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Role of *Lemna minor* L. In Phytoremediation and Reduction of Pollutants of Pulp and Paper Mill Effluents

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Abstract

Pulp and paper mill industrial effluents have pollution load in relation to their physicochemical characteristics, exceeding the standard recommended permissible limits set by various agencies. The aquatic macrophytes were found to alter the pH from alkaline to neutral pH in almost every industrial effluent. Changes in pH were dependent mainly upon the duration of the treatment methods adopted. The changes in physicochemical characteristics of paper mill effluent because of phytoremediation by *Lemna minor* L. were elaborated in this experiment. In this study, the TDS, DO, BOD, and COD values of pulp and paper mill effluent were significantly reduced by the application of *Lemna minor* L. The data demonstrated a reduction of TDS, BOD, and COD parameters dependent on concentration and duration. The results of the phytoremediation experiment are very effective for the reduction of pollutants in paper mill effluent. Data indicates that *Lemna minor* L. plants were significant in maintaining the physico-chemical characteristics of the effluent concentration at various exposure durations.

Keywords: Pulp and paper mills effluent, aquatic macrophytes, phytoremediation, BOD, COD, *Lemna minor* L.

1. Introduction

To fulfil the demands for packaging, printing, and hygiene goods, the pulp and paper sector produces about 400 million tons of paper yearly, making it a vital component of the worldwide economy. But it has a big effect on the environment, especially because production procedures like pulping, bleaching, and washing produce wastewater, or effluent. Through water pollution, eutrophication, and biodiversity loss, these effluents endanger aquatic ecosystems, soil quality, and human health if they are not properly handled. The poisoning of the Great Lakes in the middle of the 20th century is only one example of how untreated discharges have traditionally caused significant environmental harm in areas with considerable paper manufacturing, such as North America, Europe, and Asia[1]. Innovative and environmentally friendly treatment techniques are crucial as environmental sustainability gains international attention. Bioremediation, particularly using aquatic plants like *Lemna minor* L. (common duckweed), offers a promising solution for reducing pollutant concentrations in pulp and paper mill effluents. The environmental impact of the pulp and paper business includes pollution of the air, water, and solid waste. Water pollution is especially bad because of the high-water consumption (up to 60 cubic meters per ton of paper produced) and the effluents that are released thereafter, which are packed with pollutants[1].

These discharges, which come from chemical or mechanical pulping, bleaching with chlorine, and washing operations, can lower oxygen levels, kill fish, and add harmful materials to the food chain[2]. Effluent restrictions have been established by stricter rules, such as those enforced by the U.S. Environmental Protection Agency under the Clean Water Act, although compliance is still difficult, particularly in poor nations where enforcement is lax[1]. Advanced treatment techniques are required because of the sheer volume and tenacity of these effluents, which worsen pollution and water scarcity worldwide.

The complicated effluents from pulp and paper mills are distinguished by high levels of chemical and biochemical oxygen demand (BOD and COD), which signify substantial organic loads that lower the dissolved oxygen in receiving waters[3]. COD frequently surpasses 1,000 mg/L, much over permissible discharge limits, while BOD values can vary from 200 to 1,000 mg/L. Lignin and its derivatives, which are complex aromatic polymers found in wood and give it a black hue while preventing decomposition, are important pollutants. Aquatic life is at risk from these lignin compounds because they are mutagenic and androgenic[4]. Chlorine bleaching produces chlorinated organic compounds, which are bio-accumulative and carcinogenic and include furans, dioxins, chloroform, and adsorbable organic halides[1] (AOX). Heavy metals (such as Mercury, Lead, and Cadmium), sulphates, chlorides, and nutrients like nitrogen and phosphorus are examples of inorganic pollutants that cause eutrophication and toxicity[5]. Effluents may become more acidic because of gaseous pollutants like sulphur dioxide and hydrogen sulphide dissolving in them[6]. Fatty acids, resin acids, and phenols increase toxicity even more, leading to long-term consequences such as lung problems in people and endocrine disruption in animals[7]. Research shows that even at 2 % concentrations, untreated wastewater may kill fish[2]. Furthermore, lignin's dark coloring hinders light penetration in aquatic bodies, which damages photosynthesis and modifies ecosystems[4].

Physical, chemical, and biological procedures are the traditional ways of treating pulp and paper effluents. While secondary treatment uses activated sludge or anaerobic digestion to lower BOD and COD, primary treatment uses screening and sedimentation to remove suspended solids[3]. Specific contaminants like color and AOX are the focus of tertiary treatments such as chemical precipitation and advanced oxidation[8]. These approaches do have some serious disadvantages, though. Chemical and physical processes use a lot of energy and result in secondary wastes that need to be disposed of further, including sludge that contains heavy metals[9]. Only partial decolorization and detoxification are achieved by biological treatments when dealing with resistant substances like lignin and chlorinated organic[10]. These techniques are unsustainable for smaller mills because of their high operating costs, which are estimated to be between 10 and 20 percent of output costs, and the requirement for professional maintenance[9]. Alternative methods are required since effluents frequently do not satisfy regulatory levels, even after treatment.

