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INDUSTRIAL AND DOMESTIC WASTEWATER TREATMENT USING MICROALGAE AND FUNGI IN CONTEXT TO HINDON RIVER, GHAZIABAD CITY, INDIA

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ABSTRACT

Microalgae and Fungi have been studied for their absorption and adsorption properties. Much work has been reported on the absorption, adsorption, and other related uses of microalgae and fungi separately. However, little or no work so far has been reported for the use of Microalgae and Fungi along with the treatment of domestic and industrial wastewater. The present study reports the use of Microalgae or Fungi alone and in combined form (microalgae + fungi) for the treatment of domestic & Industrial wastewater. Effect of various parameters such as combination ratio of Microalgae and Fungi, contact time, adsorbent dosage and particle size of adsorbent has been studied. The potential of non-conventional extremophilic microalgae and the industrial algae-wastewater treatment concept were reviewed in this study, with a special focus on industrial wastewater sources, non-conventional extremophilic microalgae, and the industrial algae-wastewater treatment concept. It is found that there is dissolved oxygen, lead level, cadmium, chromium, Total Pesticides and out of them avg. pesticides concentration (α-endosulphan, α-BHC, β-BHC, heptachlor, heptachlor epoxide, β-endosulphan, aldrin ,BHC isomers, endosulphan sulphate) surface water quality assessment (physiochemical and biological assessments followed transparency (27 cm), pH (8.4), conductivity (447), dissolved oxygen (2.5 mg/L), biological oxygen demand (19 mg/L), chemical oxygen demand (4 mg/L), chloride (22 mg/L), turbidity (29.1-111.3 NTU), total dissolved solids (267.2-2572.9 mg/L), sulphate (39.7-169.8 mg/L), nitrate (09-259 mg /L), total alkalinity (369.0-599.3 mg/L, total hardness (247.1-466.2 mg /L), Ca-H (69.57-409.6 mg/L). And by the use of these Algae and fungi treatments, these all will be reduced approximately 70%. In Surface water quality assessment (micro invertebrate biological monitoring), we found gompheade, thiazide, spherical, Unionidae, Nereida, PI anorbidae, Chironomidae, Oligochaeta on which there biological monitoring working particle (BMWP). The range also is reduced as well as BMWP water quality class is higher from C and E. Average surface water quality assessment (heavy metal) will be found that lead (Pb), cadmium (Cd), chromium (Cr) of new Hindon colony and Arthala colony also be reduced and in surface water quality assessment (pesticide concentration) β-BHC and endosulfan also be in managing form. Using microalgal strain, the pH range has reduced 8.4 to 1.5 and deposit, and nitrogen is essential to develop the layer of lipoprotein. Nutrient deficit is common in industrial treatment systems and occasionally in municipal treatment systems. This happens when there is a nitrogen or phosphate deficiency in the influent wastewater. For every 100 mg/L of BOD consumed, bacteria require 10 mg/L of nitrogen and 1 mg/L of phosphorus, resulting in a nutritional ratio of 100:10:1. (BOD: N: P). Slime thickening can occur when bacteria do not have enough nutrition to survive.

***Keywords**: Microalgae, Fungi, Lichen, Laundry, Industrial Wastewater.*

1. INTRODUCTION

Water is one of the most important elements on the planet. Water is required for the survival of all plant and animals. If there were no water, there would be no life on earth. It covers 71% of the Earth's surface and is vital for all known forms of life. There would be no life on Earth if there was no water. It covers 71% of the Earth's surface and is essential for all known living forms. But only 2.5% of the Earth's water is freshwater. Rapid urbanization and industrialization produce massive amounts of wastewater, which is increasingly being used for irrigation in urban and peri-urban agriculture [1,2]. It generates tremendous economic activity, supports countless livelihoods, particularly for impoverished farmers, and has a huge impact on the water quality of natural bodies of water. It is becoming more contaminated as a result of industrialization and urbanization, and the risk of polluted water consumption and sanitation problems is increasing day by day in most developing countries [3]. Water scarcity is a developing problem that has a considerable detrimental impact on global economic development, human livelihoods, and environmental quality. As a result, protecting water from pollution or developing a cost-effective remedial strategy for its protection has become a critical need in today's environment. Traditional wastewater treatment methods have existed since antiquity, but they are extremely expensive and inefficient [4-10]. The new green technical methods are being introduced to overcome the conventional methods of wastewater treatment. The current research is focused on innovative green technical solutions that are proven to be superior to traditional methods; low-cost wastewater treatment employing microalgae and fungi is one of them [11-13]. A literature review was conducted to learn about new wastewater treatment methods involving microalgae and fungi, as well as their development and application in the management of natural water resources [14,15].

Water contaminated by any means of human use such as domestic, industrial, commercial, or agricultural activities, and any sewer inflow or sewer infiltration called wastewater. There is a huge difference in wastewater generation and treatment in India. According to the Ministry of Statistics and Programme Implementation, 59.3 percent of the population (rural and urban) uses sewers for wastewater disposal. Around 80 % of water in India is polluted, including most of the river. Around 674-million-liter sewage and industrial waste are drained into the Hindon River every day. Among different districts, Ghaziabad is one of the main contributors of sewage and industrial effluent, which majorly pollute the Hindon River.

