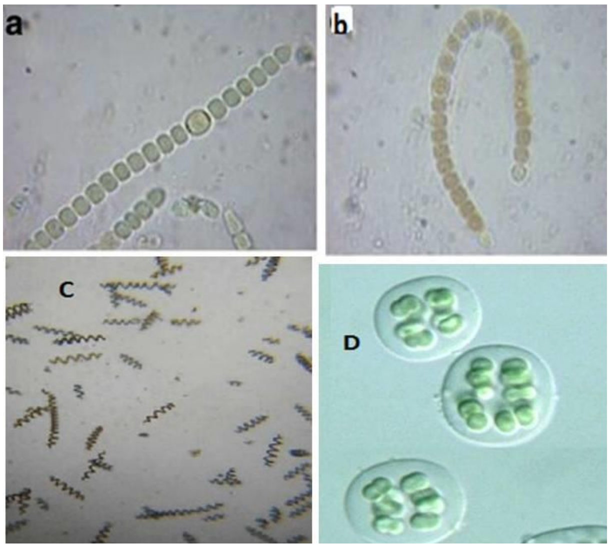

Figure-2: Isolated microalgae (Cyanophyta) from paddy fields for phycoremediation

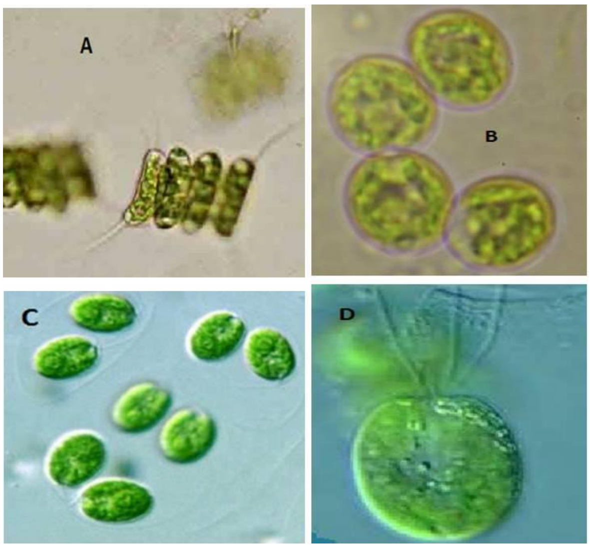

Figure-3: Isolated microalgae (Chlorophyta) from paddy fields for phycoremediation

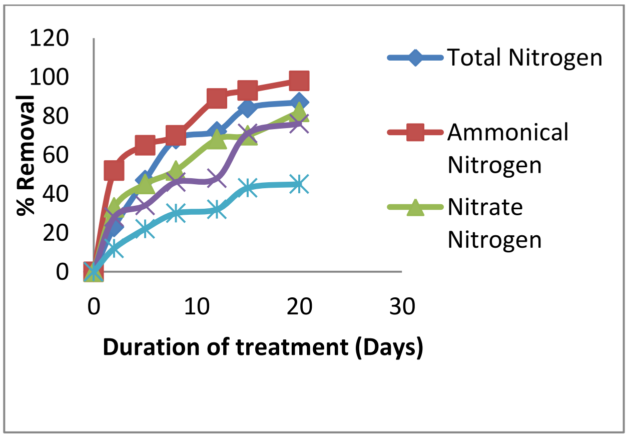

Figure-4: Nutrient removal effeciency of microalgae at RDW experimental treatment condition

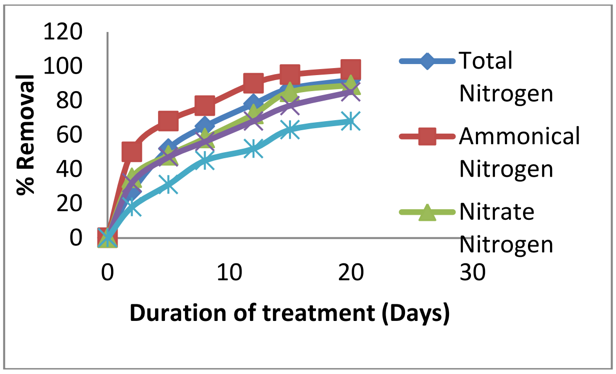

Figure-5: Nutrient removal effeciency of microalgae at RTW experimental treatment condition