Bioremediation is an economical and environmentally beneficial option that uses natural biological processes to break down or sequester contaminants. Enzymes, plants, or microbes are used to change dangerous chemicals into less dangerous ones. Bioremediation uses plant uptake or microbial degradation to target ligno-cellulosic wastes and chlorinated chemicals in pulp and paper effluents[11]. Bacillus strains and fungi like *Phanerochaete chrysosporium* have demonstrated the ability to decolorize wastewater by enzymatic degradation[12]. But because of its ease of use and other advantages, such as producing biomass for feed or bioenergy, plant-based bioremediation, also known as phytoremediation, is especially beneficial[13].

Using plants to absorb, collect, or break down pollutants in soil, water, or the air is known as phytoremediation. Because of their quick growth, large biomass output, and ability to withstand contaminated conditions, aquatic plants are perfect for treating wastewater. BOD, COD, and nutrient levels have been shown to decrease in constructed wetlands that use plants such as water hyacinth, *Eichhornia crassipes* L[14]. Among the mechanisms are rhizo-filtration (root adsorption), phyto-extraction (uptake into plant tissues), and phyto-stabilization (immobilization). Phytoremediation provides a complete solution for pulp and paper effluents by addressing both organic and inorganic contaminants. It is appropriate for sustainable wastewater management because of its advantages, which include low energy needs, aesthetic value in treatment systems, and resource recovery potential[13].

Lemna minor L.

Lemna minor L., the common duckweed, is a member of the family and order Aerales. The Lemnaceae family includes plant species that have an oval or circular shape with a leaf surface area of no more than a few square millimeters[15]. It is found on floating surface or immersed in water bodies[16]. Due to fast growth rate of duckweed, it is well suited for waste treatment purposes, as it is relatively easy for maintenance and operates such as system[17]. Duckweed plants obtained by treating water are collected from the water surface itself; duckweed grown in sewage water or livestock waste (wastewater) is not poisonous and can be used as fish and cattle feed, or as crop fertilizer[18]. For more certainty, it can be kept in safe water for a certain term or cleaned with UV-rays or ozone gas after drying[19]. Duckweed can survive from pH of 5 to 9 conditions but grow best at the range between pH 6.5 to 7.5 range, with the growth generally controlled by temperature and sunlight exceeding nutrient concentrations in the water[20]. The most suitable humidity (moisture) content of fresh duckweed growth is 95 %[21]. Plant cultivation and growth do not require a significant initial population in water bodies, because even a small amount will be enough for its quick reproduction and multiplication[22]. The most common method for rapid growth of duckweed is ensuring the water surface is calm with small to no current flow; in case the water moves too much, the plant growth will slow down[23]. If desired, cultivation can be done separately in a rectangular container at least 5 inches deep, 18 inches long, and 12 inches wide[24]. Duckweed has a great capacity to absorb nutrients, making it efficient in removing them from water and its application for treating sewage and untreated water a highly effective, commercially feasible, natural and simple method[25]. In fact, the plant has been successfully used for domestic and industrial wastewater tertiary treatment for over a decade[26].

The physiological and biochemical adaptations of *Lemna minor* L. are what give it its bioremediation effectiveness. Enzymes like peroxidases and catalase detoxify organics, and their strong root system improves rhizo-filtration by adsorbing dissolved contaminants. Its accumulation rates are higher than those of many species, and it can withstand organics, agrochemicals, and heavy metals. It promotes microbial breakdown in the rhizosphere of pulp and paper effluents, where bacterial populations supported by root exudates degrade resistant substances like lignin and phenols[27]. Eutrophication is lessened by nutrient intake, which lowers phosphate and nitrogen. Partially decomposed or volatilized contaminants are chlorinated. According to studies, exposure to diluted effluents (12.5 to 75 %) can reduce color by 50 %, COD by 60 %, and BOD by up to 70 % in a matter of days[28]. Additionally, the plant has an 80 to 90 % efficiency rate for removing phenolics and dyes from methylene blue analogues.

Sustainable development objectives are met by *Lemna minor* L. based bioremediation, which produces value-added products at a cheap cost (savings of 50 to 70 % compared to chemical treatments) and lowers the carbon footprint of the sector. Scaling this strategy might improve water quality and promote circular economies in areas like China and India, where paper mills pollute rivers[29]. Future developments might include hybrid systems that include bacteria and plants, as well as genetic engineering for improved pollution tolerance. Such technologies are essential as climate change exacerbates water stress.