Wastewater treatment by biological means (Fig 1) is merely one technique for allowing the natural breakdown of pollutants to occur under controlled settings. Last few decades, wastewater treatments by bacterial, microalgal, and fungi processes have more attention worldwide. Phosphorus, nitrogen, salt, potassium, iron, calcium, and substances such as lipids, carbohydrates, and proteins are all abundant in wastewater. Microorganisms (bacteria, algae, and fungus) utilize these chemicals as a "food" source for energy and the manufacture of necessary cell components, allowing them to maintain their lives while also helping to protect the environment by converting and removing waste from water [13]. If wastewater treatment is done under favourable conditions, it facilitates the growth of microorganisms and efficiently removes but they also "settle out "the pollutants and help in the management and treatment of wastewater. Moreover, some nuisance microorganisms not only hinder the removal of waste but are also very difficult to remove from the system [9,10].

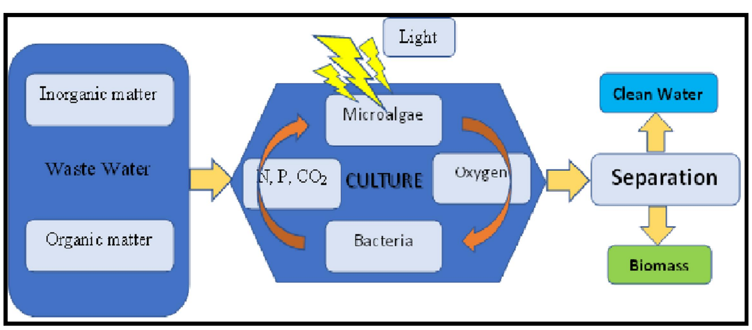

Fig 1: Wastewater treatment using microbial and bacterial culture process

2. EXPERIMENTAL

2.1 Methodology for Biological treatment of this Hindon River Contamination

The various conventional methods for wastewater treatment have been present since ancient times, but they are very costly and not economical [4-6]. So, new green technical methods are being introduced to overcome the conventional methods of wastewater treatment [7-9]. The present study is related to new green technical methods [10-15], which are proving to be superior to traditional approaches; among these, low-cost wastewater treatment utilizing microalgae and fungi is a promising option. A literature review was conducted to learn about new wastewater treatment methods involving microalgae and fungi, as well as their development and application in the management of natural water resources.

2.2 Microbiology and Activated Sludge Process (ASP)

ASP is most widely used as a biological treatment process in this niche of microorganisms that encounter and digest biodegradable materials (food) such as proteins, carbohydrates, fats, and other compounds from wastewater [16-18]. Microorganisms from the flock settle out as sludge as contaminants decay in the wastewater. In the ASP, mainly three various kinds of microorganisms dominate, namely bacteria (95%), protozoa (4%) and metazoa (1%). Activated sludge contains aerobic (require oxygen), facultative (prefer oxygen but survive some time without) and anaerobic (do not require oxygen) bacteria [19-21]. Bacteria can absorb liquid organic material directly, whereas solid particles require two-step methods such as adsorption and absorption to ingest [22,23]. Bacteria and food particles cling together in the former, and bacteria secrete enzymes that break down the food particles into smaller units that can pass through the bacteria's cell wall. Smaller dissolved food units, on the other hand, pass through the cell membrane during the absorption process [24-26].

2.3 Factors Affecting Bacteria Growth

Primary treatment (eliminating maximum of the particulates or "settleable solids" and floating off maximum of the grease) imparts crucial roles in ASP [26,27]. Not only primary treatment but several other factors affect the ASP, including oxygen availability and its utilization, mixing, PH, temperature, and nutrients. Generally to survive, aerobic bacteria require at least 0.1-0.3 mg/L of oxygen. Mixing brings bacteria, oxygen & nutrients contact with each other [28,29]. While drastic pH and temperature changes should be avoided, optimum pH is between 7.0 to 7.5 & warm temperature favours faster and fewer bacteria are required for the same job. Bacteria also require fundamental nutrients including carbon, nitrogen, and phosphorus, as well as trace amounts of salt, potassium, magnesium, and iron [29-31].

2.4 Factors Affecting Bacteria Growth

Protozoa is a single cell microorganism, and they have a variety of sizes and shapes. Evidence suggested that the percentage of protozoan is very less, about 4 %, while bacteria are 95% in an activated sludge process [11,13]. Even though their number is very less, they are recognized as bioindicators in ASP. Among different types of protozoans, ciliated one has a dominating role in ASP, and they can firmly attach or crawl over the surface of sludge and are efficient in removing the coliform bacteria through their predatory mechanism [12,13]. The protozoan is efficient in the removal of amine-containing pollutants through an ion trapping mechanism. They also assisted in the eradication of non-flocculent bacteria and extremely tiny floc that refused to settle. Protozoans have four types of feeding mechanisms (i) photoautotrophic, (ii) heterotrophic, (iii) phagocytosis, and (iv) predation.

Like bacteria, several factors influence the growth in the treatment process, such as temperature, pH, dissolved oxygen, and nutrients. Protozoans are more sensitive than bacteria and prefer ambient temperature (15-25 °C) and pH range between 7.2 to 7.4, but they can tolerate 6.0-6.8. Protozoa must need dissolved oxygen to survive. Municipal Wastewater contains sufficient nutrients. However, industrial wastewaters are mostly deficient in nutrients to support the growth of protozoa [32-37].