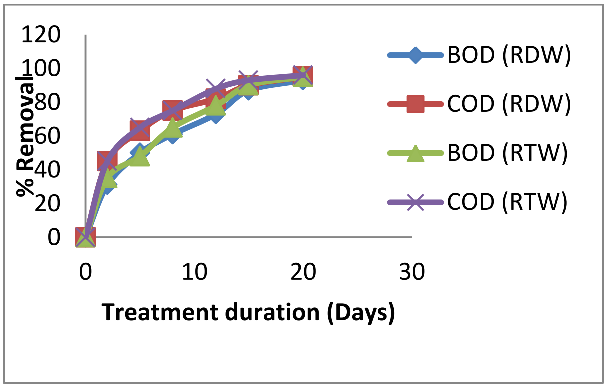

Figure-6: Organic carbon (BOD & COD) removal of microalge used in current study at RDW and RTW dilution treatments.

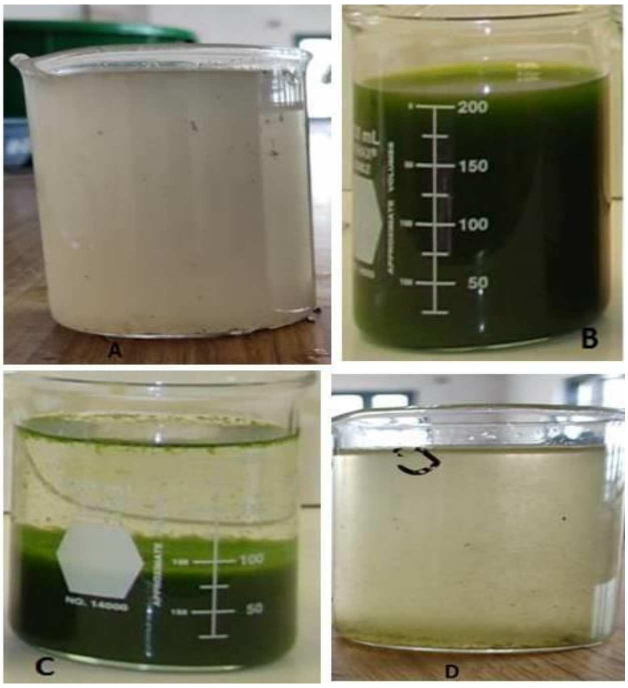

Figure-7: Turbidity and colour of rice Parboil industry wastewater before and after phycoremediation

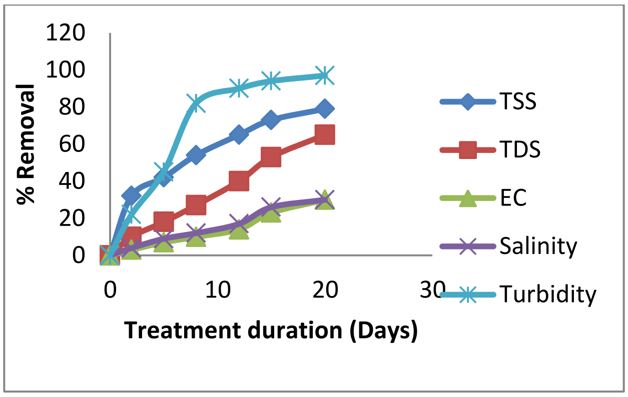

Figure-8: Solids removal effeciency of microalgae at RDW dilution experimental treatment condition

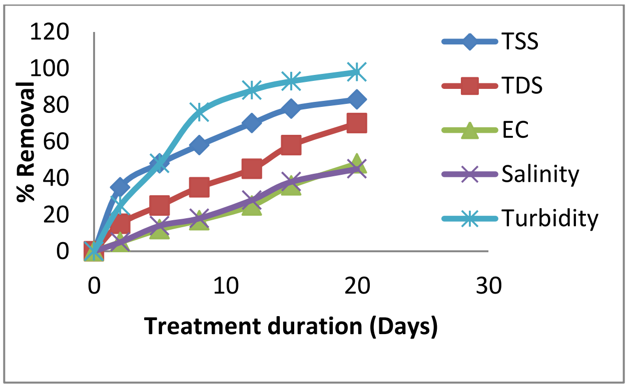

Figure-9: Solids removal effeciency of microalgae at RTW dilution experimental treatment condition

