

Utilization of Agricultural Straw as a Source of Anti Algal Agent for Control of *Microcystis aeruginosa*

K. G. Padmakumar¹, P. R. Remya², T. R. Arathi³, B. Alan⁴, T. Praseetha⁵,
Rekha Bhaskar⁶, Anjana Venu S.⁷, Athulya V Gopal⁸, Anu Pradeep P⁹

¹Director, International Research and Training Centre for Below Sea Level Farming, Kuttanad, Thottappally, Alappuzha, Kerala, India
(Corresponding Author)

^{2, 3, 4, 5, 6, 7, 8, 9}International Research and Training Centre for Below Sea Level Farming, Kuttanad, Alappuzha, Kerala, India

Abstract: Harmful algal blooms (HABs) are a regular occurrence in eutrophic water bodies and their occurrence has been increasing in freshwaters due to climate change. Microcystins are among the most prevalent and potent of the cyanobacterial toxins. As physico-chemical methods such as often employed for its control have been observed to be only with limited success as the use of copper algicides, though effective in managing HABs, often results in negative impacts such as copper toxicity and release of microcystins into surrounding water after cyanobacterial lysis. One alternative approach to the control of algal blooms involves the use of biological control agents. As conventional water treatment processes are not effective in removing these cyanotoxins in water and as it may also enhance the release of cyanotoxins contained within the producer-cells, a cheap economical and novel method for treating micro algae by allelochemicals form rice straw has been successfully demonstrated. The study has demonstrated the possible use natural material, rice straw of rice water extract for control of toxic algae *Microcystis aeruginosa* in laboratory-based investigations.

Keywords: Harmful algal blooms, Microcystin, cyanotoxins *Microcystis aeruginosa*

1. Introduction

Freshwater resources, worldwide are threatened by the presence and increase of harmful algal blooms (HAB). This is largely a direct result of human induced pollution of water bodies, either by discharge of partially treated and nutrient-rich effluents or leaching of fertilizer residue. The Special Report on the Ocean and Cryosphere in a Changing Climate (IPCC, 2019) has directly linked Harmful Algal Blooms to climate change (Gobler, 2020)

HAB's have direct implications for the use of water bodies used for recreation and as drinking water source etc. Many reports have shown the adverse effects of toxins of some such cyanobacterial micro algae on animals and humans, damaging the human organs like liver, kidney and mortality among fishes.(Carmichael, 2013)

Microcystin-LR (MC-LR) is the most toxic and the most frequently encountered toxin produced by the cyanobacteria in the contaminated aquatic environment. Microcystin -LR is a potential carcinogen for animals and humans. (Fatma et al, 2008). Water bodies that thermally warm during summer are often most susceptible to highest concentrations of cyanobacteria. During stratification, the water stratum adjoining the bottom sediments, the hypolimnion, becomes depleted of oxygen and that can help release contaminants such as ammonia, phosphorus, iron and manganese etc in a soluble form. This increase in nutrient levels further stimulates growth of cyanobacteria. Climate change linked increase in surface temperatures and precipitation patterns., also contribute to the incidence of harmful algal blooms (Moore et al., 2008). Shallow surface water sources or small water systems are at a greater risk of eutrophication as small remote communities often lack resources and infrastructure for adequate treatment of such water bodies.

Use of algicides, and chemicals commonly employed for managing HABs, often results in more serious negative impacts. Potential agents such as bacterial and predatory organisms that possess allelochemicals present a possible solution. Cereal grain straws during decomposition have been known to produce inhibitory effects on blue green algae, and hence a laboratory- based study was undertaken to investigate the efficacy of using rice straw exudates to inhibit algal growth.

This study was also intended to quantify the rate of inhibition of the growth of the bloom-forming cyanobacterium *Microcystis aeruginosa*, a toxic blue green alga that occur in freshwaters using decomposing rice straw exudates.