2.5 Metazoa and Factors Affecting its Growth

Metazoan are multicellular organisms, typically larger than protozoa and unlike protozoa, they can reproduce sexually and asexually. They are slow-growing and mainly fed to bacteria, algae and protozoa. They mainly comprise in wastewater are Rotifers, Nematodes, Tardigrades (water bear), annelids and ostracods (Daphnia), and Copepods (water flies and mites). Rotifers play an important role in the treatment of activated sludge, but they should never be the dominant in the system. They mainly remove the remanent materials in water. Rotifers are used as an indicator of water toxicity [28,34,35].

Moreover, Nematodes are seen in longer and aged or older sludges. Tardigrades (water bear) are the indicators of ammonia present in wastewater treatment. They are usually found in the same environment as rotifers and nematodes, but they survive in an extreme environmental swing. Rotifers are used in the activated sludge treatment process and mainly remove the remanent materials in water and are used as an indicator for water toxicity [13,36-38].

2.6 Process Control

A community of suspended, growing, non-flocculated bacteria, algae, or fungi is known as dispersed growth (most is bacteria as bacteria grow a "slime" layer and clump together to form a floc, they are eliminated from the liquid. Ciliates and rotifers are also capable of removing them. Inadequate floc formation is responsible for the existence of considerable scattered microorganisms [37-39]. The count will look at protozoa from the following groups: (i) Amoebae Flagellates free-swimming ciliates crawling ciliates Stalked ciliates; and (ii) Metazoa (rotifers, nematodes, water bear, *etc*.). Since amoebae and flagellates are both signs of young sludge or insufficient treatment, they are grouped together. Slime bulking occurs when bacteria create too much exocellular lipopolysaccharide, more commonly known as "slime" [12,13,40,41].

2.7 Exocellular lipopolysaccharide (slime bulking) bacteria produce an excess of substance

Gram-negative bacteria require three essential components in order to correctly form their cell wall: (i) nitrogen, (ii) phosphorus and (iii) sulphur are the three elements. The cell wall does not form properly if any of these substances are missing. For example, the formation of the phospholipid layer requires phosphorus, and the development of the lipoprotein layer requires nitrogen [42,43].

Phosphorus is required for the formation of the phospholipids layer, while nitrogen is required for the formation of the Lipoprotein layer, which is responsible for the formation of cell walls. Nutrient deficit occurs frequently in industrial treatment systems and occasionally in municipal systems. This happens when there is a nitrogen or phosphate deficiency in the influent wastewater. Bacteria require 10 mg/L nitrogen and 1 mg/L phosphorus for every 100 mg/L BOD consumed, resulting in a 100:10:1 nutritional ratio (BOD: N: P). Slime thickening can occur when bacteria do not have enough nutrition to survive [44,45].

2.8 Toxicity parameters

When a harmful material enters the treatment plant, protozoa and metazoa are the first to be affected. Something harmful has most certainly infiltrated the plant after evaluating a fresh sample of mixed liquor and seeing a bunch of dead rotifers and inactive stalked ciliates [45,46]. Toxicity is also indicated by a loss of BOD elimination and an extremely low oxygen use (dead microorganisms do not remove BOD). A bloom of flagellates can occur at any time. They prefer the secretions of dead microbes to eat. Upon healing, filamentous thickening is another sign. Filamentous bacteria recover faster than bacteria that generate flocs [47,48].

2.9 Micro algae

Freshwater makes up about 2.5% of the total volume of water, whereas the remainder is salty. Currently, most water is used in agriculture, industries, and municipalities [2,3]. The demand for freshwater more than doubled, and per capita potential availability of renewable freshwater is continuously declining. Water scarcity is well documented in the Middle East and North Africa regions [19,32]. Last few years, the proportion of polluted water has continuously increased. Water quality degradation and scarcity are major global issues [8,10]. The action plan, Agenda 21, of the UN Rio conference emphasized freshwater scarcity. Considering the above guidelines, wastewater treatment management only hopes for the world to supply fresh water and for that biological treatment (here in this research first time, we use combined relation of Microalgae and fungi for treatment) [33,34,48].

Oswald presented the concept of wastewater treatment using microalgae in 1950, among other methods. Later, this approach was developed to energy generation by algal biomass, which looked into the treatment of soy sauce effluent, including four varieties of microalgae [19], in which they used this process to ferment ethanol from biomass harvested from microalgae [40]. Various species of microalgae are currently being used by a number of scientists to remediate industrial and municipal waste [49,50]. They were represented by this reaction given below.

$$CO_2 + H_2O + \text{nutrients} + \text{light energy} \longrightarrow \text{biomass} + O_2$$

Microalgae convert water, sunlight, and CO_2 into algal biomass thanks to photosynthetic microorganisms. Microalgae are photosynthetic microorganisms that convert water, sunlight, and CO_2 into algal biomass, which is used to make biodiesel (They have been identified as a good raw material for biodiesel production because of their fast biomass output and high oil content). Unlike higher plants, microalgae do not have roots, stems and leaves. Microalgae are essential for life on earth; they produce approximately half of the atmospheric oxygen (microalgae, capable of performing photosynthesis) and use the greenhouse gas carbon dioxide simultaneously [40,41]. The use of microalgae is not limited to wastewater management but also has numerous commercial applications such as enrichment of the nutritional value of diet, an essential role in aquaculture and (i) Because of their chemical makeup, microalgae can be employed to improve the nutritional content of food and animal feed, (ii) they perform a critical role in aquaculture and (iii) they can be united into cosmetics [42].

