2025年臺灣國際科學展覽會
優勝作品專輯

作品編號　180027

參展科別　地球與環境科學

作品名稱　Reviving Resources: Harnessing Soap Nut Greywater for Sustainable Plant Growth

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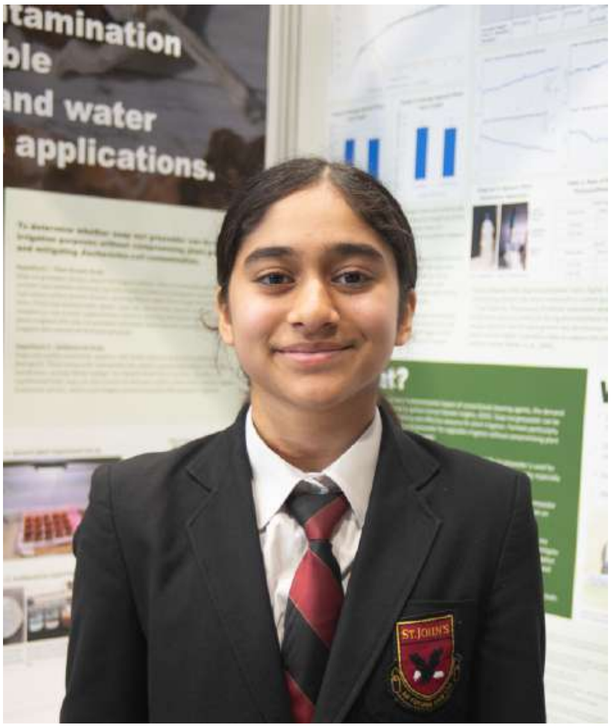


Reviving Resources: Harnessing Soap Nut Greywater for Sustainable Plant Growth

Tanvir Mundra

1. Introduction

Due to widespread water shortages, there is an increasing need for innovative water conservation strategies, such as reusing greywater from laundry. The World Health Organization (WHO) recognizes greywater as suitable for plant irrigation, but commercial laundry detergents contain synthetic chemicals that can harm both the environment and plant health.

Soap nuts, derived from the *Sapindus mukorossi* tree, offer a natural alternative. Their pericarp is rich in triterpenoid saponins, amphiphilic compounds, composed of hydrophilic sugar group and hydrophobic triterpenoid sapogenins. These saponins mimic the chemical structure of surfactants in detergents, allowing soap nuts to act as natural foaming and surface-active agents in water. As a result, soap nuts have long been used as a sustainable option for shampoo and laundry detergent in many Asian countries (Sochacki & Vogt, 2022).

Greywater, an often overlooked resource, is generated from household activities like laundry, showers, and basins. Unlike blackwater, it contains lower levels of pathogens and bacteria. However, due to a lack of awareness, greywater is frequently mixed with blackwater and directed to the same sewage treatment systems (Greywater Systems: From Recycling to Filtration, n.d.). Greywater accounts for 50-80% of a household's daily wastewater (Wong, 2011). Repurposing greywater offers a promising and sustainable solution to address water conservation challenges.

2. Purpose of Investigation

The aim of this experiment was to evaluate whether greywater from soap nuts can be safely used for irrigation without negatively affecting plant growth or contributing to *Escherichia coli* contamination.

2.1 Inspiration for the Study

My grandparents, who live in a rural community in India, have always preferred natural alternatives to synthetic cleaning products. They used soap nuts as a natural laundry detergent instead of commercial options, and like many of their neighbours, would pour the resulting greywater onto their fields. When I asked if the greywater had affected their plants, they hadn't given it much consideration. These conversations sparked my interest in exploring the impact of soap nut greywater on plant growth.

2.2 Hypothesis I - Plant Growth Study

Soap nut greywater, being a natural and organic solution, is expected to promote plant growth, as it lacks the synthetic chemicals typically found in commercial detergents. The saponins in soap nuts lower water's surface tension, potentially improving water flow through the soil around plant roots, which may enhance nutrient and water uptake. Additionally, since soap nuts are derived from the *Sapindus mukorossi* pericarp, their greywater may provide supplementary nutrients, enriching the soil. Therefore, it is hypothesised that spinach plants irrigated with soap nut greywater will show greater growth compared to those irrigated with greywater from commercial detergents.

