

Usage of lemna minor as a water phytoremediator converted into green manure

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Abstract. Synthetic fertilizers and herbicides are widely recognized for their harmful impacts on the environment. Lemna minor, a fast-growing aquatic plant, is a possible sustainable alternative due to its excellent absorption capacity and ability to be converted into green manure. Additionally, it can serve as phytoremediator to absorb toxic chemicals in polluted water. In this study, Lemna minor or duckweed was exposed to ibuprofen, glyphosate, ibuprofen and glyphosate, and a control. Ibuprofen and glyphosate were used to test the duckweeds' absorption capacity, as measured by duckweed biomass. The treated duckweed was converted into green manure by mixing it into a soil mixture. The manure, inorganic fertilizer, and a control group were then applied to kale. Duckweed-based green manure, which is an organic fertilizer, was studied to see if it could replace inorganic fertilizer. Unexpectedly, the green manure treated with glyphosate and, at times, both glyphosate and ibuprofen increased kale growth, contradicting glyphosate's intended function as an herbicide to inhibit plant growth. These findings suggest duckweed as a possible solution to low-cost wastewater treatment and eco-friendly agriculture, though further research is needed to address long-term effectiveness.

Keywords: phytoremediation, green manure, lemna minor, glyphosate

1. Introduction

Around half of the world's population is dependent on synthetic fertilizers for food. Inorganic fertilizers are composed of synthetic chemicals that supply nutrients to crops [1]. These synthetic chemicals contain macronutrients such as nitrogen, phosphorous, and potassium, which improve plant growth [2]. However, these synthetic chemicals degrade soil health over time, emit greenhouse gases, and lowers water quality [3,4].

The combined application of herbicides and inorganic fertilizers is a popular method today [5,6]. However, herbicide overuse accelerates nutrient leaching, making it hazardous for humans and animals to consume plants grown on the plot [7-9]. Akinawo's study conducted in the United Kingdom found that 70% of eutrophication came from agricultural run-off and sewage effluent, both of which contained phosphorus [10]. Eutrophication is where a body of water with overabundant nutrients (usually from agricultural runoff) causes excess growth of aquatic plants and algae [11]. In a 2005 to 2012 study conducted in Europe, only an estimate of 40 to 60% of nitrate and phosphate were used for their intended purpose of growing plants. The remaining 40 to 60% had leached into surrounding water bodies with a 0.26-0.30 TgP/yr phosphate discharge and was released into the sea. Moreover, studies have found that farmers are more likely to overuse herbicides due to convenience, cost and the immediate effectiveness [12].

In addition, heavy rainfall increases nutrient runoff, which may exacerbate the eutrophication process [13]. In Shenzhen Bay, China, researchers found that 60 to 80% of total pollution loads were caused by rainfall-runoff pollution. Their results also showed that urban rainfall-runoff pollution has an effect on the concentration of chlorophyll-A in Shenzhen Bay [14]. Inorganic fertilizers additionally release harmful greenhouse gases such as nitrogen oxide and carbon from the soil into the air [15].

An alternative to inorganic fertilizers that avoids releasing greenhouse gases is sustainable green manure. This agricultural practice involves planting a fast-growing, nutrient-rich crop on nutrient-poor land. Once the crop reaches maturity, it is plowed into the soil, where it decomposes and enriches the soil with organic matter. In this way, the nutrients are naturally recycled and the soil is replenished. As experts noted, soil fertility underpins crop yield. However, intense and improper treatment of the cultivated land increases soil degradation [16]. Repeated abuse of the land without replenishing the soil lead to poor crop yield and quality [17]. For example, monocropping depletes soil nutrients and fosters toxic buildup [16].

In a 2010 study, organic and inorganic fertilizers exhibited the same effectiveness in promoting plant growth and nutritional quality. Both outperformed compost and non-fertilized control plants. When comparing the plant shoot yield, the three commercial organic fertilizers performed the best followed by NPK inorganic fertilizer [18]. NPK is an acronym for nitrogen, phosphorus, and potassium [19]. In addition, the organic compost was able to increase soil fertility by increasing the soil organic matter by 23.3% in the second year and 0.6% in the third, improving crop yield. However, the NPK inorganic fertilizer did not increase soil fertility [20]. Therefore, organic compost was the best option overall.