2.10 Used microalgae strain

Several species of microalgae such *as Chlorella, Ankistrodesmus, Scenedesmus, Euglena, Chlamydomonas, Oscillatoria, Micractinium* and *Golenkinia, Scenedesmus, Nitzschia, Navicula* and *Stigeoclonium* used in wastewater management [47,48,51].

a. Chlorella algae strain used and a combination of microalgae (*Chlorella vulgaris* or *C. sorokiniana*) and a microalgae growth-promoting bacterium (MGPB, *Azospirillum brasiliense* strain Cd), co-immobilized in small alginate beads, was developed to remove nutrients (P & N) from municipal wastewater. Single-celled freshwater algae *Chlorella vulgaris* is effective in eliminating pollutants from wastewater even at fluctuating levels.

b. *Galdieria sulphuraria* can grow not only in neutral environments but also in highly acidic environments, down to pH 1.8.

c. *G. sulphuraria* can acidify its surroundings, By using an active proton efflux, lowering the expense of pH regulation and the risk of contamination.

d. Dunaliella efficiently removes glycerol from the wastewater and consequently reduces organic matter.

e. *Euglena* sp. growing in wastewater ponds for biofuel manufacture and treatment of wastewater particular ammonium nitrogen (NH_4-N). *Chlorella vulgaris*, a single-celled freshwater algae, effectively removes contaminants from wastewater, even at changing levels.

f. Unicellular green algae such as *Chlamydomonas reinhardtii*, Scenedesmus and nitrogen-fixing *Cyanobacterium anabaena cylindrica* are an example of algae used for biological production of hydrogen. Chlorella algae strain used and a combination of microalgae (*Chlorella vulgaris* or *C. sorokiniana*) and a microalgae growth-promoting bacterium (MGPB, *Azospirillum* baselines strain Cd), co-immobilized in small alginate beads, was developed to remove nutrients (P & N) from municipal wastewater.

3. RESULTS & DISCUSSION

3.1 Microalgae cultivation and factors affecting its cultivations: Nitrogen (N), phosphorus (P) and potassium (K) are all required for a high output of algal biomass (NPK). Wastewater is the major source of nutrients such as NPK, and the use of these nutrients by algae make the environment sustainable. A large number of algae, known as seaweed, may be grown in seawater to meet the enormous demand. Currently, two kinds of systems are used for microalgae cultivations such as (i) open raceways (ORW) and (ii) photobioreactors (PBR). ORW can be natural such as ponds or artificial, for example, the curved ground made by high-density polyethene (HDPE) or poly vinylchloride (PVC). Such kind of system generally industries are using for the production of microalgae used for foodstuff productions. PBRs, on the other hand, are a closed system made of polyethene bags soaking in a thermostatic water bath and can be tubular (TPBR) or flat panels (FPBR). The optimum pH requirement of algae growth between 8.7-8.8, lower or higher, affects the growth of algae. However, the optimum temperature is range 20-30 °C. If a temperature higher than 35 °C can be lethal for a number of algal species, if a temperature is lower than 16 °C, it decreases the progress of algae. Their growth is controlled by pH and temperature and limited by sunlight, water, carbon dioxide, and organic nutrients [40,42,48,50].

3.2 Approach for cultivation of microalgae: To maintain high algal yields, it requires a steady supply of many inorganic nutrients such as nitrogen (N), phosphorus (P) and potassium (K). The high nitrogen need is one of the concerns about future algae large-scale production. This increased demand can have a beneficial or negative impact on the nitrogen cycle, depending on how the cultivation process is managed, because nitrogen can be recycled and provided by a waste source. In this context, nitrogen requirements benefit from the use of wastes, as municipal wastewaters serve as a source of water and nutrients (C, N, and P) during the growing phase, and can be critical to the overall environmental sustainability of microalgae biofuels. The production of nutrients, which happens upstream of the algae-to-energy facility, accounts for a large portion of the life cycle burden associated with microalgae biofuels, according to LCAs [51-53]. Wastewater is the primary source of nutrients such as NPK, and the use of these nutrients by algae make the environment sustainable.

3.3 Microalgae growth survival condition: The ideal pH for most algae growth is between 8.8 and 8.7. Algae development can be slowed by maintaining a pH level of neutral or lower. The optimum temperature is range 20-30 °C. Suppose temperature higher than 350C can be lethal for no of algal species. If the temperature is less than 16 °C, it will slow down the growth of algae. Algae reproduce very quickly and need only sunlight, water, carbon dioxide, and few organic nutrients to grow. Single-celled freshwater algae Chlorella Vulgaris is effective in eliminating pollutants from wastewater even at fluctuating levels. *Chlamydomonas reinhardtii*, *Scenedesmus*, and nitrogen-fixing algae are examples of unicellular green algae. Cyanobacterium clgae like *Anabaena cylindrica* are employed in the biological synthesis of hydrogen [42,44,48].

3.4 Treatment of microbial species: There are two main processes for wastewater treatment first one is surface aerator based, and the second one is subsurface aerators, but there are some major differences noted after the analysis. In the surface method, Fourier maintenance was needed, but additional mixing reduced the time consumption, whereas, in the subsurface, it was energy-efficient and better in oxygen transformation [54-59].