2. MATERIAL AND METHODS

2.1 Study Area:

For the experiment, plants of *Lemna minor* L. were collected from a pond near the IET campus of Dr. R.M.L. Awadh University in Ayodhya. The effluent was collected aseptically in a sterile plastic container from a pulp and paper mill located in Darshannagar, Ayodhya, U.P. (India). It was transported on ice to the laboratory and stored at 4° C until further use.

2.2 Experimental Design:

In April 2025, samples of effluents were collected from the discharge point of Yash Paper Mill in Ayodhya (Faizabad). The effluent samples were stored in plastic containers at 4° C until further experimentation. *Lemna minor* L. plants were gathered from a nearby natural pond and were thoroughly washed with running tap water, followed by rinsing by distilled water, to eliminate any surface contamination. The experiment was conducted by using plastic tubs with a capacity of 10 liters each. One tub was filled with 5 liters of distilled water, while the other contained 5 liters of the Yash Paper Mill effluent. *Lemna minor* L. plants were then immersed in each tub (Fig. 1). The plants were allowed to grow, and different pollution load parameters were analyzed at intervals of 15 days over a period of 40 days.

At each specified interval, 50 mL samples were withdrawn from both tubs for analysis of various physicochemical parameters. The volume of effluent lost during sampling was replenished by adding an equivalent amount of distilled water to each tube. The analysis of the selected pollution parameters in the effluent was completed at intervals of 0, 10, 20, 30, and 40 days from the start of the experiment, using standard methods.

Figure 1: Use of the aquatic plant *Lemna minor* L. for the reduction of pollutants in pulp and paper mill effluent in laboratory

2.3 Dissolved oxygen (DO):

Oxygen present in the sample oxidizes the divalent manganese to its higher valency, which precipitates as brown-hydrated oxides after the addition of NaOH and KI. Upon acidification, manganese reverts to the divalent state and liberates iodine from KI equivalent to the DO content in the sample. The DO is calculated by the azide modification of Winkler's method.

Calculation-DO(mg/L)= $\frac{ml\ of\ titrant \times normality \times 8 \times 1000}{V}$

2.4 Biological oxygen demand (BOD):

It is defined as the amount of oxygen required by bacteria in decomposing organic material in a sample under aerobic conditions at 200 over a period of 5 days.

$C_6H_{12}O_6 + 6O_2 \longrightarrow CO_2 + H_2O$ + Microorganism

We are calculating carbonaceous BOD by the dilution method. In the first step, we calculate the initial DO of the sample, and the DO after 5 days of incubation.

Calculation - BOD (mg/L) = $\frac{(D1-D2)}{P}$

2.5 Chemical oxygen demand (COD):

The COD test determines the oxygen required for chemical oxidation of most organic matter and oxidizable organic substances with the COD test are determined by the reflux digestion method.

Calculation -COD (mg/L) = $\frac{(A-B\times N\times 8\times 1000)}{ml\ of\ sample}$

where 'A' is the volume of Ferrous Ammonium Sulphate (FAS) for the blank, 'B' is FAS for the sample, 'N' is the normality of FAS, 'V' is the sample volume, and '8000' converts units to mg/L. This measures oxygen needed for oxidation, determined by titrating unreacted dichromate after refluxing the sample with an excess oxidant.

2.6 Total dissolved solids (TDS):

Many solids are found dissolved in natural water, the common carbonate, bicarbonate, chloride, sulphate, phosphate, etc. In other words, TDS is simply the sum of the cations and anions concentration expressed in mg/L.

Calculation -TDS (mg/l) = $\frac{(W2-W1)\times 1000\times 1000}{ml\ of\ sample}$

2.7 Total suspended solids (TSS):

TSS applies to the dry weight of the material that is removed from the measured volume of water sample by filtration through a standard filter.

Calculation –TSS (mg/L)= $\frac{(W2-W1)\times 1000\times 1000}{ml\ of\ sample}$

2.8 Alkalinity:

Alkalinity is a measure of the water's ability to absorb hydrogen ions without a significant pH change. The alkalinity of a sample can be estimated by titrating with standard sulphuric acid. Titration to pH 8.3 or decolorization of phenolphthalein indicator will indicate complete neutralization of OH and ½ of CO_3, while to pH 4.5 or a sharp change from yellow to pink of methyl orange indicator will indicate total alkalinity.

