JOURNAL OF
EMERGING INVESTIGATORS

Article

Polluted water tested from the Potomac River affects invasive species plant growth

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SUMMARY
Along with global warming, water pollution has become an increasingly concerning issue. Water pollution negatively impacts ecosystems globally, as waterways are becoming unsafe to use due to fast-progressing climate change damaging bodies of water. However, more work is needed to fully understand the impact of rising pollution levels on plant growth, explicitly comparing native and invasive species. It's critical to determine if native or invasive species have an advantage over the other because ecosystems can dramatically change positively or negatively if environmental factors favor one over the other. We hypothesized that the invasive species, tiger lily, would grow faster with increasingly polluted water levels. To test this theory, we utilized the Potomac River, a polluted river bordering Washington D.C., and one of its invasive species, the tiger lily, to test invasive species' plant growth with water that was polluted at varying levels. We determined that more polluted levels of water increased plant growth of invasive species, as the tiger lily plant watered with river water grew more than those watered with boiled river water and tap water. The study reveals concerns about the tiger lily as an invasive species in the Potomac River basin. Further experimentation with other invasive species in the habitat could determine if there is a correlation between pollution and growth of other invasive plants.

INTRODUCTION

Water pollution occurs when bodies of water are exposed to toxic waste that deteriorates their water quality (1). Water contamination mainly occurs due to human activities like heating water, deforestation, agricultural chemical dumping, sewage dumping, sea vessels adding plastic to the oceans, and oil spills (2). The most important pollution indicators in the Potomac River are nitrogen, phosphorus, and sediment levels (3).

While these pollutants are decreasing, many short-term events negatively affect the environment, particularly from urban runoff, so the river pollution levels remain concerning (4). The Potomac Conservancy's 2020 Potomac River Report Card reported that the Potomac's pollution levels remain unsafe to swim or fish and therefore remain banned due to safety concerns (3). The report explains that one of the top reasons for high pollution levels is deforestation. Deforestation affects the environment's ability to manage rainwater and creates adverse short-term outcomes (3). In addition, urban runoff and stormwater systems containing sewage and untreated waste have been dumped into the Potomac River because of disorganized stormwater systems (3). If these problems are not addressed, they will further degrade the Potomac River, affecting people, animals, and ecosystems (3).

All plants have their own set of nutrients needed to grow and develop, however with polluted water levels increasing, nutrient levels are changing (2). Altered nutrient levels affect the growth and existence of species that rely on the waterway. Increases in pollution, which degrade the water quality, have the potential to kill native species leaving space for other plants to take over. These plants could include invasive species, which are species brought to a new environment, creating competition in the ecosystem. Due to their rapid growth capabilities and the absence of the natural growth regulators that native species possess, invasive species gain a distinct advantage in successfully establishing their populations within ecosystems (2). Invasive species have the potential to cause massive damage because the changing of necessary nutrients that native species rely on increases the invasive species' chances of survival and greatly decreases native species' numbers (5). Our study investigated if there is a correlation between levels of contaminated water and invasive species growth by testing the invasive species, tiger lily, in the Potomac River basin. Our study is the first to test invasive species growth utilizing the Potomac River on tiger lilies. Despite being unique, related experiments on the growth of invasive species can be compared. One experiment measured the effects of nutrients, climate change, and invasive clams on phytoplankton and cyanobacteria biomass in a river (6). The experiment tested if climate change, which increases water temperatures, would cause the destructive planktic cyanobacteria populations to increase in lakes and rivers (6). The study concluded that regulating nutrient levels effectively prevents the dangerous effects of climate change (6). Another study revealed that invasive species had more resistance to the pollutant than the native species had, concluding the need to improve the environment and rid invasive species by creating a climate benefiting native species (7).

Our study measured if polluted river water affects the growth of tiger lilies, an invasive species. Tiger lilies can multiply quickly and efficiently take over an area by out-competing other species, making them invasive (8). For these purposes, the tiger lily proved a good choice for data collection, being a present invasive species in the Potomac River basin. We hypothesized that the invasive species, tiger lily, would grow more in polluted river water than when watered with boiled river water. A tiger lily watered with tap water was used as a control. The results of the experiment supported the hypothesis. Not only was plant growth greater in the tiger lily watered by river water, but it was also more developed with longer leaves.