4.0 Surface water quality assessment

4.1 Physical, chemical and biological assessments: The water qualities of Hindon river were analyzed and the correlation between the water quality and its algae-based treatment are given in Tables 1-3.

4.2 Pesticide analysis of Hindon river samples: After analyzing the algae samples and fungi isolates in waste wastewater to know the concentration of pesticide presented in the Hindon river water samples and also need to examine the physiochemical parameters because these results are very helpful to know about the contamination level of the aquatic body (Hindon river). The results are given briefly in Tables 4 and 5.

Table 1: Fungi isolation from wastewater mud with respective pH values

S. No.	Fungi isolates	Pellet formation capability	Initial pH	Final pH
1.	*Aspergillus niger*	Positive	6.7	4.8
2.	*A. flavous*	Negative	7.0	5.7
3.	*Penicillium sp.*	Negative	6.8	5.6
4.	*Alternaria sp.*	Negative	6.5	5.7

Note: Positive means forming of pellets/negative means non-pellets formation

Table-2: Selective algae analysis (Cd, chlorophyll, carotenoids)

S. No.	Parameters	***Scenedesmus* sp.**		***Chlorella* sp.**		***Ankistrodesmus* sp.**	
1.	Cd (mg/L)	0.0	0.5	0.0	0.5	0.0	1.0
2.	Chlorophylls (µg/mL)	0.32±0.08	0.33±0.27	0.56±0.30	4.56±2.72	1.33±0.10	1.19±0.04
3.	Carotenoids (µg/mL)	0.037±0.014	0.016±0.004	0.18±0.06	1.12±0.68	0.34±0.04	0.30±0.06

Table-3 Results of sample collected from Hindon River-63-U/S of Hindon, Mohan Nagar

S.No.	Parameters	Concentration
1.	Lead (Pb)	2.2 mg/L
2.	Cadmium (Cd)	0.1 mg/L
3.	Chromium (Cr)	0.0025 mg/L
4.	Dissolved oxygen (DO)	2.2 mg/L

Table-4 Pesticide analysis of Real water samples (Hindon river)

S. No.	Pesticide	Concentration (Microorganism/L)
1.	β-Endosulfan	0.25
2.	α-BHC	2.25
3.	β-BHC	2.0
4.	Heptachlor	0.85
5.	Heptachlor Epoxide	0.03
6.	α-Endosulphan	1.1
7.	Aldrin	0.73
8.	BHC Isomers	2.13
9.	Endosulfan sulphate	0.62
10.	Total pesticide	1.47

Table5. Physico-chemical parameters of the Hindon river sample

S. No.	Parameters	Concentration	S. No.	Parameters	Concentration
1.	Transparency	27 cm	6.	Chemical oxygen demand	4 mg/L
2.	pH	8.4	7.	Chloride	22 mg/L
3.	Conductivity (EC)	447	8.	Lead (Pb)	0.412 mg/L
4.	Dissolved oxygen (mg/L)	2.5 mg/L	9.	Cadmium (Cd)	4.62 mg/L
5.	Biological oxygen demand	19 mg/L	10.	Chromium (Cr)	0.019 mg/L

4.3 Real samples analysis of groundwater: We selected the two major sites for the ground water analysis to compare the contamination level of Hindon river water and ground water and given in Table-6. These results are supporting two heavy metals Pb and Cr presented more than the permissible limit and can be hazardous for human health.

Table-6. Groundwater analysis of selected sites

S. No.	Parameter	New Hindon Colony	Arthala colony
1.	Lead (Pb)	6.063 mg/L	5.63 mg/L
2.	Cadmium (Cd)	0.06 mg/L	0.26 mg/L
3.	Chromium (Cr)	17.2 mg/L	36.31 mg/L
4.	β-BHC	1.29 microorganism/L	0.8 microorganism/L
5.	Endosulphate	1.09 microorganism/L	1.2 microorganism/L

4.4 Algae in Hindon wastewater treatment: *Chlorella, Ankistrodesmus, Scenedesmus, Euglena, Chlamydomonas, Oscillatoria, Micractinium and Golenkinia, Scenedesmus, Nitzschia, Navicula and Stigeoclonium, Galdieria sulphuraria*, also denoted as *Cyanidium caldarium*. *Galdieria sulphuraria* can grow in both neutral and extremely acidic settings, down to pH 1.8, and *G. sulphuraria* can grow in both. By using an active proton efflux, sulphuraria can acidify its surroundings, lowering the expense of pH regulation and the risk of contamination.

Chlorella protothecoides var**. *acidicola*:** It survives on the waste from fruit and vegetable processing plants. *C. sorokiniana* cells (of many species) can collect high levels of useful bioproducts such as lutein, fatty acids, and proteins, making them a good source of sustainable biomass for animal feed or biofuel generation.

Dunaliella: Efficiently remove glycerol from the wastewater and consequently reduce organic matter.

Euglena sp. growing in wastewater ponds for biofuel manufacture and treatment of wastewater particular ammonium nitrogen (NH_4-N). *Chlorella vulgaris*, a single-celled freshwater algae, effectively removes contaminants from wastewater, even at changing levels.