Calculation -Alkalinity (mg/L) = $\frac{A\times N\times 50\times 1000}{ml\ of\ sample}$

2.9 pH:

It is the negative logarithm of hydrogen ion concentration, more precisely, hydrogen ion activity. The pH changes of the effluent in the due course of growth, decolorization, and de-chlorination were measured for each set of experiments using a pH meter.

3. Result and Discussion

The efficiency of *Lemna minor* L. in scavenging contaminant indicates that the presence of such macrophytes is an important element for contaminant removal in wastewater. Hydrophytes can supply required oxygen by oxygen leakage from the roots into the rhizosphere to accelerate aerobic degradation of organic compounds in wetlands. This assumption was confirmed in the present study, since the accumulation of heavy metals was higher in plants than in water. Phytoremediation can be classified as phyto-extraction, phyto-degradation, phyto-stabilization, phyto-stimulation, phyto-volatilization, and rhizo-filtration. Rhizofiltration, also referred to as phyto-filtration, is based on hydroponically grown plants that are most efficient in removing heavy metals from water. Phyto-extraction was considered to have poor role in metal extraction, but it should be promoted.

3.1 Effects on pH:

The pH value of any sample basically depends on the nature of the sample, i.e., the acidity and basic nature of the aqueous solution. The pH measurement is useful in effluent treatment to find the design, types, and efficiency of the solution. Discharged from the effluent treatment plant has both acidic and alkaline characteristics of effluents. The untreated pulp and paper mill effluent pH value was 7.4; the final treated effluent pH was found to be 7.8 (Fig. 2). So, the pH value was basic in nature and increased due to the microbial activities[3, 30].

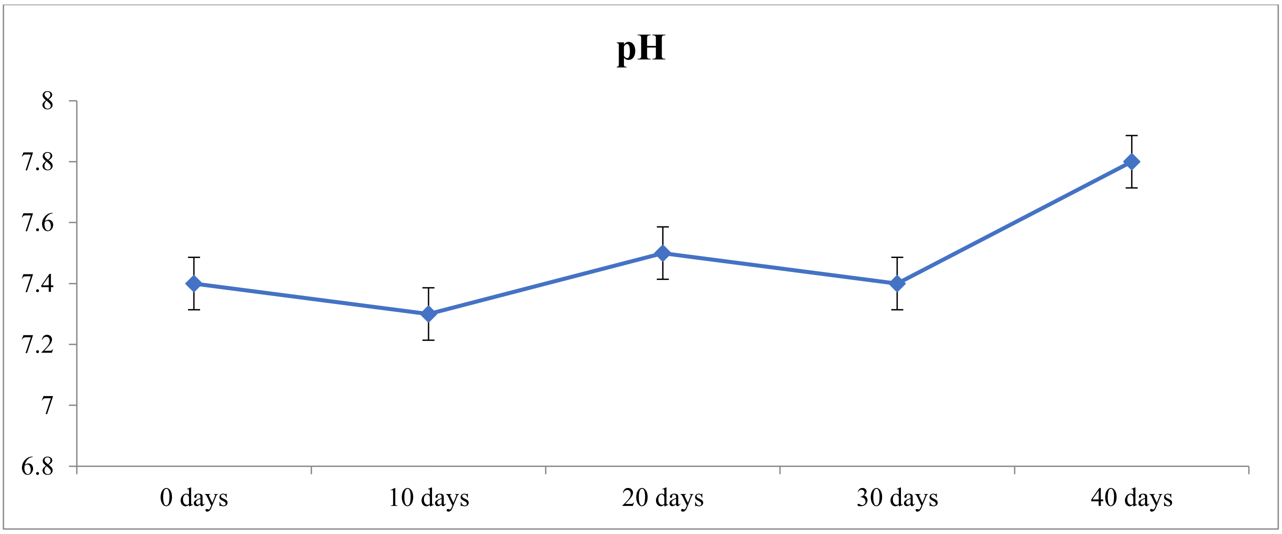

Figure 2: Graph showing the pH reduction and then again slightly rise in pH after 30 days of treatment of paper mill effluent by *Lemna minor* L.

3.2 Effects on BOD:

Biological oxygen demand (BOD) is the demand of oxygen required by microbes to degrade organic matter. It is a good parameter of water quality to assess the water quality of any sample. In the present investigation, the BOD of untreated effluent was 98.8 mg/l, and after 40 days of treatment of this effluent by *Lemna minor*, the BOD value reduced to 64.4 mg/l (Fig. 3). It shows a 65.16 % reduction. This reduction may be due to *Lemna minor,* which utilizes organic matter for its metabolic activity[31].