2.3 Hypothesis II - Antibacterial Study

Soap nuts contain amphiphilic saponins, which share a similar chemical structure to the surfactants found in detergents. The hydrophobic tails of these compounds can embed themselves in the phospholipid bilayer of bacteria membranes, disrupting the cell wall and causing leakage of cellular contents, ultimately leading to bacterial death through cell lysis. Therefore, it is hypothesised that soap nuts will exhibit antibacterial properties against *Escherichia coli* K12, potentially reducing bacteria associated with greywater irrigation.

3. Method

3.1 Variables

Independent Variable: Type of laundry greywater (soap nut greywater, commercial detergent greywater, control group)

Dependent Variables

- **Plant Growth Study:** Plant growth metrics (height, leaf length, root length, respiration rate, photosynthesis rate)
- **Antibacterial Study:** Zone of inhibition (measured by diameter)

Control Variables

- **Plant Growth Study:** Light exposure, temperature, type of potting soil, sowing depth of spinach seeds, brand of spinach seeds, equipment for measuring respiration/photosynthesis, and concentration of greywater used for irrigation.
- **Antibacterial Study:** Sterile conditions, incubation temperature, volume of bacteria per petri dish, size of paper diffusion disks.

3.2 Method I - Plant Growth Study

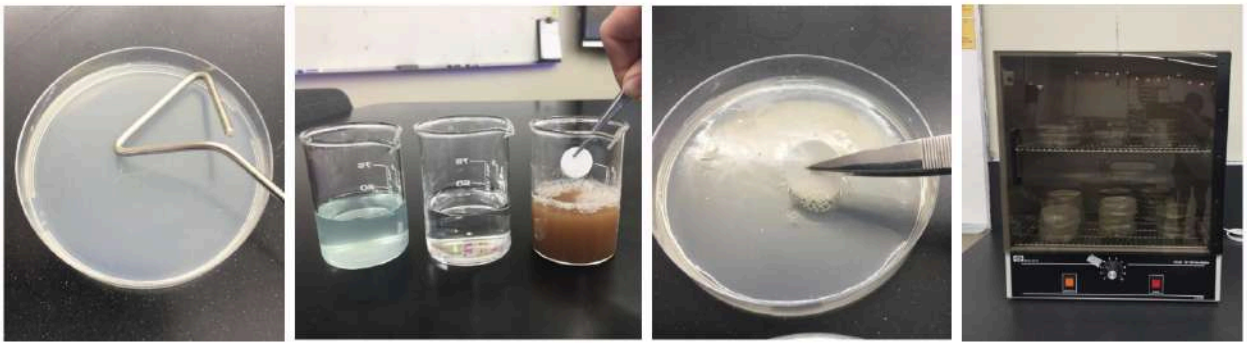
A total of 30 spinach plants were grown and irrigated with three different greywater solutions: soap nut greywater, commercial detergent greywater, and a control group using tap water, over a period of 40 days (Figure 1). Plant growth was assessed by measuring height, leaf length, root length, respiration rate and photosynthesis rate. A Vernier CO_2 gas sensor was used to detect changes in carbon dioxide levels within the respiration chamber to determine the rates of respiration and photosynthesis in the spinach plants (Figure 3). To evaluate statistical significance in plant growth, a t-Test (two samples assuming equal variances) was conducted, comparing plants irrigated with soap nut greywater against those in the control group (tap water), with p-values calculated to assess significance.

Figure 1: Spinach Plant Experiment Set-Up

3.3 Method II - Antibacterial Study

The Centers for Disease Control and Prevention (CDC) identifies *Escherichia coli* contamination in irrigation water as a significant challenge in the food industry, posing risks to consumer health and leading to economic losses for farmers, particularly when irrigating ready-to-eat produce like spinach (Irrigation water and Spinach E. coli outbreak, 2022). Despite the regulations set by the Canadian Council of Ministers of Environment and national water quality guidelines, *E. coli* contamination in irrigation water often exceeds recommended limits (Irrigation Water Quality and Mitigating Food Safety Risks, 2022). To assess whether soap nuts could help reduce *E. coli* contamination in greywater used for irrigating spinach plants, the Kirby-Bauer disk diffusion susceptibility test was conducted. This test measured the diameter of the inhibition zone for *Escherichia coli* K12 when exposed to different greywater treatments (soap nut greywater, commercial detergent greywater and a control group) in vitro (Figure 2).