4.5 Fungi in wastewater treatment: Traditionally biological wastewater treatment is limited to bacteria; however, fungi have been recognized for wastewater management in the last few years. Phylogenetic analysis suggested that *Basidiomycota* and *Ascomycota* are the utmost rich phyla, and *naumovozyma*, *pseudotomentella*, *derxomyces, ophiocordyceps, pulchromyces and paecilomyces* dominating genera found in the wastewater treatment setting. One of the unique characteristics of fungi is their ability to biodegrade trace organic contaminants and organic materials. Besides that, they are also involved in membrane biofouling, bulking and foaming. For example, filamentous fungi (White-Rot-Fungi) are well documented in flocculation, solids and pathogens reduction and removal and degradation of toxic compounds. Lignolytic enzymes of fungi enable them to tolerate high concentrations of toxic materials such as dye, polyaromatic hydrocarbon and xenobiotic compounds. Fungi also play an important part in trickling filters in aerobic biological wastewater treatment systems. The trickling filter is made up of higher amounts of bacteria, fungi, algae, and fungal biomass. Unlike in ASP, a symbiotic association of fungi and algae occupy a significant part of the active biomass of the trickling filter. However, some pathogenic fungi also impart their role in wastewater treatment, such as Candida involved in biofilms formation through surface adhesion and extracellular polymers. Glomus species drive microbial diversity and secrete polysaccharides and sugars via their hyphae. In comparison to bacteria, fungi would offer more benefits. The biomass produced during the treatment process has more potential value than bacteria in ASP. The treatment and management of bacterial biomass account for 40-65 % of operational costs. The fungal biomass has valuable proteins, organic acids, and other metabolites sources. Besides, fungi have the capacity to cope with adverse environmental conditions. *Pleurotus ostreatus* and *Trichoderma harzianum* has been used in brewing industries to remove nitrogen phosphorus [12,13,15].

4.6 Growth conditions: Important factors such as pH, temperature, hydraulic and solids retention time, non-axenic and axenic operation, and others that affect the fungal growth. Fungal communities are also affected by dissolved organic carbon (DOC), PO_4^{3-}, nitrate and nutrients such as Ca, Zn, Cu, Mn and Mg. Besides, excessive growth of mycelia gives difficulty in culturing the fungi [60-63].

5.0 Green characteristics of wastewater treatment by means of microalgae and fungi

Wastewater treatment with a combination of algae and fungi demonstrates its potential for environmental greening and appropriateness for use in green technical solutions. It follows the green chemistry principle. Exclusion of nutrient wastes and heavy metals is more efficient when done together than when done separately, and it is cost-effective, with a low energy requirement and a large production of useful biomass (contains more than 50% oil in its biomass and provides 10-100 times higher biomass and fuel yields than comparable energy crops), as well as a reduction in sludge formation. They can be cultivated in conditions that are inappropriate for conventional crops [11, 64,65]. A novel fungus palletization-assisted bio flocculation technology used for wastewater treatment and harvesting of valuable algae.

5.1 Future prospect: For their symbiotic relation

Lichen: Lichens are slow-growing organisms consisting of a fungus and photosynthetic alga/ or cyanobacteria, two different organisms stably living together symbiotically. However, fungus dominates over algae/ or cyanobacteria. Lichen does not have a root system waxy cuticle, and hence they obtained most of the nutrients directly from the atmosphere through the dry or wet deposition. Lichens have been widely used as an indicator of air pollution monitors because they strongly bind and accumulates metals [46,48].

Recent growing evidence demonstrates that lichens are widely used in wastewater treatment, especially in the removal of heavy metals. Moreover, the nonliving biomass of lichen is more efficient than living in removing the metals ions because the living plasma membranes inhibit the entry of metals into cells. However, the living biomass of *Cladonia thalli* has greater efficiency than dead biomass in the removal of zinc. Below is the list of lichen and their properties [53].

Lichens are symbiotic organisms in which a single fungus (mycobiont) coexists with one or more algae species (phycobionts), some of which are nitrogen (N) fixers (cyanolichens), *e.g*. members of the blue-green algae. The fungus gives the algae structure, while the algae, in turn, supplies energy and assimilates it through photosynthesis. The fungus, characteristic of lichen symbioses, synthesizes numerous secondary compounds. Lichens get practically all of their nutrients from the air by absorbing them across their whole surface. Unlike vascular plants, they lack a cuticle and no mechanism of limiting nutrient intake, allowing for unrestricted passage of gases and solutions across cell surfaces. Furthermore, their surface area to mass ratio is very large, and their assimilation capacity is limited. As a result, they are extremely sensitive to changes in atmospheric chemistry and deposition, making them excellent sensors of such changes. Lichens come in a variety of growth types.