Plants typically used for green manure such as legumes take up land and resources that could have otherwise been used to grow the desired crops. As the world's population steadily increases, the food industry has to increase production to accommodate. However, it remains a huge challenge to produce more on limited land and is bound to become a greater problem in the future [16].

Duckweed might be the key to solving the problem [21]. *Lemna minor*, also known as duckweed, is a small aquatic plant that does not compete with food crops and has strong absorption capacity [22]. The aquatic plant responds extremely well to increases in nutrients, often triggering its own eutrophication with rapid growth in abundance [23-25]. Other features of the plant include that it adapts well to a range of climate conditions, has the capacity to absorb a lot of nutrients, and can easily be collected from small bodies of water [26]. Researchers suggested using *Lemna minor* in places that have an overabundance of nutrients and later turning it into agricultural manure [27]. The captured nutrients such as nitrogen and phosphorus can be reintroduced into the soil as an organic fertilizer [24,25].

Often in waterways and in bodies of water, pollutants and pharmaceuticals can be found [28]. As the world's third most consumable drug, ibuprofen was found to be at levels ranging from 1417, 15 to 414, and 5.0 to 280 µg/L in China, Korea, and Taiwan, respectively in surface waters [29,30]. In addition, roundup, which contains glyphosate, has increased in popularity as one of the most widespread herbicides in the world. When tested in short-term exposure and at the lowest content found in water, glyphosate was found to affect duckweed [31]. As the food production industry expands, the usage of agricultural related chemicals increases, which emphasizes the need for designing technology to remove these chemicals, including from the waterways [28]. Several researchers have tested and discovered that duckweed could be used as a treatment to pick up anthropogenic pollutants and convert it into plant biomass before the chemicals enter other waterways [32]. However, because duckweed is a good absorber, there is a possibility that it has the ability to take in toxic chemicals. If the duckweed absorbs these pollutants, the duckweed-based manure could negatively affect crops.

Yet, such property of duckweed may still be leveraged to purify polluted water. In developing nations, water and sanitation services are severely inadequate especially compared to countries such as the United States. This also increases the risk of hygiene-related diseases and as a result, millions die from otherwise preventable illnesses [33]. The lack of proper water treatment facilities in developing countries such as Pakistan remains a major cause of mortality with 0.2 to 0.25 million children dying every year due to diseases developed from drinking contaminated water [34]. The practice of using duckweed as both a phytoremediator and green manure can be used in developing nations and places without functional water treatment facilities. Lemnaceae, the subfamily *Lemna minor* belongs to, can be found almost anywhere on Earth, except in deserts [35]. Duckweed is therefore cost effective and easily accessible [26]. Therefore, *Lemna minor* could act as both a water remediation and agricultural manure which is a sustainable alternative to inorganic fertilizers.

Despite recent growing knowledge of duckweed as a green manure and as a treatment, this study examines whether duckweed can absorb pollutants and pharmaceuticals and their impact on growing crops. The central hypothesis is that if the contaminated *Lemna minor* green manure amendment has a wet plant biomass greater than or equal to the inorganic fertilizer, then the green manure could be coupled with the duckweed, which can also function as a water phytoremediator. The experiment answers these two main questions: (1a) Which treatment(s) the duckweed was grown with affected the kale biomass? (1b) What treatment had the greatest positive influence in promoting agricultural crop growth? (2) What is the difference between growing an agricultural crop with inorganic or organic fertilizer? The control duckweed treatment is speculated to have the greatest increase in duckweed growth and that organic fertilizer would outperform inorganic fertilizer.

2. Methods

2.1. Duckweed propagation set-up

One species of duckweed, *Lemna minor*, was used for this experiment. The duckweed was provided by the Kohler Environmental Center (KEC) in Wallingford, CT. The optimal range of temperature to keep duckweed is between 63.5 to 86 Fahrenheit [36]. The duckweed was separated into 8 tanks to propagate with temperatures around 66.5 Fahrenheit. The temperature was taken by a digital thermometer with an external sensor and was purchased from Amazon. The tank was made out of glass and was 20 x 10.5 x 12.5 inches. Each tank was filled with water until the 3 inch mark. The water was put through a reverse osmosis system to remove unwanted particles and to add nutrients. Four tanks shared one grow light set at 12 hours

purchased from Amazon, which is a total of 2 lights. An additional 2 tanks were placed in the greenhouse at a temperature of 74.3 Fahrenheit and tested to see if it could propagate as well as a tank under grow lights.

