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# Wastewater to Wetlands: Turning the Tide with Azolla Ferns

# Wastewater to Wetlands: Turning the Tide with Azolla Ferns

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**Abstract**. Water pollution is a major problem exacerbated by untreated wastewater discharged into the environment, leading to eutrophication and algal blooms. This research at the American University of Beirut explores the potential of using *Azolla pinnata*, an aquatic fern, to rid wastewater from ammonium ($NH_4$-N) and soluble reactive phosphorus (SRP), which are the main contributors to eutrophication. A controlled phytoremediation experiment conducted at the Advancing Research and Enabling Communities (AREC) center in the Bekaa valley showed that *A. pinnata* can decrease $NH_4$-N and SRP in the primary treated wastewater by 98.2% and 96.4% respectively, within 20 days. The color and odor of treated wastewater reverted to the characteristics of fresh water, making this recycling method highly sustainable due to its relatively low cost. The prospective project would be scaled to the university's farm level by constructing artificial wetlands at AREC using wastewater generated by the farm facilities. The harvested *Azolla* can be used as animal feed and/or as a green fertilizer. Successfully reintroducing the precarious wetlands in that arid region would alleviate the stress on aquifers and replenish many endemic species currently on their way to extinction. As a result, the university would be treating its wastewater in a sustainable way while contributing to greening the landscape slowly transfigured by desertification.

**Keywords:** Phytoremediation, Waste Water, Azolla, Eutrophication, American University of Beirut

## 1. Introduction

The pressure on freshwater resources is intensifying, leading to their rapid exhaustion[1]. The overuse of water in some areas and misuse in others is leading to water pollution and increasing scarcity[2]. Water stress is a global issue that is exacerbated by the growing population, expanding urbanization, widespread industrialization and alarming climate change patterns. The Sustainable Development Goals (SDG) indicator 6.4.2 on global water stress increased from 17 percent in 2017 to 18 percent in 2018[3]. Freshwater biodiversity decreased 81 percent between 1970 and 2012 due to misuse, overuse and exploitation of water bodies[4]. According to the UN World Water Development Report, two thirds of the world's population currently live in areas that experience water scarcity for at least one month a year[5]. Moreover, about 500 million people live in areas where water consumption exceeds the locally renewable water resources by a factor of two[6].

Besides water scarcity, water pollution is another factor contributing to the deteriorating global water status. According to the Organization for Economic Co-operation and Development (OECD), at least half the world's population suffers from polluted water. Excessive fertilization, particularly nitrogen (N) and phosphorus (P) contaminates soil and water resources[7]. Nitrogen and phosphorus are the main contributors to the eutrophication of freshwaters[8, 9]. Eutrophication results in algal blooms that produce harmful toxins such as neurotoxins, hepatotoxins, cytotoxins, dermotoxins and endotoxins, which can have detrimental impacts on humans, animals and the ecosystem[10, 11]. Over 80 percent of the wastewater worldwide is discharged into the environment without treatment. Discharging improperly treated wastewater can have numerous negative impacts on human health, the environment and socioeconomic development. The U.S. Environmental Protection Agency (EPA) estimates that 46% of the world water bodies are polluted and inappropriate for human use[12].

Sustainable water management requires treating wastewater as a resource, rather than a waste to be disposed of[13]. This is important in the framework of the 2030 Agenda for Sustainable Development, where SDG target 6.3 emphasizes the need for reducing pollution and improving the management and treatment of wastewater. In contrast to freshwater, wastewater is a widely available and ample resource. It is characterized by year-round availability and the possibility of recovering energy and nutrients. Therefore, recycling wastewater can have a positive impact on food security and climate change adaptation, but also in terms of environmental sustainability, economic development and societal benefits[14, 15].