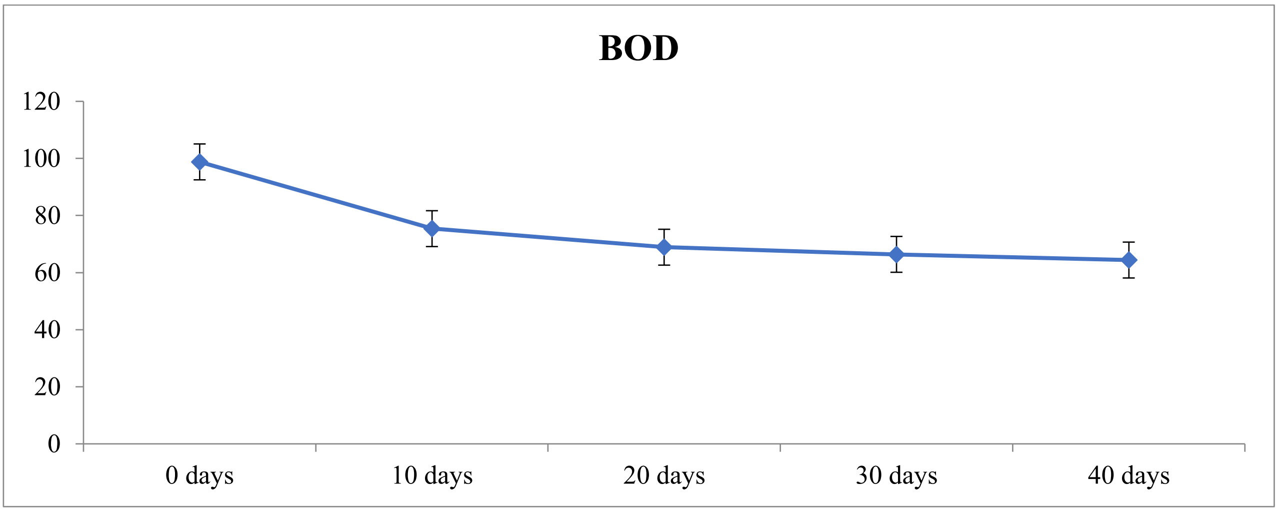

Figure 3: Graph showing the BOD reduction in paper mill effluent after treatment with *Lemna minor* L.

3.3 Effects on Total suspended solids (TSS):

The undissolved matter present in water or wastewater is usually referred to as suspended solids. In the present investigation, the TSS of untreated effluent was 35.6 mg/l, and after treatment of this effluent by *Lemna minor* L., the TSS value reduced to 25.8 mg/l (Fig. 4). It shows a 72.3 % reduction. This reduction may be due to the photosynthetic activities of water plants by smothering benthic organisms[32, 33].

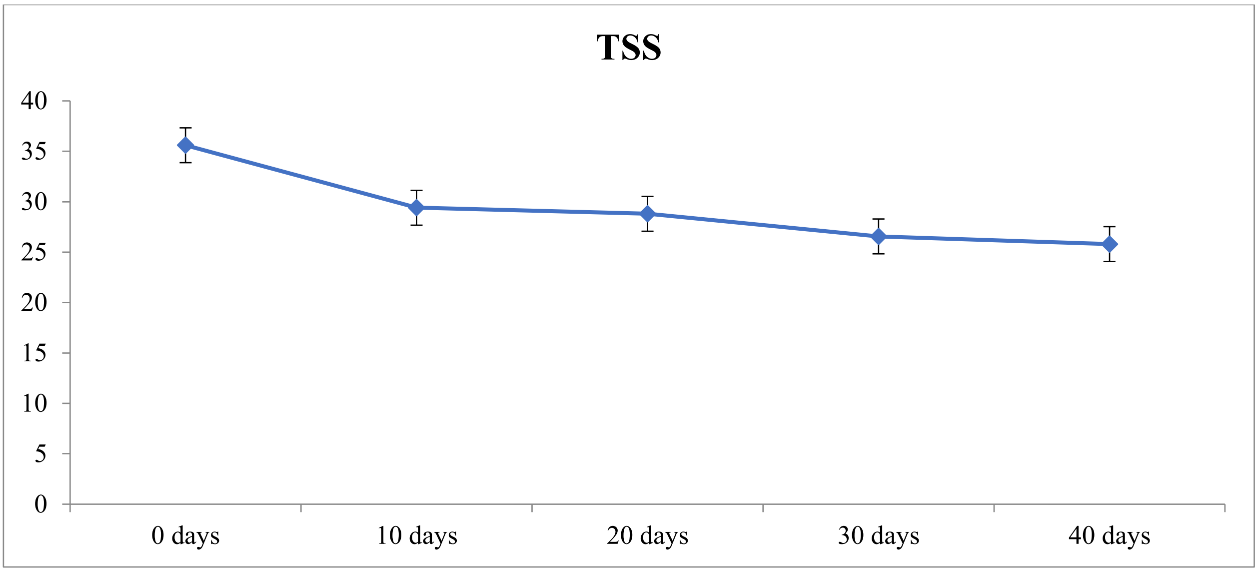

Figure 4: Graph showing the TSS reduction in paper mill effluent after treatment with *Lemna minor* L.