2.2. Duckweed container treatments

After 5 weeks, the duckweed was separated into 16 Sterilite 6 Qt. Storage Boxes (34.6 cm x 21 cm x 12.4 cm). There were four replicates per treatment. The 6-quart container could hold a total of 1 gallon of water. The water went through the same reverse osmosis system. An average of 59.90g of water were added to each container, not including any treatments nor duckweed. Additionally, 8mL per 6-quart container of Thrive's All in One Fertilizer was given to all 16 containers as duckweed requires nutrients to grow [36]. The aquatic fertilizer, purchased from Amazon, contained nitrogen, phosphate, potash, magnesium, sulfur, boron, copper, iron, manganese, molybdenum, and zinc. The application rate was determined based on the nitrogen concentration listed on the bottle, in order to simulate nitrogen levels typically found in untreated sewage entering a municipal wastewater facility. A range from 20-85mg/L of nitrogen were found so 50mg/L of nitrogen were allotted per container [37].

Four of the containers held 1 tablet of ibuprofen. The ibuprofen was purchased from Amazon containing 200 tablets with 200mg per tablet. 1 tablet was put into a beaker with 1L of water and mixed using a magnetic stirrer. After 5 minutes, 12mL of the mixture was allocated to each container. The amount of ibuprofen was based on the concentration of ibuprofen found in aquatic ecosystems in several countries. Another four contained 6 fluid ounces of glyphosate. The glyphosate was purchased from Home Depot as a 35.2oz Concentrate Weed and Grass Killer Roundup Concentrate Plus. 990mL of water and 10mL of roundup were mixed using a magnetic stirrer for 5 minutes. 13.0μL were allocated for each container. Four different containers contained a mixture of both 6 ounces of glyphosate and a 200mg tablet of ibuprofen. A different set of four contained both glyphosate and ibuprofen containing the same amounts of treatments. The last four only had Thrive fertilizer and did not have any other additives. This was the control of the experiment. An average of 59.71 grams of duckweed were placed into each container.

2.3. Kale pots

The green manure used in the final treatment pots for kale consisted of 40 g of treated duckweed and 133.57 g of soil amendment. The soil amendment was prepared as a mixture of peat moss, vermiculite, and perlite in a 2:1:1 ratio. Black Gold Natural & Organic Canadian Sphagnum Peat Moss, Sta-Green Organic Vermiculite, and Viagro Horticulture Perlite were purchased and provided by the KEC.

After the soil amendment was prepared, blue curled scotch kale seeds were planted in each of the starter pots, with 3–4 seeds sown at a depth of ¼ inch. A total of 40 containers were filled with the soil mixture, and 3–4 kale seeds were distributed per pot. The Vates Blue Scotch Curled non-GMO heirloom seeds were obtained from Gardeners Basics.

There were four replicates for each treatment: inorganic fertilizer, glyphosate green manure, ibuprofen green manure, combined glyphosate and ibuprofen green manure, untreated green manure, and soil only. In total, 24 plastic pots (6 × 4 inches) were filled to the 3.5-inch mark with the soil amendment for the final kale treatments. After three weeks of duckweed treatment, 133.6 g of wet duckweed biomass was measured and applied to the top of each kale pot. The contents were mixed with a shovel to incorporate the duckweed into the soil. Over the course of three days, the decomposing duckweed was turned to accelerate the green manure process. Pot placement was randomized using an Excel sheet randomizer. The inorganic fertilizer was composed of the soil mixture and 5mL of Miracle-Gro's All Purpose Plant Food mixed with a gallon of water, which was sprayed on the pot.

2.4. Biomass measurement

After four weeks of treatment, the plants were harvested and their wet biomass was measured. The total wet biomass per pot was weighed in grams, after which the roots were severed to obtain the aboveground biomass. The belowground wet biomass was then calculated by subtracting the aboveground biomass from the total wet biomass.

The plants were placed into paper bags labeled with the respective treatment names and subsequently dried in an oven to determine the dry kale biomass. After 24 hours of drying, the bags were removed, and the previously separated roots and leaves were measured to obtain the total dry biomass, dry aboveground biomass, and dry belowground biomass.