2. Materials and Methods

A Laboratory study was undertaken utilizing algal rich waters from a eutrophied open temple pond at Sankara Narayana Moorthy temple (9° 24''N & 76°21'01''E) located close to the TD Medical College campus at Vandanam, Ambalappuzha North panchayath, where a massive bloom was reported during January- February 2022. (Fig 1)



Figure 1: Location Map

Water quality parameters and cyanobacterial concentration and the bloom incidence were monitored by collecting water samples both from surface and mid depths by a Vandorn sampler, when the pond was afflicted with a massive algal bloom. The water samples were preserved at 4°C till analysis. The physicochemical parameters of the water in the open pond where the bloom appeared were monitored. pH, Salinity, Alkalinity, Hardness, Free CO₂, Dissolved Oxygen, Phosphate, Nitrate, and qualitative and quantitative assessment of algal count were estimated after APHA, (2005). Temperature was measured using a Mercury Thermometer and turbidity of water was measured by Secchi disc method. pH of water was measured electrometrically by Eutech pH meter. Salinity of water sample was estimated by Oakton SALT 6+ salinity meter and the same also confirmed titrimetrically as per Mohr-Knudsen method. Dissolved Oxygen (DO) was determined by the modified Winkler method. The alkalinity of the sample and free CO₂ was estimated as per standard procedures. Phosphate was estimated after Fonselvis and Carlberg (1972) and Nitrites and Nitrates quantified as per procedures described by Mullin and Riley (1955) after APHA (2005).

Samples for algal cell enumeration were collected from mid depths from the temple pond and immediately preserved in 'Lugols solution' as per methods described by Saraceni and Ruggiu (1969) and Thronsdon (1978) and cell counts were enumerated by using a modified Sedgwick-Rafter cell as recommended by Lund et al., (1958) and Frontier (1972). The algal count in the experimental tanks was monitored by microscopic examination and the algal cells were identified during counting., list of species and their abundance was evaluated.

As part of the algal control plan, *in vivo* bioassay was undertaken by exposing the algal rich water to two different concentrations of decomposing rice straw@3g/L (T1) and

@6 g/L (T2) dry weight. For this, fresh paddy straw was collected and was cut into pieces approximately 15 cm in length, and placed loosely in transparent experimental tanks filled with filtered and cleaned soil substrate from the pond. The experiment was set up in five replicates by placing the decomposing straw @ approximately 20 cm below surface in the tanks so the algae were close contact with decomposing straw after Newman (1994). Straw bundles were allowed to rot so that the allelopathic exudates released in the process were utilized for its effect on the growth of algae. In order to compensate reduction of oxygen caused by decomposing straw and to limit release of sediment bound phosphate from the bottom soil substrate that might promote the sustained eutrophication, violent aeration of water was ensured by employing an aquarium aerator.

The effect of rice straw on the water quality parameters viz., dissolved oxygen, pH, Nitrate-Nitrogen (NO₃ - N), Ammonia, Hardness, Alkalinity, Phosphate -Phosphorus (PO₄-P), Temperature, Turbidity and Algal density in the two treatments with five replicates and control were assessed. The algal concentration remaining in each tank was enumerated at 24 hour interval by using Lugols iodine and formalin as fixative. Counting was carried out in Sedge wick rafter chamber using an Olympus i20 binocular microscope. The algal clearing rate in the tanks exposed to decomposing straw and control were quantified and algal abundance monitored to evaluate the treatment effect over time.

3. Results

The pond water looked highly turbid with alkaline pH at 9 and free carbon dioxide was not detected. The air and the water temperature in the pond was 29°C and 28.5°C respectively. The Dissolved Oxygen (DO) was very low, 3.6 mg/l. The water hardness in the pond water was 35mg/l and total alkalinity was 117.5 mg/l. Phosphate and Nitrate concentrations were very low observed only in traces and free Ammonia in the pond was observed to be 1.0 mg/l. The algal concentration was 3 x10⁶ No/ l, predominantly comprised of *Microcystis aeruginosa* (*M.aeruginosa*).

The water temperatures in the experimental tank varied from 25.5-26.5^o C in T1 i.e., 3g/L and 25.0-27.0^oC in T2 i.e., 6g/l. pH of water in the experimental tanks remained almost alkaline, at 8.5 to 9 throughout the study. The total alkalinity of water varied from 50 to 100 mg/l in both systems. The water hardness ranged from 25 to 37.5 mg/l in the T1, and was marginally low ranging from 12.5 to 25.00 mg/L in the T2. Dissolved oxygen was maintained high in the beginning 5.1 to 5.5 mg/l, which declined to 4.1 mg/l in T1 and it fluctuated between 3.7 - 4.0 mg/l declined to 2.4 mg/l by 5th day in T2. The decline in DO level from the third day onwards with the progress of decomposition process has been most conspicuous.