Figure 2: Antibacterial Study Experiment Set-Up



Inoculate petri dishes with *Escherichia coli* K12 using spread plate technique.

Dip paper disks in greywater solutions for Kirby Bauer Disk Diffusion Susceptibility Test.

Place paper disks on inoculated petri dishes.

Place petri dishes in incubator set at 25 degrees celsius.

4. Results and Analysis:

4.1 Plant Growth Study Results

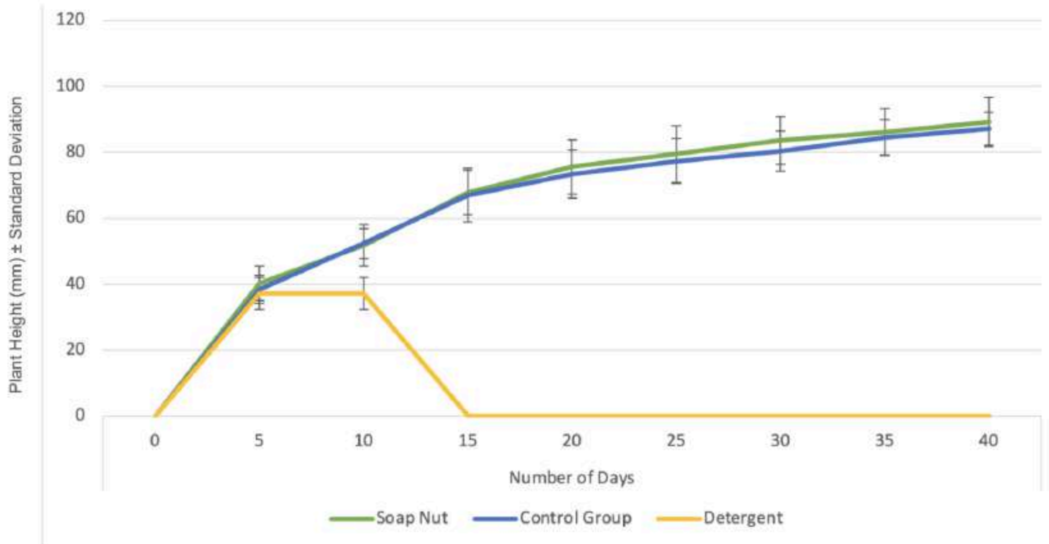
The data supported the hypothesis of the plant growth study. Spinach plants irrigated with soap nut greywater showed significantly greater plant height ($p < 0.05$) compared to those irrigated with commercial detergent greywater by day 10 (Graph 1 and Table 2). However, there was no statistically significant difference in plant height, leaf length or root length ($p > 0.05$) between the spinach plants irrigated with soap nut greywater and those irrigated with tap water (control group), indicating that soap nut greywater supports similar plant growth to tap water (Graph 2, Graph 3 and Table 1).

Spinach plants irrigated with soap nut greywater exhibited higher rates of respiration and photosynthesis compared to the control group (Graph 4, Graph 5, Graph 6, Graph 7 and Table 3). These processes are essential for plant growth, as cellular respiration breaks down glucose produced during photosynthesis into ATP, which provides the energy necessary for growth and development. Growing plants have increased respiration rates to meet the demands of heightened metabolic activity (Meller et al., 2004).

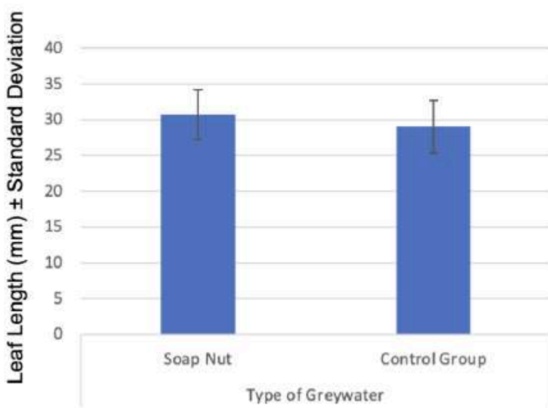
Soap nut greywater, a natural and organic solution, promoted plant growth due to its amphiphilic saponins. These saponins reduce the surface tension of water droplets, allowing water to flow more efficiently through the soil near the plant root zone, thereby enhancing nutrient and water uptake (Liu et al., 2024).