3.4 Effects on Total dissolved solids (TDS):

TDS is a measure of the combined content of all inorganic and organic substances contained in a liquid in molecular, ionized, or micro-granular (Colloidal solution) suspended form. In the present investigation, the TDS of untreated effluent was 1450 mg/l, and after treatment of this effluent by *Lemna minor*, the TDS value reduced to 1090 mg/l. It shows a 75.17 % reduction (Fig. 5). This reduction may be due to the decrease in the concentration of suspended solids[32, 34].

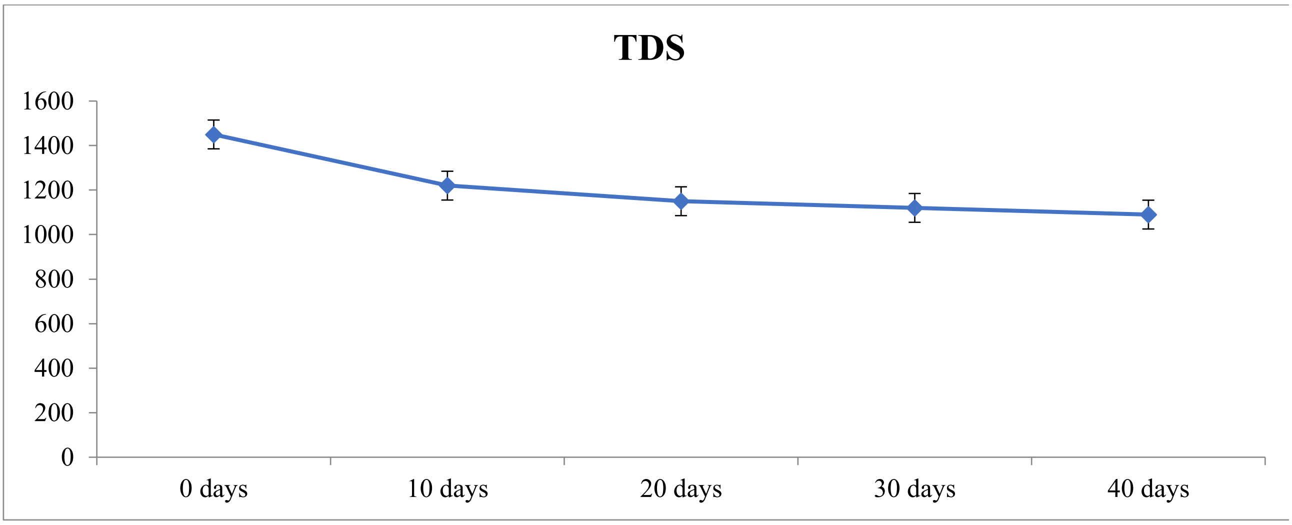

Figure 5: Graph showing the TDS reduction in paper mill effluent after treatment with *Lemna minor* L.

3.5 Effects on Dissolved oxygen (DO):

DO analysis measures the amount of Gaseous oxygen dissolved in an aqueous solution. It is a good parameter of water quality. In the present investigation, the DO of untreated effluent was 2.9 mg/l, and after treatment of this effluent by *Lemna minor*, the DO value increased up to 6.5 mg/l. It shows a 232.14 % reduction (Fig. 6). This increase may be due to the *Lemna minor*, which oxygen gets into water by diffusion from the surrounding air through aeration and a waste product of photosynthesis[34].