RESULTS

In our study, we investigated the effects of polluted water on the growth of invasive species by watering three tiger lilies with three different sources of water: tap, boiled river water, and river water. Plant growth was measured every 10 days, starting from the first signs of emergence from the soil for a 45-day test period. We observed physical characteristics and found that the river-watered tiger lily had the longest and the most leaves. When measured, the longest leaf on the river-watered tiger lily was 6.91 cm, the boiled river-watered tiger lily was 4.58 cm, and the tap-watered (control) tiger lily was 2.53 cm (data not shown). The river-watered tiger lily also had three plants out of its bulb compared to boiled river watered with one and tap water with two **(Figure 1)**. The higher number of plants originating from the bulbs in the river-watered group might be attributed to the delay in planting the bulbs, a few weeks after arrival.

Over the testing period, all tiger lily plants experienced growth in height. However, the tiger lily watered with river water demonstrated the fastest growth with Day 15 (first signs of change) at 1.3 cm and ending on Day 45 at 17.2 cm, consistently exhibiting the highest growth on each day of recording. which occurred every 10 days after Day 15 **(Figure 2)**. Tap water, the control of the experiment, also had a high level of growth. However, the tap-watered tiger lily never passed the river-watered tiger lily in height. The tap-watered tiger lily began on Day 15 at 0.3 cm in height and ending on Day 45 at 16.4 cm. The boiled river-watered tiger lily experienced the slowest growth, at 0.6 cm on Day 15 to 10.4 cm on Day 45.

We also conducted soil and water pH tests. For soil testing, we compared the river-watered plant soil to the tap-watered plant soil and noticed slight differences. The river-watered tiger lily soil had a pH of 7.5 compared to tap water's pH of 7 **(Table 1)**. We also measured nutrients in the soil on Day 25 of the experiment, so the shifts already present in soil levels demonstrate the water sources' impact on the plants. Soil testing for nutrients measured nitrogen, phosphorous, and potassium levels in all three soil pots. The trial had five levels of nitrogen, phosphorous, and potassium, from lowest to highest: depleted, deficient, adequate, sufficient, and surplus. The river-watered tiger lily, compared to the tap-watered tiger lily soils, had the same nitrogen levels (N1 deficient), slightly more phosphorous (P1 deficient compared to P0 depleted), and marginally more potassium levels (K2 adequate compared to K1 deficient). Finally, we also compared each water source's water pH test, resulting in river water with a pH of 6.0, tap water with a pH of 7.0, and boiled river water with a pH of 6.5 **(Table 2)**.

The results from the Two-Factor Without Replication ANOVA generated from plant height growth for each tiger lily, revealed the experiment was statistically significant

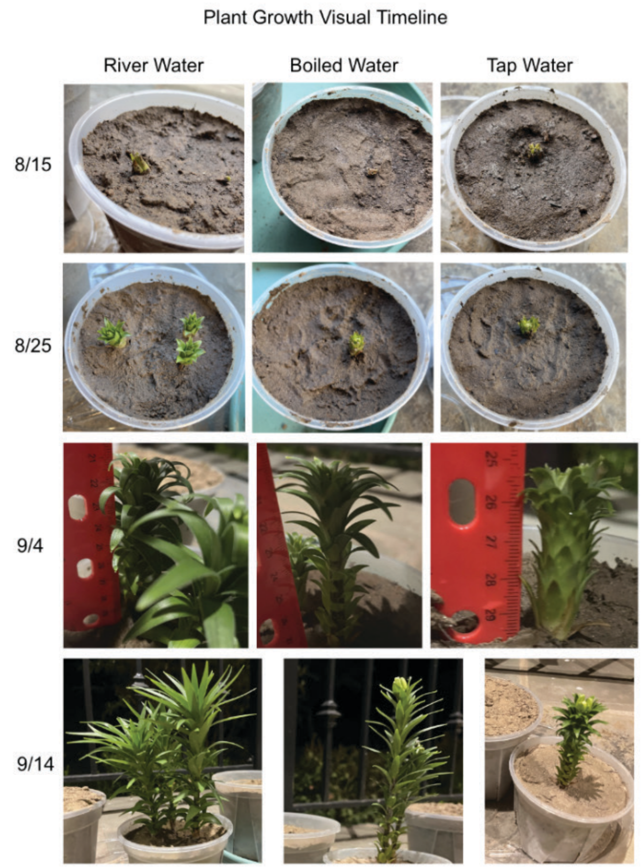

Figure 1: Visual timeline of river- and boiled river-watered tiger lily plants and control tap-watered tiger lily plant. The images begin Day 15 from the first signs of growth. Following are Day 25, Day 35, and Day 45. The river-watered tiger lily began having faster growth than the other 2, but signs of all are seen on Day 15. At the end of the experiment, Day 45, all plants have grown, but the river-watered tiger lily has the tallest plant growth and appears the healthiest physically.