In contrast, chemicals in commercial detergent caused stunted growth in spinach plants, leading to phytotoxicity, which damaged root cell walls and hindered nutrient and water uptake. This resulted in cell exosmosis, visibly wilting plants, and irregular stem thickness. Spinach plants irrigated with commercial detergent greywater ultimately died prematurely (Figure 4).

Graph 1: Effect of Greywater Solutions on Spinach Plant Height Over Time



Graph 2: Average Spinach Plant Leaf Length



Graph 3: Average Spinach Plant Root Length

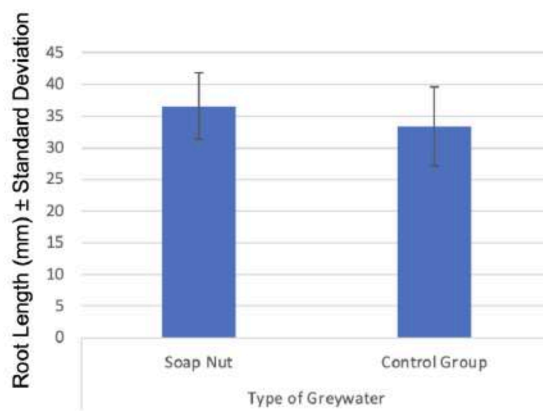


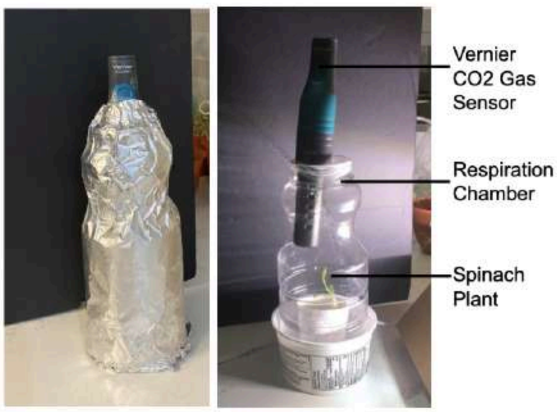
Table 1: Effect of Greywater on Average Spinach Plant Height, Leaf Length, Root Length, Rate of Respiration and Photosynthesis on Day 40

Greywater	Average Height (mm) ± Standard Deviation	Average Leaf Length (mm) ± Standard Deviation	Average Root Length (mm) ± Standard Deviation	Rate of Respiration (ppt/min)	Rate of Photosynthesis (ppt/min)
Soap Nut	89.3 ±7.53	30.7 ±3.50	36.6 ±5.27	0.01403	-0.034270
Control Group	87.2 ±5.07	29 ±3.68	33.4 ±6.26	0.01154	-0.02103
Statistical t-Test Analysis	$p = 0.474$	$p = 0.304$	$p = 0.232$	N/A	N/A

Table 2: Effect of Greywater on Average Spinach Plant Height on Day 10

	Soap Nut	Control Group	Commercial Detergent	Soap Nut vs. Commercial Detergent t-Test	Soap Nut vs. Control Group t-Test
Average Height (mm) ± Standard Deviation	51.9 ±6.3	52.4 ±4.6	37.3 ±4.8	p = 1.575E-05 $p < 0.05$	p = 0.844 $p > 0.05$

Figure 3: Spinach Plant Respiration Apparatus



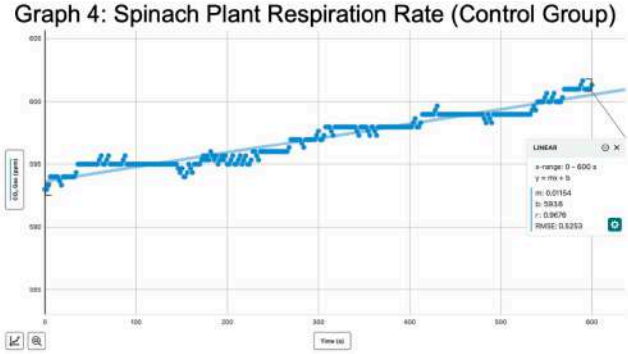
Rate of Respiration   Rate of Photosynthesis