Table 1: Treatment with Straw

Parameters	T1 (3g/l)					T2 (6g/l)				
	1	2	3	4	5	1	2	3	4	5
Water Temperature(°C)	25.5	26	26	26.25	26.5	25	25.5	26	27	27
pH	8.5	9	8.5	9	9	9	9	9	9	9
Dissolved Oxygen(mg/l)	5.1	5.5	4.25	4.45	4.1	3.7	4.05	2.9	2.8	2.4
Alkalinity(mg/l)	50	50	50	100	100	50	100	100	100	100
Hardness(mg/l)	25	25	37.5	37.5	31.25	12.5	25	25	25	25
Nitrate (mg/l)	0	0	0	0	0	0	0	0	0	0
Ammonia (mg/l)	0.1	0.1	0.1	0.3	0.3	0.3	0.3	0.3	0.4	0.5
Transparency(cm)	1.3	1.5	1.4	1.6	1.5	1.5	1.7	1.6	1.8	2.05
Colony count($10^6 \times \text{N/L}$)	1.3	1	0.98	0.95	0.8	0.95	0.69	0.5	0.4	0.2

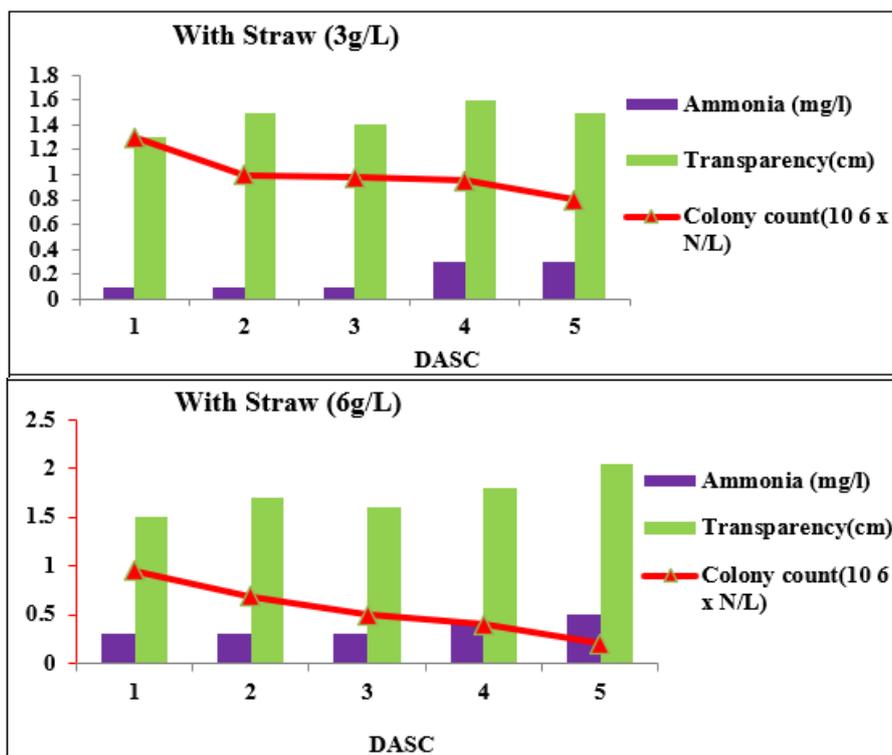


Figure 2: Treatment with Straw

Reactive NO_3^- -N and PO_4 -P concentration in the experimental tanks were almost absent throughout the trial. However, the concentration of ammonia increased to 0.3 mg/L in T1 while in T2 it rapidly increased to 0.5 mg/L apparently contributed by the exudates from the decomposing straw. Ammonia was totally absent in the control system (Table 1).

The cell count of *M.aeruginosa* in the experimental system declined from 1.3×10^6 to 0.80×10^6 Nos/l in T1 while in T2 the cell counts declined from 0.95×10^6 to 0.20×10^6 Nos/l. The percentage reduction of algal concentration ranged from 24.34 % (T1) to 78.94 % (T2) in 5 days (Fig 2 & 3). The significant decline in cell counts in T2 is apparently contributed by the enhanced concentration of exudates. In contrast, in the control system devoid of straw, the algal count did not exhibit any significant variation. The transparency of water ranged from 0.13-2.05 cm and with the progressive decomposition of straw and decline in algal density, improvement in transparency in both the treatments was perceptible. When the straw was first placed in the experiment tanks, water during the initial days looked apparently brown in color. Algal abundance represented by population density measured to evaluate the treatment effect

over time indicated drastic decline of algal density presumably contributed by straw exudates.