Wastewater treatment technologies have seen substantial improvements since the invention of the aerated systems such as the activated sludge system. Physical processes like settling and filtration, and chemical procedures like disinfection and coagulation have been efficiently used to restore polluted water, but they are costly and non-feasible[16]. Decentralized wastewater treatment systems, such as constructed wetlands, are becoming a viable option for many countries because the investment cost of these treatment facilities represents only 20–50% of conventional treatment plants, with even lower operation and maintenance costs. Therefore, natural or biological treatment technologies are important to complement the limitations of the existing ones. Phytoremediation is a possible complementary technology that emerged 300 years ago being efficient and eco-friendly[17].

The term ‘‘phytoremediation’’ comes from the Greek word “phyto” = plant, and Latin ‘‘remedium’’ = restoring balance. It describes the ability of plants to remove, uptake or stabilize contaminants from soil, water or air[8, 10]. Most phytoremediation studies have focused on cleaning contaminated soils, but less attention has been given to purifying polluted water. Many aquatic plants have been studied for their potential in removing heavy metals and organic pollutants from contaminated water[18]. Macrophytes, which are macro aquatic plants, occur naturally in wetlands, shallow lakes, ponds and streams (Yin Sim Ng, 2016). They are promising in phytoremediation initiatives because of their potential to improve the biological, chemical and physical properties of wastewater, particularly in shallow water columns, by absorbing and concentrating organic and inorganic pollutants from heavily polluted waters (Yin Sim Ng, 2016).

This research aims to fill such research gaps around phytoremediation initiatives in Lebanon by using *A. pinnata* to purify treated wastewater from specific pollutants, namely ammonium ($NH_4$-N) and soluble reactive phosphorus (SRP). *A. pinnata* used in this experiment is a free-floating macrophyte, known as a hyper-accumulator because of its fibrous root system and large biomass[18]. Establishing a pilot wetland is another objective of the project. Such a trend would help mitigate several aspects of climate change in the area[19, 20]. Namely, the disappearance of endemic species, and attrition of the “bottleneck site” migratory bird routes, deeply affected by the fragile wetlands ecosystem in this arid region[20-22]. The American University of Beirut’s pioneering research helped change several unsustainable practices in Lebanese agriculture since the establishment of its experimental farm centre in the Bekaa valley [23].

Haphazard wastewater management, intensive use of agrochemicals and exploitation of groundwater resources have led to severe water scarcity and increased deterioration of the water quality in Lebanon. Besides, this has led to the extinction of many endemic species due to water scarcity. The situation has negatively affected the quality of life of the inhabitants and the economy[24]. The cost of water degradation in Lebanon was estimated at about US$120 million per year or 0.71 percent of annual GDP[25].

## 2. Methodology

### 2.1. Description of the Study Site and Experimental Design

The experiment was conducted at the American University of Beirut's center for Advancing Research Enabling Communities (AREC)in a greenhouse covered with a green net and a white plastic shield to maintain the proper light requirements of the plants. AREC is located at Haush-Sneid in the Bekaa Valley, around 1000 meters above sea level, 80 km from Beirut and 25 km northeast of Zahle. The first season of the experiment extended from the 6$^{th}$ of April till the 24$^{th}$ of May where the temperatures ranges between 19ºC and 30ºC during this period. The experiment consisted of five treatment and three replicates of each treatment: Negative Control (NC), Positive Control (PC), Primary Treated Wastewater (PW), Secondary Treated Wastewater (SW) and Tertiary Treated Wastewater (TW). A complete randomized design was adopted to place all the replicates in the greenhouse.