Table 3: Rate of Respiration / Photosynthesis Results

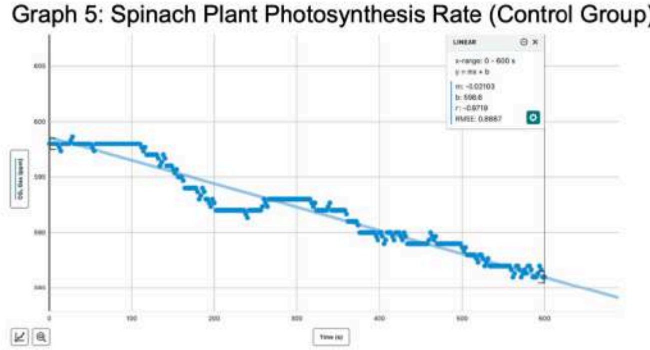
Control Group	
Spinach Plant	Rate of Respiration/Photosynthesis (ppt/min)
In the Dark	0.0115
In the Light	-0.0210

Soap Nut	
Spinach Plant	Rate of Respiration/Photosynthesis (ppt/min)
In the Dark	0.0140
In the Light	-0.0343

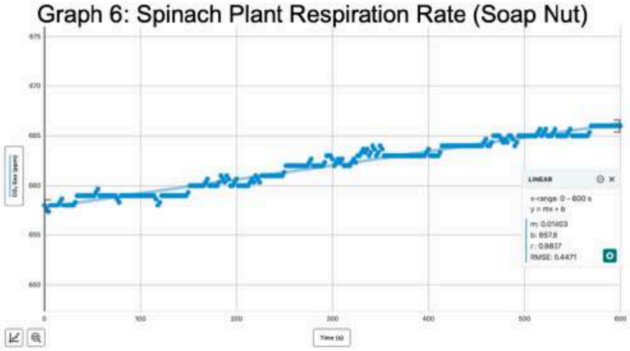
Graph 4: Spinach Plant Respiration Rate (Control Group)



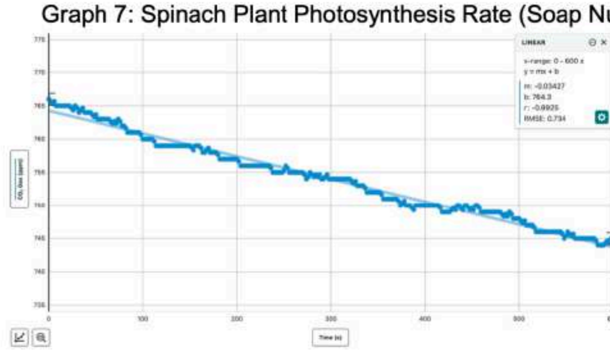
Graph 5: Spinach Plant Photosynthesis Rate (Control Group)



Graph 6: Spinach Plant Respiration Rate (Soap Nut)



Graph 7: Spinach Plant Photosynthesis Rate (Soap Nut)



4.2 Antibacterial Study Results

The data supported the antibacterial hypothesis. Soap nuts demonstrated antibacterial properties against *Escherichia coli* K12, as indicated by a clear zone of inhibition around the diffusion disk compared to the control group. This suggests that soap nuts can help reduce bacterial contamination risks associated with reusing greywater for irrigation (Table 4, Table 5).

The antibacterial action of soap nuts is attributed to the amphiphilic nature of saponins, which, like surfactants in detergents, contain hydrophilic and hydrophobic components. The hydrophobic tails of saponins can embed themselves in the phospholipid bilayer of bacterial cell membranes, causing disruption of the cell wall and leakage of cellular contents. This degradation of the bacterial cell wall leads to cell lysis and bacterial death. Saponins also form micelle-like structures around proteins from the disrupted membranes, similar to how conventional cleaning agents function (Kunatsa & Katerere, 2021).