Soil Tests	pH	Nitrogen	Phosphorous	Potassium	Temperature (Celsius)
River water soil	6.5	N1 deficient	P1 deficient	K2 adequate	27.11
Tap water soil (control)	7	N1 deficient	P0 depleted	K1 deficient	27.28

Table 1: Soil tests of pH, nutrients, and temperature for the river- and tap-watered plants. The pH, nitrogen, phosphorous, and potassium tests were done with the Luster Leaf Rapitest Soil Test Kit. The temperature tests were done with a soil thermometer. For the N, P, and K tests, the kit had five levels of N/P/K levels. From lowest to highest: depleted, deficient, adequate, sufficient, surplus. All trials occurred on Day 25 of experimentation.

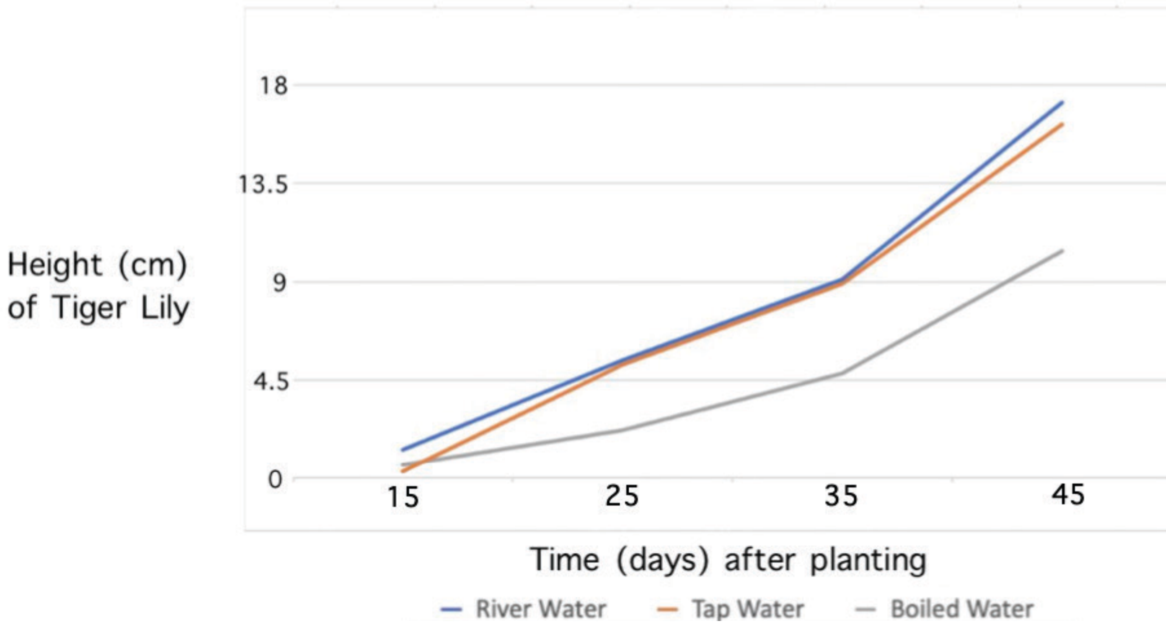

Figure 2: Timeline of the growth of *Lilium lancifolium* over the 45-day test period between the river-watered, boiled river-watered, and tap-watered plants. The plant watered with river water began growing the fastest and ended with the most plant growth on Day 45 (rows *p*-value = 0.025 and column's *p*-value = 0.00015).