4. Discussion

M.aeruginosa is a single-celled blue green algae, or cyanobacterium, that occurs naturally in surface waters. It can proliferate to form dense blooms and mats in warm, turbid, and slow-moving waters. Highest biomass occurs in waters that are high in nitrogen or phosphorus (eutrophic waters).

Laboratory studies on utilization of straw exudates for inhibition of toxic algae *M. aeruginosa* establishes the algicidal activity of paddy straw. This activity was apparently linked to the synergistic effects of various phenolic compounds in the rice straw (Iredale et al. 2012)

Microcystins (MCs) are the most prevalent and potent of the toxins produced by *Microcystis* and the toxin microcystin can cause liver damage and dysfunction in humans and animals (Rebecca Burton, 2019) and can move through the aquatic food web, exposing fish, shellfish, as well as the people who consume them. Ingestion or inhalation of water containing

dense bloom material has been reported to cause vomiting, nausea, headaches, diarrhea, pneumonia and fever. Cyanobacteria prefer warm waters and excess nutrients, including nitrogen and phosphorus. Elevated concentrations of microcystins produced by the algae *M. aeruginosa* was linked epidemiologically to an outbreak of human hepatocystitis (Falconer et al., 1983).

It is reported that *Microcystis* contain the genes needed to synthesize the potent hepatotoxin, microcystin, known as “fast death factor” (Bishop et al., 1959) generally very stable compounds, resistant to chemical breakdown, persistent in natural waters for weeks to several months (Sivonen and Jones, 1999).

Although high concentrations of inorganic nutrients (e.g., NO₃, PO₄) have been implicated for such blooms, the nutrient concentrations were negligible in the algal rich water monitored after the bloom outbreak periods. This is in agreement with several earlier studies that HAB cells can obtain their nutrition through the utilization of organic compounds either in dissolved or particulate form (Taylor & Pollinger 1987, Berge et al. 1996). Numerous researchers have also noted that cyanobacterial carbonic anhydrase gives *Microcystis* an advantage in the use of bicarbonate as a carbon source and it is presumed that cyanobacteria are also well adapted to high CO₂ concentrations.

The most direct control method for microcystin removal from water bodies involves the use of chemical treatments such as algaecides, including copper compounds etc., often resulting in more serious negative environmental impacts. As ruptured or damaged cells may release intracellular toxins into the surrounding water, Chemical control methods and usage of reactive algaecides is reported to release toxin from broken algae cells. This necessitates the use of expensive removal processes such as activated carbon and/or oxidative ozone and chlorine (Haider et al., 2003).

Throughout the study, the pH of water in the open pond and the experimental tanks were highly alkaline with total alkalinity ranging from 50 to 100 mg/l. *Microcystis* bloom events commonly increase surface water pH to well above 9 as the cyano bacterium rapidly consumes available inorganic carbon. It is believed that the success of *Microcystis* in raising the pH create conditions unfavorable for other phytoplankton, e.g., the siliceous frustules of diatoms become soluble, as Si is likely incorporated at lower rates under these pH conditions. As blooming algal populations photosynthesize, carbonic acid, the dissolved form of carbon dioxide more rapidly removed from the water the pH is naturally raised. There was no significant difference in water pH among treatment and the control tank system. Incidentally high pH is a significant requisite that favored blooming of these blue green algae.

Increased aeration facilitated helped enhanced dissolved oxygen in the study. It also helped re suspension of the algal biomass within the water column. As decomposing straw lowers DO in the water body as it decomposes and consume dissolve oxygen, the lower dosage of straw found effective shall be recommended. Some authors do not recommend using straw due to the consumption of dissolved oxygen

during the decomposition process (Chorus and Mur (1999). Appropriate straw activity is only produced if the straw is rotting in well aerated conditions.

High summer water temperature measured on a huge range 26-27.5° C as a factor apparently influenced the decomposition process with, no significant difference among the replicate treatments. In fact, temperatures apparently provided conditions that favored the cyanobacterial out breaks *Microcystis* populations has been reported to grow faster at warmer temperatures (Paerl et al., 2008).