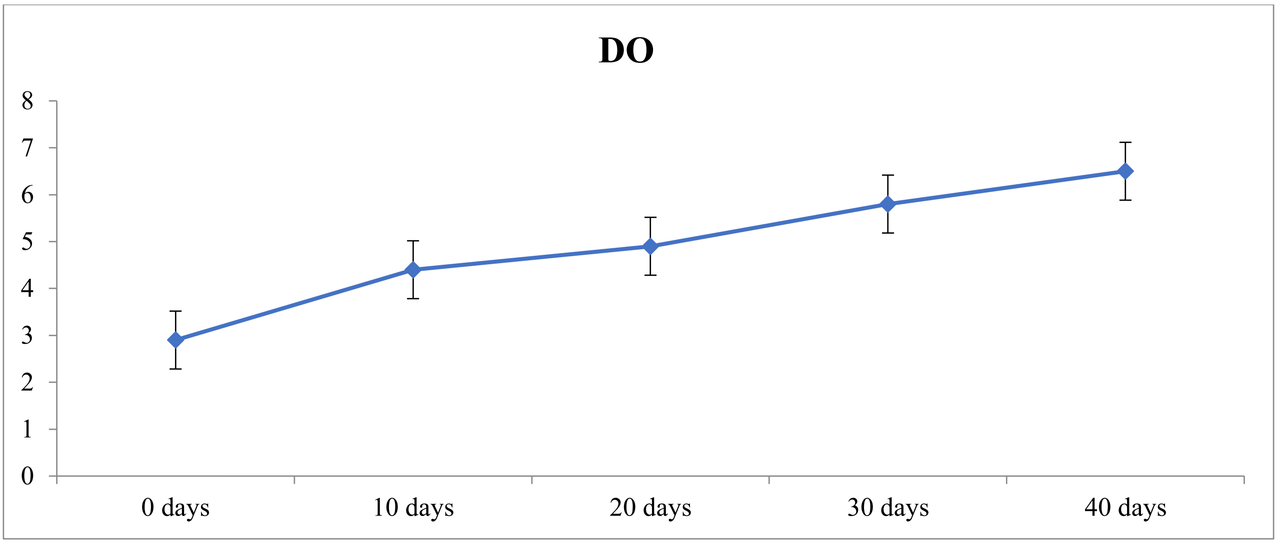

Figure 6: Graph showing the rise in DO activity in paper mill effluent after 20 to 40 days of treatment with *Lemna minor* L.

3.6 Effects on Alkalinity:

Alkalinity is a quantitative capacity of an aqueous solution to neutralize the acidity of any substance. In the present investigation, the untreated effluent was 425.6 mg/l, and after treatment by *Lemna minor*, the alkalinity value increased to 458.2 mg/l due to the increase of pH of paper mill effluent (Fig. 7). It shows a 7.11 % increase as compared to before and after treatment by *Lemna minor*. Alkalinity was highly dependent on presence of our extensive use of sodium hydroxide ions in the pulp and paper industry[35]

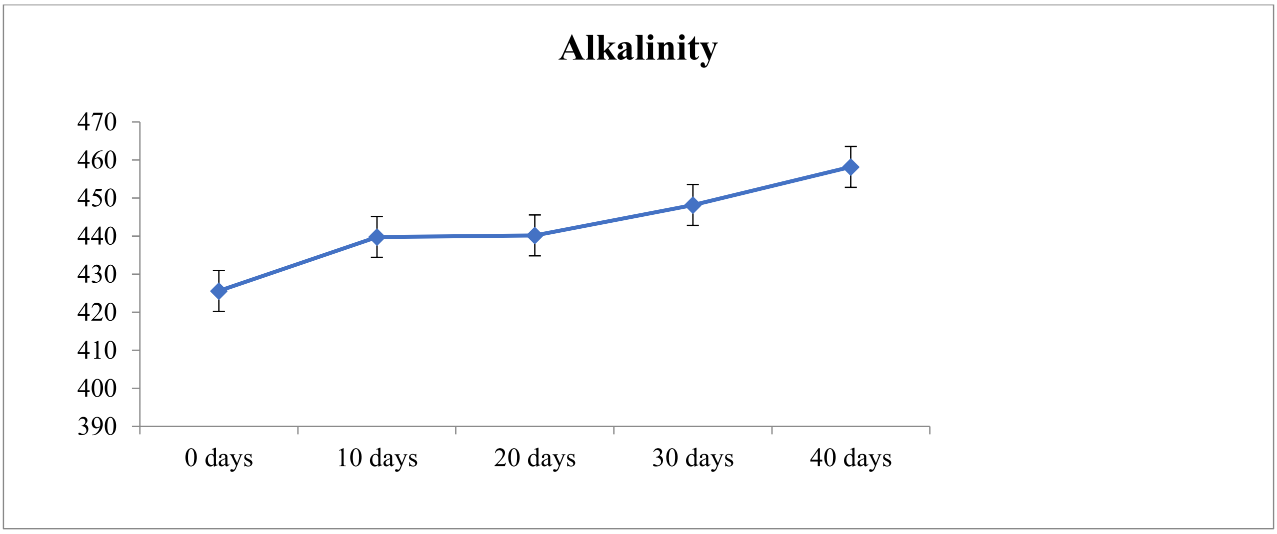

Figure 7: Graph showing the slight rise in alkalinity during beginning of 10 days treatment in treated paper mill effluent by *Lemna minor* L. and it again occurred after 20 to 30 days of treatment.