(Figure 2). The F value for the rows (river water, boiled river water, and tap-watered tiger lilies) was 7.26 (p = 0.025). The F value for the columns (dates of recording height) was 46.8 (p = 0.00015). Both p-values were found to be less than the pre-defined alpha value of 0.05 before the experimentation, demonstrating statistical significance. In addition, the Turkey's Test, a posthoc statistical analysis, was run after the ANOVA. Results showed that comparing the river to tap (control) data there was no statistical significance but comparing river to boiled and tap (control) to boiled, mean is significantly different. From the statistical results, the null hypothesis — the tiger lily being watered with boiled water and the tiger lily being watered with river water experienced the same level as growth — was proven incorrect, which then supported the alternative hypothesis: the tiger lily will experience the most growth with the river water compared to the tiger lily being watered with the more purified less polluted boiled water.

DISCUSSION

The results of our study demonstrate that polluted waterways can increase the growth of certain invasive plant species. The tiger lily watered by polluted river water from the Potomac River had the most plant growth over the 45-day testing period compared to the tiger lily watered with boiled river water, a less polluted level of river water, and the tap water, a non-contaminated and non-polluted source. These results establish a correlation of faster plant growth with higher water pollution. While our experiment is unique in studying the correlation between invasive species and climate change as represented by water pollution, other experiments conducted on similar topics, including a study on invasive clam species, concluded that managing nutrition levels prevents invasive species growth, agreeing with our study (6). In terms of limitations, slower growth for the boiled water could have been because microorganisms were killed when we boiled the water. The absence of microorganisms in the boiled water would limit plant growth because microorganisms help the decomposition process in the soil to release the necessary nutrients for plant growth (9).

Water Tests	pH
River water	6.0
Tap water (control)	7.0
Boiled water	6.5

Table 2: Tested pH levels of the river, tap, and boiled water. The water pH tests were done with the VARIFY Complete Water Test Kit. Tests occurred on Day 25 of experimentation.

Our results showing that the invasive species, tiger lily, has faster growth from a polluted water source suggests that the invasive species' necessary nutrients are provided in more contaminated levels of water. In the river water, potassium and phosphorous levels were more abundant **(Table 1)**. Potassium and phosphorous levels being plentiful suggests that an increase in potassium and phosphorous stimulates plants growth in invasive species, which is alarming because if polluted water levels increase invasive species' growth rate, they will overtake native species faster, suggesting native species are at a higher risk. While there are no similar studies to amplify this conclusion, studies tracking the effects of invasive species on water quality have found that invasive species decrease water quality by increasing runoff and erosion (10). The additional impact of invasive species further harming water quality adds to the risk factor of increased invasive species growth. To validate the suggestions of our experiment, further testing should repeat the experiment with replicates of each condition to allow for a more accurate and significant statistical result. Also, repeating the experiment with an additional group of a native species can determine if invasive and native species respond differently to the levels of nutrients in polluted water to discover if polluted water assists invasive species in dominating an environment.

MATERIALS AND METHODS

Our experiment was conducted outside under a porch roof. Soil was collected from the Potomac River's Riverside at 38.967833, -77.202417 and used to fill 3 plastic 6-inch-deep plant pots. Once soil filled the entire pot, a 4.5-inch hole was dug in the center of each pot, and a tiger lily bulb, purchased from American Meadows, was placed in the hole and covered back up with the soil. The experiment was not done in replicates. Starting on the first day of planting, and then every five days, 170 mL of river water was used to water the first pot, 170 mL of boiled river water, which was boiled for 3 minutes to purify, was used to water the second pot, and 170 mL of the control tap water was used to water the third pot. The tiger lily plants were watered every 5 days around 8 p.m., when the soil temperature across all three pots averaged 21.11 degrees Celsius. Once the first signs of emergence from the soil were observed, on Day 15, plant height was measured every ten days (4 recordings in total). On Day 25, soil tests were conducted with the Luster Leaf Rapitest Soil Test Kit. Photos every 10 days, starting on Day 15, were also compiled together. To validate the findings, a Two-Factor Without Replication ANOVA and the Turkey's Test were conducted to confirm the experiment to be statistically significant.

ACKNOWLEDGMENTS

We would like to thank Fairfax County for granting us a permit to conduct our project. We would also like to thank I-Min Chao for her support and accompaniment during collecting materials for testing. Finally, we would like to thank JEI.

Received: November 25, 2022
Accepted: April 12, 2023
Published: September 20, 2023

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