2.5. Statistical methods

R was used to analyze the data and produce visualizations. Differences between the initial and final duckweed wet biomass were analyzed using two-way analysis of variance (ANOVA) followed by boxplots and an interaction plot. The significance level was considered at $p < 0.05$. The p-value was significant, so the null hypothesis was rejected. Differences between the kale growth after treatment application were analyzed using two-way ANOVA followed by boxplots and an interaction plot. The significance

level was considered at $p > 0.05$. The p-value was not significant, so the null hypothesis could not be rejected. Differences between inorganic fertilizer and green manure were analyzed using one-way ANOVA. The significance level was considered at $p > 0.05$. The p-value was not significant, so the null hypothesis was kept.

3. Results

3.1. The effect of treatments on duckweed growth

The duckweed in each container was weighed (in grams) at the time of distribution and again before preparing the green manure. One can see slight differences in mean in all cases except for duckweed grown with ibuprofen (Figure 1A and Figure 1B). Duckweed grown with glyphosate showed a significant difference ($p < 0.05$), however duckweed grown with ibuprofen did not show a significant difference ($p > 0.05$). The combination of glyphosate and ibuprofen induced statistically significant growth of duckweed compared to controls. There is a strong correlation showing that duckweed growth increases depending on the presence of glyphosate (Figure 1C).

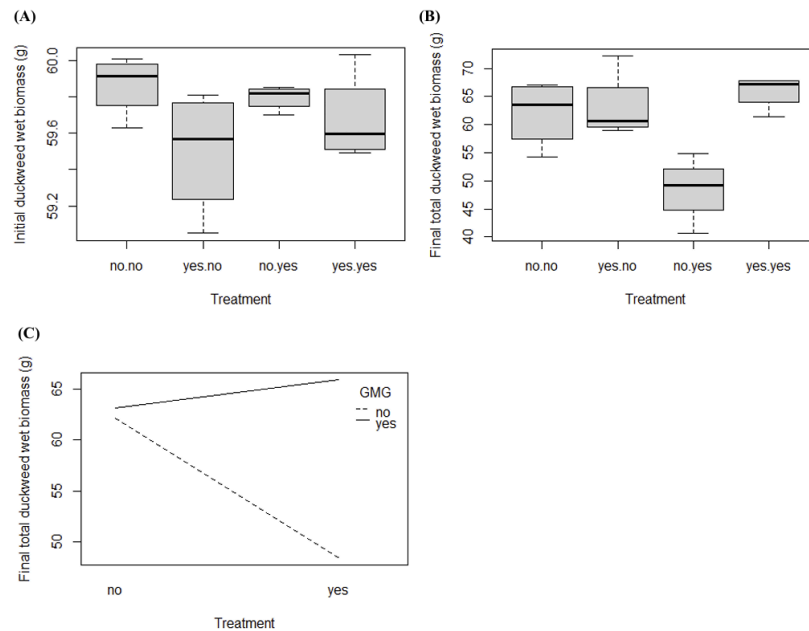


Figure 1. Changes in duckweed (*Lemna minor*) wet biomass by taking (A) initial wet biomass prior to treatment and (B) final wet biomass after treatment (control, glyphosate, ibuprofen, and both glyphosate and ibuprofen). (C) is an interaction plot for all treatments

3.2. The effect of treatments on kale using wet biomass

After a few weeks of watering with or without treatment, the total, aboveground, and belowground wet kale biomass per pot was weighed in grams. There was no statistically significant between treatment conditions ($p > 0.05$, Figure 2A, Figure 2B). In contrast to the duckweed (Figure 1C), application of green manure treated with ibuprofen alone appeared to increase kale growth, however when paired with glyphosate, there was a slight decrease in biomass. The application of glyphosate alone had the highest total biomass, which is unexpected since glyphosate is typically used as a herbicide.

Kale was severed at the root–stem junction, and the leaves were collected for measurement of above-ground wet biomass. Glyphosate had a significant effect on the above ground kale growth ($p < 0.05$, Figure 2C). Duckweed treated with combined application of glyphosate and ibuprofen induced kale growth ($p < 0.05$). However, ibuprofen alone did not induce significant increase in the kale biomass ($p > 0.05$). (There needs to be statistical bars/astericks on the graphs). The below kale wet biomass were not significantly different across conditions ($p > 0.05$, Figure 2D).