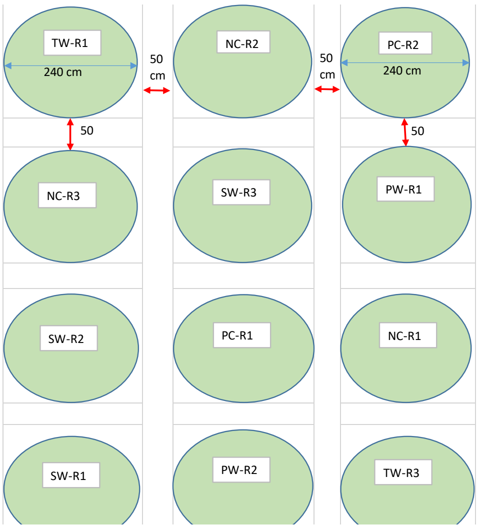


**Figure 1.** Experimental layout randomized on Excel (R1, R2 and R3 represent replicate 1, 2 and 3 of each treatment

**Figure 2.** (a)Azolla ferns, and (b)the media of the experiment taking place at AREC Farm

### *2.2. Source of Treated Wastewater and A. pinnata*

The *A. pinnata* plant material used in this study was freshly collected from a farm in South Lebanon. Fifteen improvised plastic pools (240 cm diameter and 20 cm depth) were used to mimic ponds. They were filled with 1000 L of treated wastewater (TWW), collected from the Zahle Wastewater Treatment Plant and transported to AREC using a special municipal wastewater truck that was flushed with clean water between the different treated wastewater shipments.

### *2.3. Wastewater Parameters Analysis*

The study is limited to the following physical parameters: color, odor, electrical conductivity (EC) and pH. Color and odor were assessed subjectively using a pre-set scale. EC and pH were measured using a Hach multimeter 'HQ40d' (HACH Company, Loveland, Colorado, USA). Assessed chemical parameters include $NH_4$-N and SRP. The phosphorus was determined using the ascorbic acid method APHA 4500-P according to the American Public Health Association. The analysis was carried out periodically on day 0 (baseline), day 20 and day 56. *A. pinnata* was harvested and weighed fresh on a weekly basis from all the pools.

### *2.4. Data Analysis*

Two-way Analysis of Variance (ANOVA) was applied to conduct the data analysis using the Statistical Package for Social Sciences (SPSS), version 22.0. Differences were deemed significant for a P-value less than 0.05.

## 3. Results

### 3.1. Physical Properties of Wastewater

(Negative Control (NC), Positive Control (PC), Primary Treated Wastewater (PW), Secondary Treated Wastewater (SW) and Tertiary Treated Wastewater (TW).)

The change in water color and odor over the three sampling dates is shown in Table (1). Color and odor are given scores based on a pre-set scale. The color scale ranges from 0=extremely turbid and black to 4=clear and transparent water. Both of the control treatments (NC and PC) had a color score of 4, which is higher than the score of all other treatments. As for the odor, the scale ranges from 0=extremely smelly to 5=odourless. Similarly, PC and NC treatments had the highest odor score whereas all the three levels of TWW had lower scores. On day 0, the PW treatment has the lowest color and odor scores. After 20 days, color and odor scores of the PC and NC treatments remained nearly unchanged, whereas those of the PW, SW and TW treatments improved. Color and odor scores of the SW and TW increased to 4 becoming similar to the control treatments. As for the PW, the color score increased to 2 (slightly turbid and green), and the odor score to 3 (burns smell). On day 56, the color and odor scores of the PW, SW and TW became similar to that of the NC except that the odor score of NC was slightly higher, while the PC witnessed a decrease in the odor score.

Table 1: Change in the color and odor scores in the five treatments: PW, SW, TW, PC and NC, over the three sampling dates.
Negative Control (NC), Positive Control (PC), Primary Treated Wastewater (PW), Secondary Treated Wastewater (SW) and Tertiary Treated Wastewater (TW).

| | Average Color and Odor Score over three sampling dates | | | | | |
|---|---|---|---|---|---|---|
| | **Sampling Date** | **PW** | **SW** | **TW** | **PC** | **NC** |
| **Color** | Day 0 | 1 | 2 | 3 | 4 | 4 |
| | Day 20 | 2 | 4 | 4 | 4 | 4 |
| | Day 56 | 4 | 4 | 4 | 4 | 4 |
| | | | | | | |
| **Odor** | Day 0 | 0 | 1 | 1 | 5 | 5 |
| | Day 20 | 3 | 4 | 4 | 5 | 4 |
| | Day 56 | 4 | 4 | 4 | 3 | 5 |