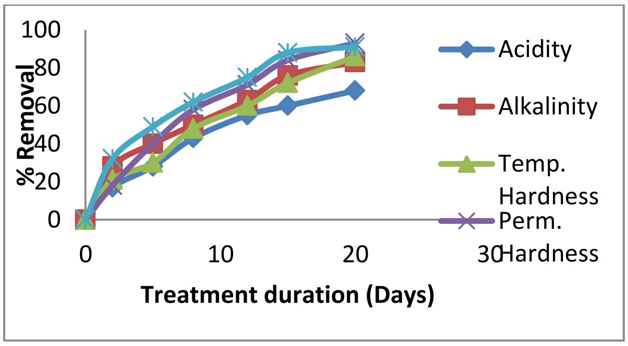

Figure-10: Acidity, alkalinity and hardness removal efficiency of microalgae at RDW dilution remediation condition

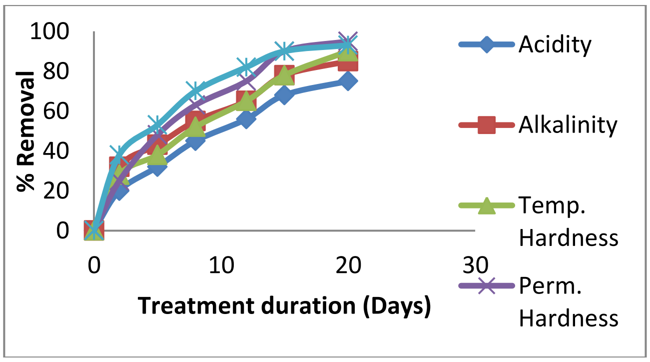

Figure-11: Acidity, alkalinity and hardness removal efficiency of microalgae at RTW dilution remediation condition

Table-1: Soil quality parameters (soil used for seed germination and growth analysis)

S. No	Parameter	Analyzed value
01	pH	7.2
02	EC	0.78 µmhos/cm
03	Moisture (%)	3.2
O4	CEC	172 meq/100 g
05	Nitrate Nitrogen	0.19 mg/ 100 g
06	Nitrite Nitrogen	0.12 mg/100 g
07	Phosphorus	0.53 mg/100 g
08	Sulfates	4.3 mg/ 100 g
09	Potassium	77 mg/ 100 g
10	Calcium	1.2 mg/ 100 g
11	Magnesium	0.7 mg/ 100 g

Table-2: Water quality parameters (water used for irrigation)

S. No	Parameter	Analyzed value
01	pH	6.9 to 7.2
02	EC	130.00 µmhos/cm
03	Salinity	0.03 ppt
O4	Dissolved oxygen	9.72 mg/L
05	Nitrate Nitrogen	0.84 mg/L
06	Nitrite Nitrogen	0.02 mg/L
07	Total hardness	84 mg/L
08	Sulfates	23 mg/L
09	Chlorides	2.7 mg/L
10	Calcium	17 mg/L
11	Magnesium	8 mg/L

Table-3: Physicochemical analysis of rice parboiling industry wastewater

S. No	Parameter	Value	S. No	Parameter	Value
01	pH	8	**16**	Hydroxide + Carbonate	0
02	Conductivity	3.36	**17**	Temporary hardness	246.17
03	Salinity	2.9	**18**	Permanent hardness	335
04	TDS	2.17	**19**	Total hardness	88.3
05	TSS	176	**20**	Turbidity	280
06	DO	0.5	**21**	NH_4-Nitrogen	2.3 mg/L
07	BOD	1062	**22**	NO_3-Nitrogen	1.79 mg/L
08	COD	1645	**23**	Total Nitrogen	4.23 mg/L
09	Acidity	500	**24**	Soluble phosphate	1.03 mg/L
10	Alkalinity (A)	133.33	**25**	Total Phosphate	1.83 mg/L
11	Hydroxide. A	0	**26**	Chromium	-
12	Carbonate. A	0	**27**	Copper	-
13	Bicarbonate. A	133.33	**28**	Arsenic	-
14	Phenolphthalein. A	0	**29**	Lead	-
15	Carbonate + Bicarbonate	133.33			

Table-4: Effects of RRW and phycoremediated RDW, RTW wastewater on germination & growth indices of Bengal gram (Chickpea).

S. No	Treatment	GR (%)	GP (%)	GI	VI
01	Control (Bore well)	96.06 ± 1.87	90.06 ± 0.67	39.01 ± 0.70	25.62 ± 0.52
02	T1 (RRW)	44.00 ± 2.00	38.21 ± 1.92	11.75 ± 0.66	9.38 ± 0.77
03	T2 (RDW)	93.78 ± 1.92	85.63 ± 0.60	28.11 ± 0.86	22.45 ± 1.18
04	T3 (RTW)	98.63± 0.60	88.11 ± 3.85	25.72 ± 0.36	24.28 ± 0.46

The germination, values of growth indices represent the mean ± SE (n = 5). Values obtained by ANOVA concluded that there is significantly different impact in germination index between controls and treatments at $P < 0.05$.