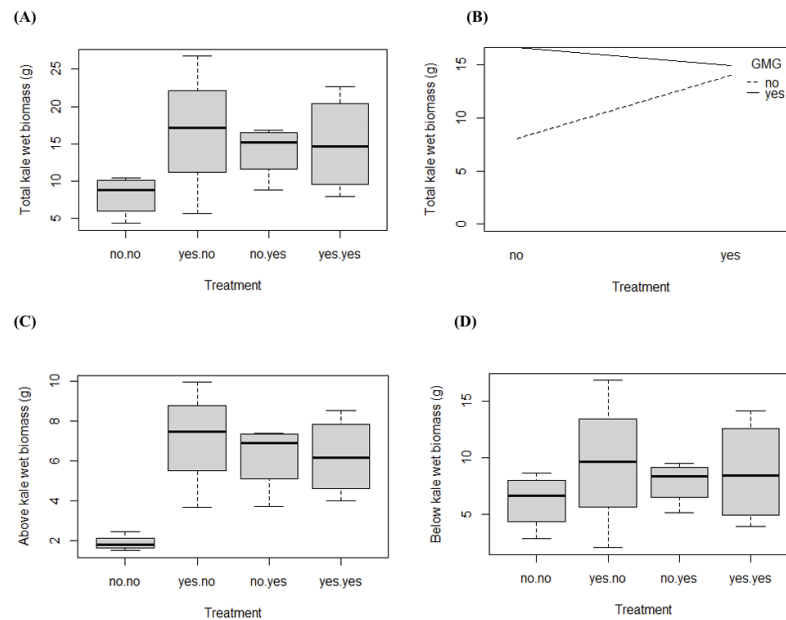


Figure 2. Changes in kale wet biomass by taking (A) total wet biomass after treatment and an (B) interaction plot across all treatments. (C) is the above ground wet biomass boxplot after treatment (control, glyphosate, ibuprofen, and both glyphosate and ibuprofen). (D) is the below ground wet biomass boxplot after treatment

3.3. The effect of treatments on kale using dry biomass

After noting the wet kale biomass, the plants were dried in an oven to determine the dry biomass in grams. The total, aboveground, and belowground dry kale biomass per pot was then weighed. Similar to figures 2A, 2B, and 2D, nothing was statistically significant in figures 3A and 3B ($p > 0.05$). Figure 3B showed a trend similar to figure 2B, albeit at a smaller scale due to water loss.

The stems and leaves were separated from the roots to measure the aboveground biomass. In Figure 3C, no statistically significant differences were observed ($p > 0.05$). The belowground biomass was calculated by subtracting the aboveground kale biomass from the total kale biomass. However, no statistically significant differences were detected in Figure 3D ($p > 0.05$), indicating that the treatments did not have a measurable effect on belowground biomass under the conditions of this experiment.

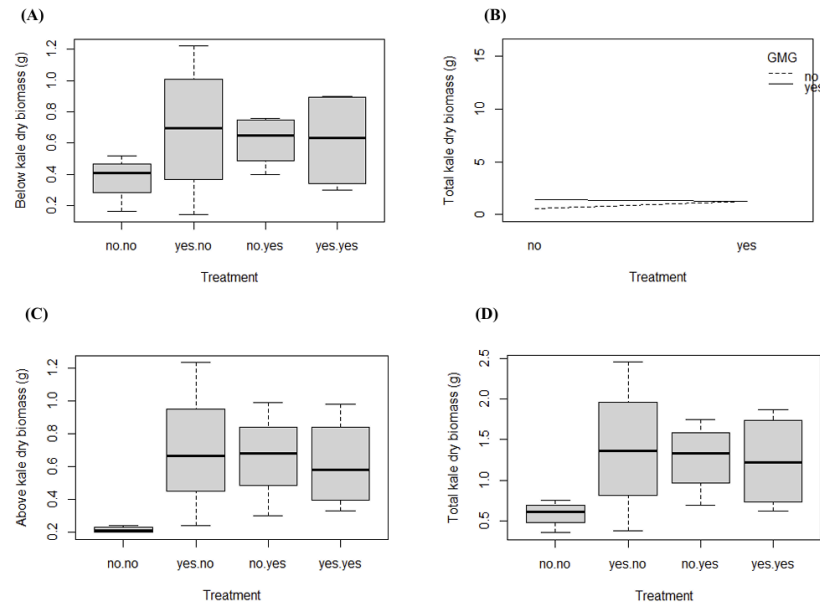


Figure 3. Changes in kale dry biomass by taking (A) total wet biomass after treatment and an (B) interaction plot across all treatments. (C) is the above ground wet biomass boxplot after treatment (control, glyphosate, ibuprofen, and both glyphosate and ibuprofen). (D) is the below ground wet biomass boxplot after treatment