Use of allelochemical has been demonstrated as an eco-friendly method for control of algal growth (Shahabuddin, et al., 2012). The use of decomposing barley (*Hordeum vulgare*) straw for the control of algae and cyanobacteria has been a subject of considerable interest and investigations since early 1990s. Several studies have confirmed the effectiveness of barley straw treatment for control of algal blooms (Islami & Filizadeh 2011). The observed effects, is attributed to the production of antibiotics by the fungal flora responsible for the decomposition of straw. Release of phenolic compounds such as ferulic acid and coumaric acid from decomposing straw cell walls is also. (Newman, 1994)

Straw on decomposition lead to release of lignin which is on decomposition release fungal enzymes leading to the formation of mixture of compounds that get transformed to fulvic and humic acids. These humic substances are called dissolved organic carbon or DOC, When exposed to light release humic substances in of dissolved oxygen and hydrogen peroxide is eventually formed. Continuous supply of right DOC create hydrogen peroxide and other oxidizing agents that controlling algae (Gammal (2009), Lembi (2001). The use of agricultural straw for algal bloom control has been studied for more than 30 years. The isolation and discovery of anti algal allelochemicals, and the sequence of physiologic events that contribute to the species-specific inhibitor phenomenon has been a concentrated research. (Kokoette, 2020)

Though studies using cereal straw to prevent algal growth have been concentrated on barley straw, use of other cereal straws like rice straw also has been investigated as effective substitutes. In the present study, exposure to decomposing rice straw extract 5 days significantly inhibited the growth of *M. aeruginosa* stored for in a concentration-dependent way. (Gammal et al., (2009) observed that rice straw extract is an environment-friendly biomaterial for controlling the algal bloom of *M. aeruginosa* in natural water after 9 days of treatment. In open field investigations, when residues from the first year were left in the paddies. (Rice et al., 1981). Apparently, the decomposing straw acts as an algistat, rather than an algaecide, as straw per se does not itself kill existing algal cells but prevents the growth of more algae.

The dissolved phosphorus and nitrate nitrogen concentration was almost nil from the beginning and did not differ significantly even towards the end. Although nutrient requirement varies among algal species, the ideal nitrate-to-phosphate ratio for mass multiplication of algal cell is reportedly close to 10:1, and concentrations of individual

nutrient requirements vary among algal species (Downing and McCauley, 1992).

The identification that rice straw is an effective remediation material for inhibition of the growth *M. aeruginosa* observed in the study implies that it has the potential to be used as an environment-friendly biomaterial for control of algal bloom of *M. aeruginosa* in eutrophic waters. Undoubtedly, it is a “clean” form of algal control method.

A critical factor in the outbreak of such harmful algal bloom development is abundant nutrient supply, the major nutrients being nitrogen, and phosphorus the most direct solution to stopping cyanobacterial bloom events is to reduce nutrient loading. Proactive approaches to control blooms such as long-term management strategies for mitigation of nutrient input assumes significance.

The study demonstrates that use of decomposing rice straw is most effective and a reliable method in inhibiting blue green algal menace. Although the inhibitory chemical produced on straw decomposition, responsible for algal remediation is elusive, the observations suggest that treatments using straw exudates is a home remedy for preventing algal growth (Lembi, (2001).

Microscopic examination also indicated that not only drastic reduction of algal density after application of rice straw, but algal cell content also dropped significantly within a week. The microscopic images of water sample in the experimental tank taken on the 5th day with decreased cell density is given in Fig. Undoubtedly the study signifies the need for detailed investigations on the mode of algal inhibition by cereal straw and the toxicity effects on blue-green algal biomass.

The results illustrates that the decrease of algal count was related to allelochemicals discharged from rice straw, which apparently help suppress growth of the algal species. Hence, this technique is a way to reduce the cost and reduce the use of herbicides. Two dosages were tested in this study. Further the study endorses the observations that a lower dosage of straw (3g/L) rotting at well aerated conditions is efficacious. Evidently it is a cheap economical and novel method for treating fresh waters afflicted with *Microcystis* blooms. Results of the study demonstrate that decomposing rice straw is an effective and reliable method in inhibiting blue green algal menace.