Color scale: 0=extremely turbid and black, 1=slightly turbid and grey, 2=slightly turbid and green, 3=Not turbid and light green, 4=colorless and clear
Odor scale: 0=extremely smelly, 1=slightly smelly with burns, 2=fishy smells, 3=burns smells, 4=grassy smells, 5=odourless.

The pH did not differ significantly among treatments (Table 2). It ranged between 6.88 and 7.54 throughout the 56 days. The pH increased in all treatments on day 20, then it increased slightly or remained unchanged on day 56 of the experiment. On day 0, the average EC of PC and NC was similar decreasing to 564 µS/cm and 512.33 µS/cm, respectively, on day 20. The EC of the PW was the highest of all treatments (954.33 µS/cm) on day 0. And that of SW and TW was 738.33 µS/cm and 751.33 µS/cm, respectivel. Similar to the trend observed in the control treatments, average EC decreased in all treated wastewater treatments after 20 days and increased again on day 56 in all treatments except in PW which decreased further to 657 µS/cm.

Table 2: Average pH and EC (µS/cm) in the five treatments: PW, SW, TW, PC and NC, over three sampling dates.
Negative Control (NC), Positive Control (PC), Primary Treated Wastewater (PW), Secondary Treated Wastewater (SW) and Tertiary Treated Wastewater (TW).

| | Average pH and EC (µS/cm) over three sampling dates | | | | | |
|---|---|---|---|---|---|---|
| | **Sampling Date** | **PW** | **SW** | **TW** | **PC** | **NC** |
| **pH** | Day 0 | 6.92 | 6.93 | 6.88 | 6.95 | 6.93 |
| | Day 20 | 7.32 | 7.54 | 7.48 | 7.24 | 7.28 |
| | Day 56 | 7.39 | 7.46 | 7.5 | 7.34 | 7.22 |
| | | | | | | |
| **EC (µS/cm)** | Day 0 | 954.33 | 738.33 | 751.33 | 820.33 | 811.67 |
| | Day 20 | 701.67 | 594.33 | 598.33 | 564.00 | 512.33 |
| | Day 56 | 657.00 | 721.67 | 787.33 | 686.50 | 638.00 |

### 3.2. Chemical Properties of Wastewater

The SRP was significantly greater in PW on day 0 compared to all other water types (Table 3), which had very low SRP concentration close to zero. On day 20, the average SRP decreased significantly in the PW by 96.4%. This is evident in Figure (1) that reflects a significant decrease in SRP between day 0 and day 20, but not day 56. SRP also had a decreasing trend in the PC treatment from 1.111 ppm on day 0 to 0.008 ppm on day 56. The SW and NC treatments did not change significantly over the duration of the experiment.

As for $NH_4$-N, its concentration on day 0 was significantly greater in PW compared to the other types of water (Table 4). On day 20, the average $NH_4$-N decreased significantly in the PW by 98.2%. This reduction in $NH_4$-N concentration in PW is represented in Figure (2). The reduction is significant between day 0 and day 20, but not day 56. The decrease in $NH_4$-N was not significant in SW, TW and NC between the three sampling days. Yet, it was significant in PC between day 0 and day 20 only.