3.4. The effect of fertilizer on kale using wet biomass

Inorganic fertilizer was prepared using Miracle-Gro, while organic fertilizer consisted of untreated green manure. At the end of the kale growth period, the wet biomass per pot was collected and weighed in grams. As shown in Figures 4A, 4B, and 4C, no statistically significant differences were observed between the inorganic and organic fertilizer treatments ($p > 0.05$). This indicates that, under the conditions of this study, organic green manure performed comparably to the commercial inorganic fertilizer in supporting kale growth. The lack of significant differences may also suggest that the experimental scale and duration were insufficient to capture subtle treatment effects, highlighting the need for larger sample sizes, longer growth periods, and more detailed nutrient analyses in future studies.

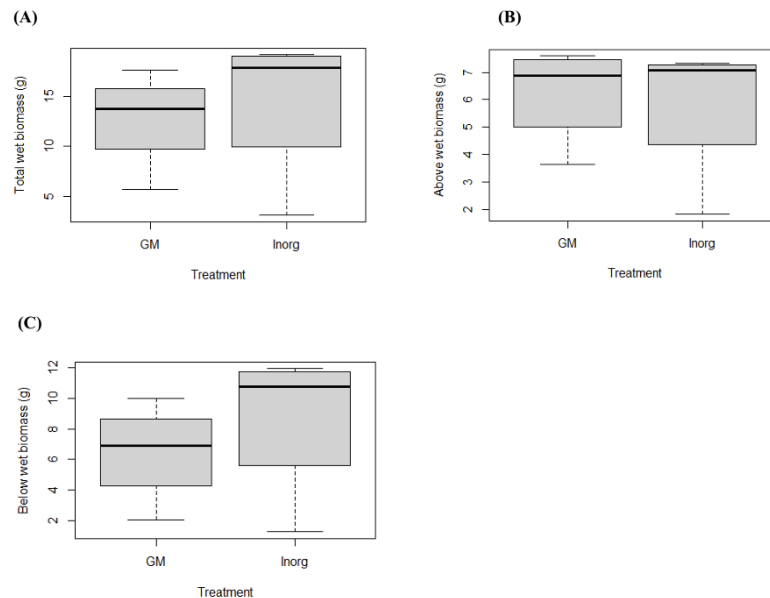


Figure 4. Comparing inorganic and organic fertilizer (green manure) by showing changes in (A) total, (B) above ground, (C) below ground kale wet biomass

3.5. The effect of fertilizer on kale using dry biomass

After the wet biomass was recorded, kale plants were dried and their dry biomass was determined in grams per pot. Similar to the wet biomass results, no statistically significant differences were detected among treatments for total, aboveground, or belowground dry biomass (Figures 5A–C; $p > 0.05$). These results suggest that, within the timeframe and scale of this study, organic green manure and inorganic fertilizer provided comparable effects on kale growth. The absence of clear differences may reflect the short duration of the experiment, the relatively small sample size, or external factors such as pest pressure. While the data indicate that both fertilizer types can sustain crop growth in the short term, further investigation using longer experimental periods and more detailed nutrient analyses will be needed to determine whether differences emerge under extended cultivation.