5. Conclusion

The promising potentials of using agricultural straw as source of anti-algal agents, including the effectiveness of each major straw type has been elucidated in this study. Although the use of agricultural straw for algal bloom control has been studied for past three decades, the present study identifies paddy straw as a more practical and economical means of bio remediation tool against Use of agricultural straw in the field of algal inhibitions is urgent and meaningful option. As open-field straw burning constitute a serious air pollution problem and a source of emissions and is even prohibited in some regions. The observation that the algal inhibitors released from decomposing straw originate possibly by the oxidation of

phenolic extracts facilitated by aeration of the decomposing straw, is a crucial information. The identification that utilization of paddy straw is a new weapon against some of the toxic species, is a hot topic of further studies in hydro ecology.

Although experiments were carried out as a laboratory model, and the specific functional mechanisms of allelochemicals elusive, the understanding of its ecological significance as a safe alternative to chemical control is of immense value. The study shows that the allelopathic effect of agricultural straws are algistatic and not algicidal, therefore the present study consider the application approach to be more of a proactive nature. It is hoped that the study will encourage more works that would not only illustrate the general characteristics and physiology of algal species, but also encourage and explore their allelopathic pathways that will identify the specific allelochemicals and their species specificity. The study identifies agricultural straws have high potential of being effective as an environmentally safe agents for the control of nuisance algae.

Acknowledgement

The authors are grateful to Shri. P. Prasad, Hon’ble Minister for Agriculture and Farmers Welfare, Kerala and Chairman Executive Committee, IRTCBSF for constant encouragement and support. We are deeply indebted to the Principal Secretary to Government and Agriculture Production commissioner, Government of Kerala for guidance valuable suggestions. The authors are thankful to Smt. Majimol, for field and laboratory level helps and the staff of the International Research and Training Center for Below Sea Level Farming, Kuttanad for helps and support to the conduct of the study.

Data Availability

The materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes, without breaching participant confidentiality.

References

- [1] Anderson DM, Cembella AD, Hallegraeff GM. (2012) Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. *Ann Rev Mar Sci.*, **4**, 143-76.
- [2] APHA. (2005) Standard methods for the examination of water and waste water, American Public Health Association, Washington, DC, *APHA-AWWA-WEF*.167
- [3] Berg D.J. , S.W. Fisher, P.F. (1996) Landrum **Clearance and processing of algal particles by zebra mussels (*Dreissena polymorpha*)** J. Gt. Lakes Res., **22** 779-788
- [4] Bishop CT, Anet E, Gorham PR. (1959) Isolation and identification of the fast-death factor in *Microcystis aeruginosa* NRC-1. *Can J Biochem Physiol.*, **37**, 453–471.
- [5] Carmichael, W.W. (2013) Human Health Effects from Harmful Algal Water blooms: A Synthesis. Report