The algal matt is sandwiched between the fungus and leafy, round lobes with root-like structures (rhizines); Bushy/fruticose, little shrub-like mounds sprouting up from the ground or beard-like, small tangles hanging down, only linked to the substrate at their bases, having a circular cross-section with a central algal core; or crustose, strongly adhering to the substrate, *e.g*. tree bark, stone, with the algae dispersed. All lichens except fruticose grow slowly, at a rate of 0.5 to 5 mm y^{-1}, as determined by the enlargement of their circles; fruticose lichens grow vertically and swiftly, at a rate of up to 2 cm y^{-1}. If left undisturbed and on a suitable long-lived substrate, lichens can exist for hundreds of years. Lichens make up a major amount of the overall species richness and vegetative biomass in upland/alpine habitats. Lichens are vital to ecosystem activities such as biogeochemical cycling and carbon storage. Not all lichens are sensitive to the same pollutants, and some can be rather resilient. Sulphur, nitrogen, acidity, halogens (*e.g*., fluoride), heavy metals, and ozone are all susceptible to lichens. Many lichen species, such as

Usnea articulate, have become extinct in wide parts of lowland Britain as a result of industrialization. High SO_2 levels were thought to be the primary reason, but habitat degradation, particularly in ancient woods, has also contributed to population declines in some species. Shrubby and leafy lichens are the most sensitive, whereas crustose lichens are the most resistant. Many shrubby and leafy lichens, such as Ramalina, Usnea and Lobaria species, have had their ranges shrink as a result of industrialization. Some lichen species have spread more broadly than they did a century ago, and these species are more tolerant of the acid conditions caused by SO_2 deposition, *e.g.* some species of *Bryoria*, *Parmeliopsis*, *Pseudevernia* and *Rinodina*.

SO_2 and fluoride pollution (local to some big sources, such as aluminium smelters) dominated air pollution chemistry in the 1970s and 1980s. In big towns and cities, or surrounding industrial complexes, a lichen zone pattern has been identified that corresponds to the mean levels of SO_2. The presence of specific lichen species on tree bark can reveal normal SO_2 levels. If there are no lichens present, for example, the air quality is poor, and only crusty lichens, such as *Lecanora conizaeoides* or *Lepraria incana*, can endure poor air quality in terms of SO_2 leafy lichens like *Parmelia caperata* and *Evernia prunastri* may thrive in moderate to good air, while uncommon species can thrive in regions where the air is pure, *e.g. Usnea articulata* or *Teloschistes flavicans* may grow areas where SO_2 levels are high, *Chaenotheca chrysocephala*, *Cladonia digitata*, *Evernia prunastri*, *Lecanora pulicaris* and *Phlyctis argena* are unlikely to be found. Many lichens are returning now that SO_2 levels are significantly lower, but they may not always recolonize in the same magnitude or location; one cause for this could be nitrogen pollution. Many lichens are returning now that SO_2 levels are significantly lower, but they may not always recolonize in the same magnitude or location; one cause for this could be nitrogen pollution.

5.2 Nitrogen pollution/acidification:

Nitrogen restriction can affect lichens growing in distant, pristine habitats, especially fast-growing speciesLichens can utilise both kinds of reactive nitrogen (NH_4^+ and NO_3^-), however nitrate uptake appears to be limited to the lichen's fungal portion (mycobiont). Long-distance nitrogenous air pollution is a major determinant of the prevalence of acidophyte (acid-loving) lichen species and poses a serious danger to natural populations. Among the explanations for acidophytes' sensitivity to N molecules are:

5.2.1 Effects of NH_4^+ and NO_3^- in precipitation:

Overgrowth of competing species, primarily algae, but also mosses and other N-tolerant lichens, is a result of eutrophication. Acid rain was frequently cited as a contributing factor in the reduction of (acid sensitive) Lobarion populations. Lowering bark pH as a result of wet, acidic deposition, on the other hand, may not be the primary cause of species composition change; some of it could be attributed to nitrogen at a concentration of 10 mg N L^{-1}, NH_4^+ in precipitation can have a deleterious effect on *Bryoria capillaris*, *Bryoria fuscescens*, *Imshaugia aleurites* and *Chaenotheca ferruginea*, leading them to all but disappear. By contrast *Lecanora pulicaris* becomes more common above 1.0 mg N L^{-1}. At > 0.2 mg N L^{-1}, two species, *Cetraria pinastri* and *Usnea hirta*, are highly susceptible to NO_3^-, although *Bryoria fuscescens* benefits from nitrate. Evidence suggests that the concentration of nitrogen, particularly as ammonium, maybe more important than the overall N dose. Although, in the longer term, N load may become important due to nitrogen accumulation [45,52,54].

5.2.2 Epiphytic lichens as indicators of nitrogen air quality:

Epiphytic lichens, or those that grow on trees (dead or alive), have been extensively investigated in terms of pollution (SO_2, nitrogenous gases and acidity). Lichens on Atlantic oak trees that grow in locations with a lot of rain but not a lot of dry deposition (gaseous pollution) appear to be very sensitive to nitrogen. The lichen flora of oak trees growing in agricultural areas in Europe and the United Kingdom has shifted from communities dominated by species that favour acid bark to communities dominated by species that tolerate and thrive by N. High ammonia levels in the Netherlands have resulted in the extinction of acidophyte (acid-loving) species, leaving ecosystems dominated by nitrophytic species. Ammonia deposition caused the bark pH to rise, resulting in a drop in Hypogymnia physodes abundance across the country. In the Netherlands, the pH of Quercus bark has been positively associated with the NH_3 content in the air, rising by nearly two pH units at high NH_3 levels. Recent research has discovered lichens on oak and birch trees across the UK that are sensitive to, or tolerant of, rising nitrogen dioxide concentrations. The decline in N-sensitive lichen species and the increase in N-tolerant lichen species growing on the bark of these trees can be used to quantify the response to increased atmospheric N pollution in the field.