Table 4: Antibacterial Effects of Soap Nuts and Commercial Detergent on *Escherichia coli* K12

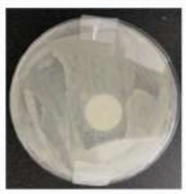
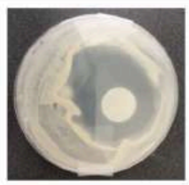
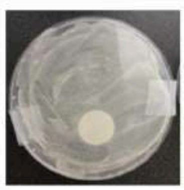
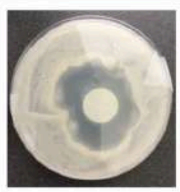
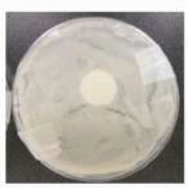
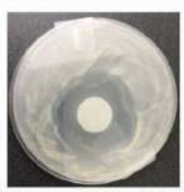
	Soap Nut	Control	Detergent
Trial 1			
Trial 2			
Trial 3			

Table 5: Average Inhibition Zone Diameter by Soap Nut and Commercial Detergent Against *Escherichia coli* K12

	Soap Nut	Commercial Detergent
Average Zone of Inhibition Diameter (mm)	23.33	50.67
σ Standard Deviation	2.52	4.16

4.3 Conclusion

In conclusion, soap nut greywater is a sustainable alternative for plant irrigation, promoting plant growth comparable to tap water while avoiding the harmful effects of commercial detergents. Irrigating plants with soap nut greywater not only supports healthy plant development, but also helps reduce bacterial contamination risks. This practice exemplifies sustainable development and contributes to water conservation efforts.

5. Applications

As consumers become more conscious of the environmental impact of conventional cleaning products, the demand for sustainable alternatives is increasing (Global Soap Nuts Extract Market Insights, 2024). Soap nut greywater offers a sustainable, eco-friendly, and cost-effective option for plant irrigation.

Farmers, particularly in drought-prone areas, can use soap nut greywater for vegetable irrigation without compromising plant growth, while also reducing the high cost of irrigation water. In regions with warm climates and dry soil, poor water infiltration and surface runoff are common challenges. The bio-based surfactants (saponins), present in soap nut greywater lower water surface tension, improving infiltration into the soil and enhancing irrigation efficiency particularly in arid conditions.

In residential settings, implementing greywater systems can lower household water bills by using soap nut greywater for landscape irrigation instead of fresh water. This also reduces the demand on public freshwater supplies, which is critical in regions facing water scarcity and drought.

On a global scale, particularly in underserved communities, replacing commercial detergent greywater with soap nut greywater can decrease the volume of laundry wastewater entering sewage treatment facilities. This, in turn, reduces the strain on municipal treatment plants, lowers financial costs, and mitigates the environmental pollutants released from these systems.

In North America, Escherichia coli contamination in irrigation water is a widespread agricultural challenge (Irrigation Water and Spinach E. coli Outbreak, 2022). Using soap nut greywater for irrigation may help reduce *E. coli* contamination, offering an economically viable, natural alternative to current irrigation practices. This approach could enhance the sustainability of global food systems and reduce the risk of foodborne outbreaks.

This innovative project, using natural resources like soap nuts and greywater, contributes to a greener future by promoting both water conservation and sustainable agriculture.

6. Further Studies

Future research could focus on conducting a soil microbiome analysis to assess the impact of greywater on microbial biomass, which is a key indicator of soil health. Additionally, increasing the concentration of soap nut greywater could be explored to evaluate its effects on plant growth and its antibacterial properties more thoroughly.

7. Acknowledgments

I would like to extend my sincere gratitude to my mentor, Professor Anne Lacey Samuels from the Department of Botany at the University of British Columbia (UBC), for generously sharing her expertise and guidance with me through a virtual meeting. Special thanks to the science teachers at St. John's School for their supervision and for providing the necessary materials and space to conduct the experiment. I am also deeply thankful to my family for supporting me throughout this journey.

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Greywater recycling is the main purpose of this project to evaluate the soap nuts can be safely used for irrigation without negatively affecting plant growth or contributing to E-coli contamination. This project conducted with plan growth study and antibacterial study. The application of soap nut in greywater presents a good behaviour for plant irrigation and promoting plant growth. The use of natural resources, soap nut, contributes fora more sustainable future.