- prepared for the International Joint Commission (IJC), by the Health Professions Advisory Board (HPAB), 56
- [6] Chorus I and Mur L. (1999) Preventative Measures. In: Chorus I., Bartram J., Ed. Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management. Published by E & FN Spon on behalf of the World Health Organization. 235-273
- [7] Downing, J. A., & McCauley, E. (1992) The nitrogen: phosphorus relationship in lakes. *Limnology and Oceanography*, **37**(5), 936-945
- [8] Effiong Kokoette, Jing Hul, Caicai Xu, Tao Tang, Haomin Huang, Jiangning Zeng and Xi Xiao.2020. Sustainable Utilization of Agricultural Straw for Harmful Algal Blooms Control: *A Rev. J. of Rene. Mat.*, 461-483.
- [9] Falconer IR, Beresford AM, Runnegar MTC (1983). Evidence of liver damage by toxin from a bloom of the blue green alga, *Microcystis aeruginosa*. *Med. J. Austr.*, 511-514
- [10] Fonselius S.H. and S. Carlberg. (1972) Determination of dissolved inorganic phosphates. In co operative Research Report.
- [11] Frontier S. (1972) Calcul del' erreur Sur un comptage de zooplancton. *J.Exp. Mar. Biol. ECOL.*, **12**
- [12] Gobler, C.J. (2020) Climate Change and Harmful Algal Blooms: *Insights and perspective*, *Harmful Algae*. Volume **91**, 101731
- [13] Gurbuz Fatma, AE. Geoffrey and A. Codd. (2008) Microcystin Removal by a Naturally-Occurring Substance: Pumice Article in Bulletin of Environmental Contamination and Toxicology · *Bull Environ Contam Toxicol.*, **81**,323–327
- [14] Haider S, Naithani V, Viswanathan PN, Kakkar P. (2003) Cyanobacterial toxins: a growing environmental concern. *Chemosphere.*, **52**(1),1-21.
- [15] IPCC. (2019) Special Report on the Ocean and Cryosphere in a Changing Climate. Eds., Pörtner, D.C. Roberts, V. MassonDelmotte, P. Zhai, M. Tignor, E. Poloczanska, K. Mintenbeck, A. Alegria, M. Nicolai, A. Okem, J. Petzold, B. Rama, N.M. Weyer (eds).
- [16] Iredale RS, McDonald AT, Adams DG. (2012) A series of experiments aimed at clarifying the mode of action of barley straw in cyanobacterial growth control. *Water Res.*, **46**,6095–6103
- [17] Islami, H.R., and Y. Filizadeh. (2011) Use of barley straw to control nuisance freshwater algae. *Amer. Water Works Assoc. J.*, **103**,111-18.
- [18] Lembi, C. A. (2001) Barley Straw for Algae Control., <http://www.btny.purdue.edu/Pubs/APM/APM-1-W.pdf>
- [19] Lund, J.W.G., C. Kliplin and E.D. Le Cren. (1958) The inverted Microscope method of estimating algal numbers, and the statistical basis of estimation by counting. *Hydrobiologia.*, **11**, 143- 170
- [20] Moore, S.K., Trainer, V.L., Mantua, N.J. (2008). Impacts of climate variability and future climate change on harmful algal blooms and human health. *Environ Health.*, **7**, S4
- [21] Mostafa El Gammal & Daa Abdel Reheem Al-Kenawy. (2009) Environment-friendly biomaterial (Rice Straw extract) for controlling the algal bloom of *Microcystis aeruginosa* in Natural water in Abbassa and Ryan water in Egypt, *Abbassa inte Jo for aqua Cul.*, ISSN-1687-7683, 171-181
- [22] Mullin, J.D. and Riley J.P. (1955) The Spectrophotometric determination of nitrate in natural waters, with particular reference to sea water. *Anal. Chem. Acla.*, **12**, 464-480
- [23] Newman, JR (1994) Report on the control of growth of *Microcystis aeruginosa* by decomposing barley straw and the development of a bankside straw digester. LACR-Aquatic Weeds Research Unit, Broadmoor Lane, Sonning-on-Thames, Reading RG4 6TH, UK.
- [24] Paerl, H.W. and J. Huisman. (2008) Blooms Like It Hot. *www.sciencemag.org. science* **Vol.320.4**. Published by AAAS, 57
- [25] Rebecca Burton (2019) State of the Science for Cyanobacterial Blooms (*Microcystis* species) in Florida. A summary document from the 2019 Harmful Algal Bloom State of the Science Symposium. Florida Sea Grant and UF/IFAS, Florida Sea Grant Harmful Algal Bloom webpage at: www.flseagrant.org/habs
- [26] Rice, E.L., C.-Y. Lin, and C.-Y. Huang. (1981) Effects of decomposing rice straw on growth of and nitrogen fixation by rhizobium. *J. of Chem. Eco.*, **7**, 333-44.
- [27] Saraceni, C. and D Riggju. (1969) Techniques for sampling water and phytoplankton. In: A manual on methods for measuring primary production in aquatic environments, *IBP, Handbook No.12*. (Eds) R.A. Vollenweider, J.F. Talling and D.F. Westlake, Blackwell Scientific publication, Oxford
- [28] Sivonen, K. and Jones, G. (1999) Cyanobacterial Toxins. In: Chorus, I. and Bartram, J., Eds., Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring, and Management, E & FN Spon, London, 41-111.
- [29] Shahabuddin, A. M., M.T. Oo, Y. Yi, D.P. Thakur, A.N. Bart and J.S. Diana. (2012) Study about the Effect of Rice Straw Mat on Water Quality Parameters, Plankton Production and Mitigation of Clay Turbidity in Earthen Fish Ponds. *World J. of Fish and Mar. Sci.*, **4**, (6): 577-585,
- [30] Taylor, F. J. R., & Pollingher, U. (1987) Ecology of diano flagellates. In F. J. R. Taylor, (Ed.), The biology of dinoflagellates. London: *Blackwell Scientific Publications*
- [31] Throndsen, J. (1978) Preservation and Storage. Phytoplankton manual monographs on oceanographic methodology,6, (Ed.) E.A. Sournia, UNESCO, Paris, 69-74