3.7 Effects on chemical oxygen demand (COD):

Chemical Oxygen Demand is the measure of the amount of oxygen required to break down both organic and inorganic matter. In the present investigation, the untreated effluent was 544 mg/l, and after treatment of this effluent by *Lemna minor*, the COD value reduced to 474.4 mg/l. It shows 87.2 % (Fig 8). This reduction may be due to the destruction of organic substances as well as the self-purification capacity of the water body[31].

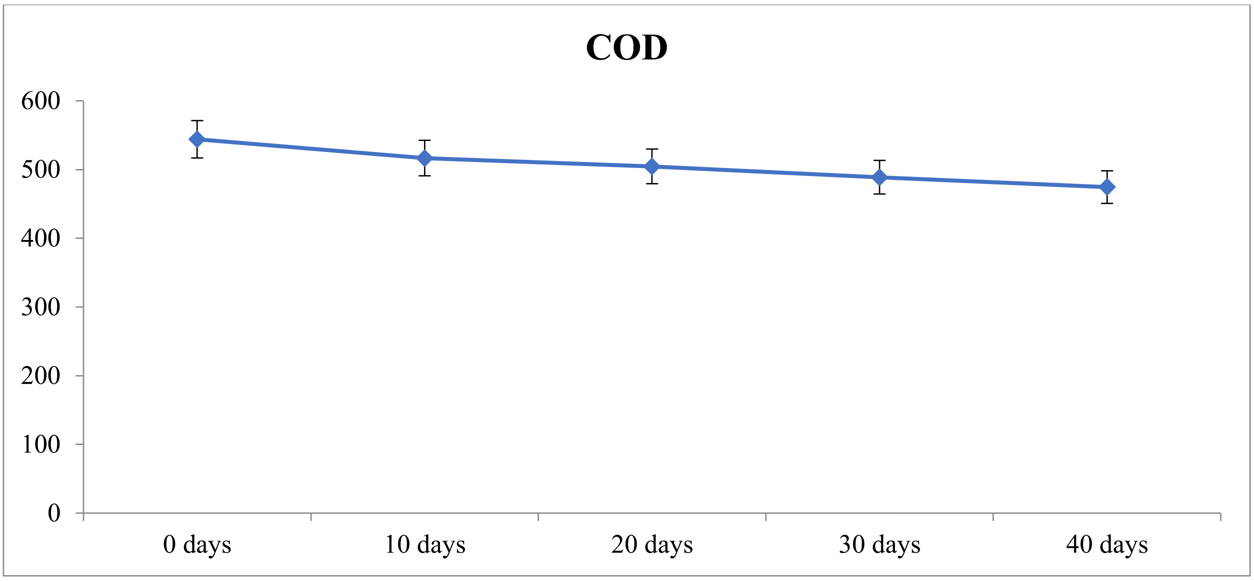

Figure 8: Graph showing the reduction in COD of paper mill effluent treated with *Lemna minor* L.

4. Conclusion

Nowadays, water contamination caused by industrial effluent having heavy metals is a major problem worldwide. Both wastewater and un-sufficiently treated industrial water contribute continuously to degrade the environment. In contrast to organic contaminants, heavy metals persist and are likely to accumulate in the environment through food chains etc. and causing different types of damages. To overcome their effects, conventional remediation technologies, such as chemical precipitation, reverse osmosis, ion-exchange, and solvent extraction, have disadvantages, including incomplete metal removal, being quite expensive, and the generation of toxic sludge, which requires disposal. Hence, 'Phytoremediation' has proved to be a viable option to purify water contaminated with heavy/trace elements since it is cost-effective and has a positive impact on the environment. This is an alternate technology in which small-scale wastewater treatment can be achieved. With increasing time, the concentration of the pollutants decreases. However, beyond attainment, *Lemna minor* L. ceases to contribute towards pollution removal. The variation in parameters caused by phyto-remediation of industrial effluents cannot exceed a finite limit and a maximum on the first day of the experiment. So, the use of such plants in the treatment of industrial effluents having heavy metals as contaminants is a safe and cost-effective technique for sustainable prospects.

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Authors' Contributions: RK and SV conducted the experiment and wrote the original draft of the paper. AKS conceptualized and supervised the project, while PK handled the editing and corrections and helped in writing original draft. Additionally, RV, SV, and PK were involved in the various investigation of the study. All authors have read and agreed to the final version of the manuscript.

Data Availability Statement: All data generated is included in this article.

Conflict of interest: The authors declare that there is no conflict of interest regarding this research work.

Ethical Statement: This study involved plant materials and environmental samples only. No experiments were conducted on human participants or live animals. All experimental procedures complied with relevant institutional, national, and international guidelines for plant research and environmental safety. The authors confirm that no ethical approval was required for this study.

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