5.2.3 Impacts of nitrogen on Terricolous (ground-living) lichens:

In a three-month N addition study, the effect of applied N concentration and load on thallus chemistry and growth of five terricolous alpine lichen species was examined. Thresholds for effects identified in that study indicate a low critical load for terricolous lichen communities (7.5 kg N ha^{-1} y^{-1}) and show that present N concentrations in UK cloud water may be harmful to N sensitive species' growth. Some Cladonia species have been found to be extremely sensitive to reactive N deposition in heathlands and bogs, especially when the N is deposited as gaseous ammonia. Because lichens have a low N status in nature, they are more sensitive to ammonia deposition, which is taken up until concentrations inside the algal cells and the surrounding environment approach equilibrium. Furthermore, many lichen thalli are acidic (pH 3-5), which aids in the deposition of alkaline gas. Growing C. portentosa downwind of an ammonia source first developed a reversible pink tinge, then bleached, then greened, indicating algal expansion and eventual death [12,13,15].

The relationship between N pollution and the presence of a variety of lichens was investigated for critical semi-natural habitats (acid grassland, bog, heathland, calcareous grassland) using the British Lichen Society's database for 10 km hectads (10 km × 10 km), allowing predictive modelling of responses to increasing N deposition.

5.2.3 Mosses and their importance in pollutant sink:

Mosses are the anatomically and morphologically basic plants. Moss growth is less polarized than that of vascular plants, and hibernation or the death of the developing point can induce buds lower down the stem. Many mosses have root-like structures called rhizoids, and some mosses, like vascular plants, can be classified as calcifuges or calcicoles, depending on whether they prefer acid or calcareous circumstances. Mosses may grow in a wide variety of environments, including those with harsh climates, and some species have amazing tolerances to heavy metals, such as copper. These mosses absorb metals from the atmosphere and the substrate over a broad surface area, trapping and concentrating them. Mosses, like lichens, obtain nutrients through atmospheric deposition, however they are less reliant on this nutrient source than lichens. Mosses are one of the most pollutant-sensitive parts of the plant, and they can be affected by both acidity and nitrogen, which are prevalent in today's human deposition. Many mosses, like lichens, have become extinct in urban/industrial settings, such as the Lower Tyne valley [16,17].
Mosses play a crucial role in nutrient cycling because they effectively capture reactive nitrogen deposition in specific environments, such as bogs and heathlands, preventing it from leaking into the pore water and rendering it unavailable to the roots of higher plants. The ability to sequester nitrogen, on the other hand, is dependent on the nitrogen load, and systems can quickly become saturated. Some mosses, such as those found on bogs, Sphagnum spp., have evolved 'liaisons' with nitrogen-fixing microorganisms in order to supplement their nitrogen supply while also providing carbon. In pristine ecosystems with very low nitrogen deposition, nitrogen fixation is a major source of nitrogen (ammonium ion).

Both nitrogen-sensitive and nitrogen-tolerant mosses have been identified in studies *Eurynchium praelongum* and *Brachythecium rutabulum* have been reported to sustain exceptionally high tissue nitrogen concentrations of at least 4% in studies around ammonia-affected woods. *Pleurozium schreberi* has been shown to be nitrogen sensitive in various investigations and to have a nitrogen concentration threshold, benefiting from low nitrogen inputs that do not exceed the threshold. Species belonging to the major peatland genera Pool species tend to be the most tolerant of N, likely due to the lower ionic concentrations in these moist settings, whereas hummock formers are the most N sensitive. Wet N doses more than 24 kg N ha^{-1} y^{-1} can damage *S. capillifolium* and limit its cover, according to *in situ* N manipulation studies on this species.

6.0 Future Prospect of use of algae and fungi:

Wastewater treatment with a combination of algae and fungi demonstrates its potential for environmental greening and appropriateness for use in green technical solutions. Symbiotic association of fungi and algae (Lichen) may occupy a significant role in wastewater treatment. The most commonly available lichen and their bio-absorption possibilities are given in Table-7. Segregation of nutritional wastes and heavy metals is more efficient and cost-effective when done together, with a low energy need and a big amount of useable biomass produced, which reduces sludge formation. They can be cultivated in conditions that are unfavorable for conventional farming.

Table 7. Lichens and their absorption capabilities

S. No.	Name of Lichen	Bioabsorption of heavy metals
1.	*Parmelia tiliaceae*	Pb(II) and Cr(III)
2.	*Cladonia furcate*	Ni(II) and Pb(II)
3.	*Hypogymnia physodes*	Co(II)
4.	*Cetraria islandica* (L.)	Au(III)
5.	*Usnea longissimi*	Cu(II)
6.	*Pseudevernia furfuracea*	Ni(II) and Cu(II)
7.	*Evernia prunastri*	Pb(II) and U(IV)
8.	*Cladonia thalli*	Pb(II), Cu(II) and Zn(II)
9.	*Pseudevernia furfuracea*	Zn(II)

7.0 Conclusion

In this study, we analyzed the physio-chemical parameters as well as heavy metals and pesticide in both the algae and water samples. After concluding the remarkable presence of the metals and pesticides in Hindon river. Algae treatment can be helpful for balancing of the pH value of contaminated surface water. The approach and the possibilities in the wastewater treatment can be more beneficial if we use this algae-based treatment. In real sample analysis, we found better results in the contaminated wastewater. Lichen and its positive impacts are also highlighted the scope of green methodology for wastewater treatment.

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