Table-5: Effects of RRW and phycoremediated RDW, RTW wastewater on seedling growth of chickpea at t= 15 days.

S. No	Treatment	Shoot length (Cm)	Root Length (Cm)	Fresh Weight (gm)	Dry Weight (gm)
01	Control (Bore well)	12.30 ±0.46	4.53 ±0.31	0.4± 0.01	0.24 ± 0.01
02	T1 (RRW)	6.50 ± 0.31	1.89 ± 0.23	0.19 ± 0.01	0.07 ± 0.01
03	T2 (RDW)	20.34 ±0.01	10.23 ±0.39	0.82 ± 0.01	0.42 ± 0.01
04	T3 (RTW)	22.19 ±0.06	10.58 ±0.23	0.86 ± 0.01	0.45 ± 0.01

The values represent the mean ± SE (n = 4). Values obtained by ANOVA concluded that there is significantly different impact in germination index between controls and treatments at $P < 0.05$.

Table-6: Effects of RRW and phycoremediated RDW, RTW wastewater on seedling growth of chickpea at t= 30 days.

S. No	Treatment	Shoot length (Cm)	Root Length (Cm)	Fresh Weight (gm)	Dry Weight (gm)
01	Control (Bore well)	17.25 ± 0.03	6.58 ± 0.46	0.58 ± 0.01	0.26 ± 0.01
02	T1 (RRW)	Seedling dried	Seedling dried	Seedling dried	Seedling dried
03	T2 (RDW)	28.34 ± 0.05	12.78 ± 0.39	1.01 ± 0.01	0.73 ± 0.01
04	T3 (RTW)	35.50 ± 0.06	14.12 ± 0.01	1.16 ± 0.01	0.84 ± 0.01

The values represent the mean ± SE (n = 4). Values obtained by ANOVA concluded that there is significantly different impact in germination index between controls and treatments at $P < 0.05$.

Table-7: Effects of RRW and phycoremediated RDW, RTW wastewater on the photosynthetic pigment contents of Bengal gram/chickpea.

S. No	Age of the crop	Treatment	Chl-a	Chl-b	Chl-a +b
01	15 days	Control (Bore well)	2.29 ± 0.06	0.81 ± 0.05	3.10 ± 0.01
02		T1 (RRW)	0.78 ± 0.03	0.12 ± 0.01	0.90 ± 0.01
03		T2 (RDW)	4.25 ± 0.02	1.01 ± 0.07	5.26 ± 0.01
04		T3 (RTW)	4.33 ± 0.02	1.07 ± 0.01	5.40 ± 0.01
01	30 days	Control (Bore well)	3.60 ± 0.03	1.18 ± 0.08	4.78 ± 0.04
02		T1 (RRW)	Seedling dried	Seedling dried	Seedling dried
03		T2 (RDW)	5.26 ± 0.03	1.25 ± 0.01	6.51 ± 0.01
04		T3 (RTW)	5.38 ± 0.02	1.68 ± 0.01	7.06 ± 0.01

The values represent the mean ± SE (n = 4). Values obtained by ANOVA concluded that there is significantly different impact in germination index between controls and treatments at $P < 0.05$.

Table-8: Effects of RRW and phycoremediated RDW, RTW wastewater on the contents of osmolytes and enzymes (Total carbohydrates, protein and peroxidase enzyme activity).

S. No	Age of the crop	Treatment	Total Carbohydrates	Total Proteins	POD enzyme activity
01	15 days	Control (Bore well)	5.08 ± 0.03	2.79 ± 0.06	11.02 ± 0.09
02		T1 (RRW)	2.06 ± 0.09	1.23 ± 0.04	1.59 ± 0.09
03		T2 (RDW)	5.44 ± 0.07	3.53 ± 0.08	15.45 ± 0.08
04		T3 (RTW)	5.99 ± 0.03	3.67 ± 0.02	20.54 ± 0.01
01	30 days	Control (Bore well)	6.94 ± 0.03	3.26 ± 0.04	19.67 ± 0.03
02		T1 (RRW)	Seedling dried	Seedling dried	Seedling dried
03		T2 (RDW)	7.32 ± 0.07	5.12 ± 0.06	26.77 ± 0.09
04		T3 (RTW)	7.79 ± 0.03	5.23 ± 0.01	29.67 ± 0.05

VI. ACKNOWLEDGMENT

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