Table 3: Change in the concentration of SRP (in mg/L) in the five treatments: PW, SW, TW, PC and NC, over the course of 56 days.
Negative Control (NC), Positive Control (PC), Primary Treated Wastewater (PW), Secondary Treated Wastewater (SW) and Tertiary Treated Wastewater (TW).

| | Average SRP Concentration (mg/L) ± SE* | | | | |
|---|---|---|---|---|---|
| | **PW** | **SW** | **TW** | **PC** | **NC** |
| **Day 0** | 8.06±0.25 Aa** | 0.028±0.004 Ba | 0.06±0.01 Ba | 1.23±0.12 Ca | 0.007±0.003 Ba |
| **Day 20** | 0.29±0.07Ab | 0.06±0.001 Aa | 0.05±0.01 Aa | 0.3±0.03 Ab | 0.05±0.005 Aa |
| **Day 56** | 0.15±0.12 Ab | 0.005±0.002 Aa | 0±0 Aa | 0.013±0.009Ab | 0.02±0.01Aa |

*SE is the standard error
** Values are deemed significant for p-value < 0.05. Different capital letters refer to significant values within the same row and different small letters refer to significant values within each column.

Table 4: Change in the concentration of $NH_4$-N (in mg/L) in the five treatments: PW, SW, TW, PC and NC, over the course of 56 days.
Negative Control (NC), Positive Control (PC), Primary Treated Wastewater (PW), Secondary Treated Wastewater (SW) and Tertiary Treated Wastewater (TW).

| | Average $NH_4$-N Concentration (mg/L) ± SE* | | | | |
|---|---|---|---|---|---|
| | **PW** | **SW** | **TW** | **PC** | **NC** |
| **Day 0** | 27.70±0.67 Aa** | 0.91±0.01 Ba | 0.62±0.02 Ba | 1.06±0.05 Ba | 0.58±0.03 Ba |
| **Day 20** | 0.59±0.3 Cb | 0.13±0.01 Cb | 0.13±0.05 Ca | 0.33±0.11 Ca | 0.24±0.03 Ca |
| **Day 56** | 0.72±0.11 Db | 0.42±0.04 Dab | 0.31±0.01 Da | 0.32±0.038 Da | 0.81±0.28 Da |

*SE is the standard error
** Values are deemed significant for p-value < 0.05. Different capital letters refer to significant values within the same row and different small letters refer to significant values within each column.

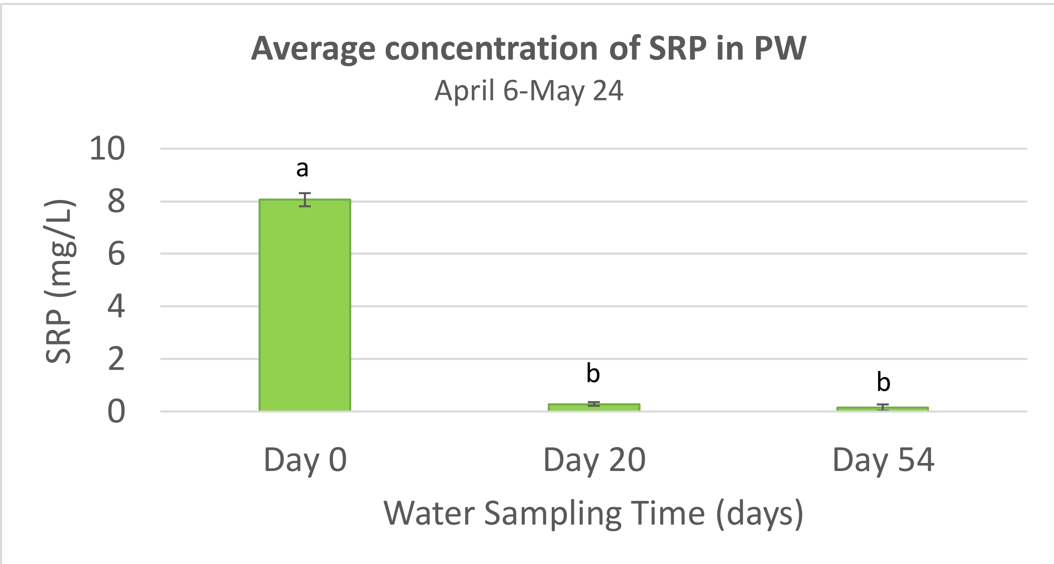


Figure 3: Average SRP concentration (mg/l)
Soluble reactive phosphorus (SRP), in primary treated wastewater (PW) over the 3 sampling (error bars are the standard error of the mean, n=3). Different letters stand for significant differences among different sampling dates.