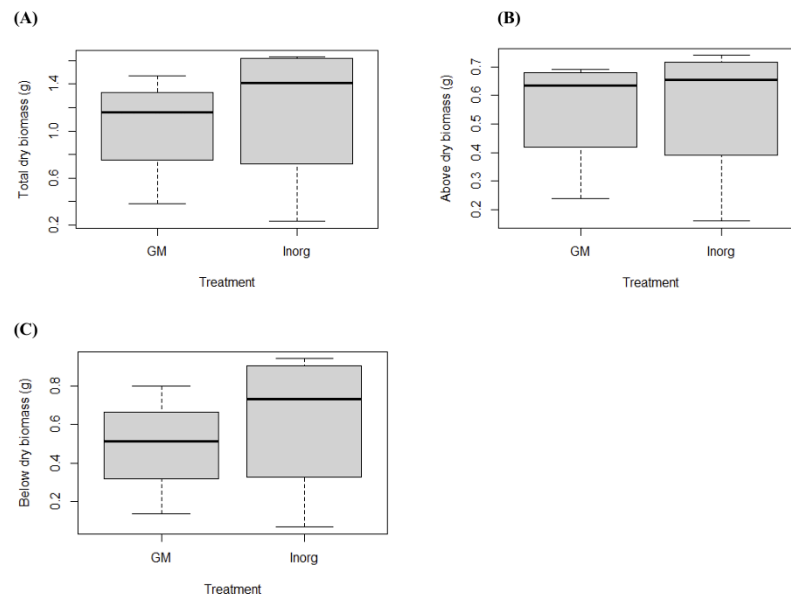


Figure 5. Comparing inorganic and organic fertilizer (green manure) by showing changes in (A) total, (B) above ground, (C) below ground kale dry biomass

4. Discussion

4.1. Treatment interactions with duckweed and kale

Glyphosate, which is an active ingredient in Concentrate Weed and Grass Killer Roundup Concentrate Plus, was created with the purpose of killing weeds and grass. The herbicide has been found at high concentrations in run-off such as $54 \mu\text{g} \times \text{L}^{-1}$ in Australia and $40.8 \mu\text{g} \times \text{L}^{-1}$ in Canada [31]. In addition, ibuprofen, which is a pain reliever medicine, has been found at average concentrations of 0.98, 1417, and 15 to $414 \mu\text{g/L}$ for Canada, China, and Korea, respectively in surface waters [30]. Plants such as *Lemna minor* or duckweed have been looked into as potential wastewater treatment filtration systems [38]. When exposed to glyphosate, there was an increase in growth compared to when exposed to ibuprofen, which decreased the amount of growth. However, when combined, there was an increase in growth higher than when exposed to ibuprofen alone. The interaction was statistically significant.

After application of the treatments on kale, there was statistically significant p-value in the above ground wet biomass. Glyphosate and the combination of glyphosate and ibuprofen had p-values less than 0.05. Glyphosate was shown to increase kale growth; however, ibuprofen was not able to change growth clearly. The interaction between the two meant that ibuprofen can change plant growth, but only when glyphosate is present. Thus, ibuprofen has the ability to modify how glyphosate affects the plant. The effect of ibuprofen on crop growth needs to be further evaluated. Unfortunately, the rest of the graphs were all statistically insignificant with p-values being greater than 0.05.

4.2. Implications

The presence of aphids during the last few weeks of the kale part of the experiment could have hindered the growth of the kale due to selected residing on certain individual plants. One of the inorganic fertilizer plants died and had two plants instead of three which was the standard number per pot. The lack of significance could be because of the small size of the experiment and would

need to be done on a larger scale. Larger concentrations of duckweed to create the green manure would most likely change the p-value and produce more accurate results. Furthermore, the wet and dry biomass of individual kale plants were not measured separately; data collection was conducted based on the pot as a whole. Although each average pot mass could be divided into the number of individual plants, it would not be accurate. Analyzing individual kale plants would allow better insight into the effects of the treatments had on duckweed and kale. Contrary to the experiment, glyphosate was not supposed to increase plant growth but rather decrease it [31].

Overall, the data suggests that glyphosate positively influences kale growth. Further research is needed to assess long-term effects and individual interactions between kale and the treatments. A wider range of treatment levels such as glyphosate and ibuprofen will need to be explored as well.

5. Conclusion

This study aimed to analyze the impact glyphosate and ibuprofen had on the growth of *Lemna minor*, a fast-growing, native aquatic plant. Application to kale in two different types of fertilizer which were inorganic fertilizer or green manure. The findings showed that glyphosate and the mixture of the two popular wastewater pollutants can positively influence kale growth. These results suggest that duckweed may utilize toxic chemicals in polluted water to promote plant growth and may serve as both a phytoremediator and a sustainable green manure. Future experiments should focus on taking a more detailed approach in addressing long-term approaches, larger experimental size, limiting access to predators, and examining individual plants. Glyphosate is meant to decrease and not increase plant growth. In conclusion, research have suggested that *Lemna minor* is a good candidate for acting as a potential wastewater filter to pick up pollutants. It also has the potential to be applied to other agricultural crops and replace harmful industrial practices.

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