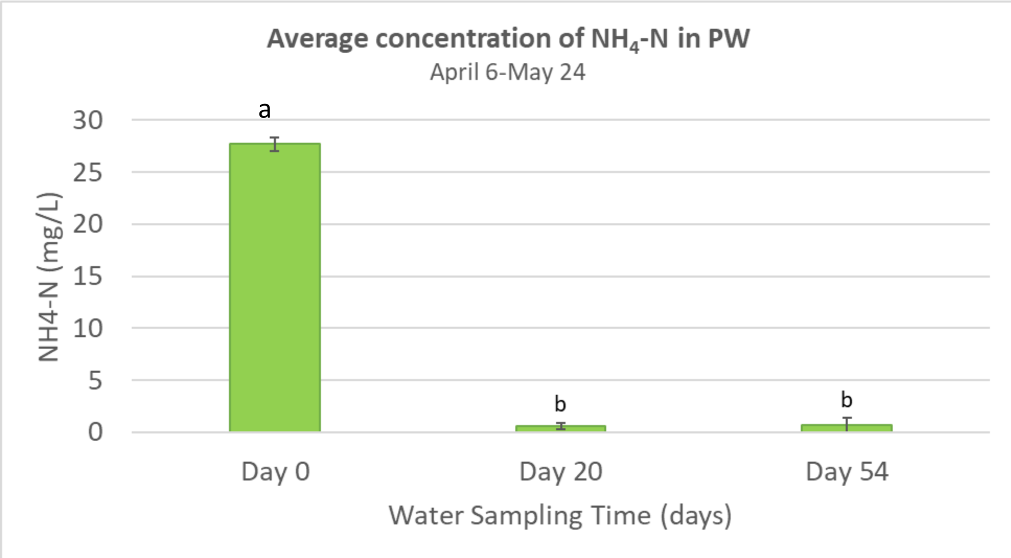


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Figure 4: Average $NH_4$-N concentration (mg/l)
Showing Ammonia (NH4-N) in primary treated wastewater (PW) over the 3 sampling dates (error bars are the standard error of the mean, n=3). Different letters stand for significant differences among different sampling dates.

## 4. Discussion

The results of this preliminary experiment showed that the color and odor of all types of TWW has noticeably improved after growing *A. pinnata* in the water. These results are somewhat comparable to a study that showed that the unpleasant odor of wastewater completely disappears and its transparency increases in a 28-day biological treatment experiment using *A. filiculoides*[26].

Azolla pinnata had no effect on the pH of water within the duration of the experiment. The pH of all types of water below 7 on day 0 did not obstruct the growth of *A. pinnata* because as reported earlier by *A. pinnata* can thrive within a pH range of 3.5 to 10[27]. The pH increased with time throughout the 56 days experiment, and it ranged between 6.9 and 7.54. The increase in pH is possibly due to the settling of the soil particles that were originally attached to the plant roots. It is also linked to the plant residues being released into the water.

## Conclusion

In conclusion, preliminary results show that *A. pinnata* is a promising agent for treating wastewater in Bekaa in an eco-friendly and sustainable way. This form of biological wastewater treatment can act as a complimentary technology to remove pollutants from water and allow a safe reuse of the recycled water. It would also contribute to alleviating water stress and reducing water pollution in the country. The next step is implementing this project at the American University of Beirut's farm, AREC, to treat the wastewater generated by the university facilities sustainably. The long-term goal is mitigating the habitat loss of threatened endemic flora and fauna species that water scarcity in the Bekaa region is exacerbating.

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