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Negative Effects of Pollution on English Daisy (*Bellis perennis*) Height and Flower Number

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Summary

River pollution is an increasingly serious issue that can lead to an imbalance in an ecosystem. Chemicals found in fertilizers and pesticides, such as phosphate and nitrogen, often end up in nearby water and can have a negative effect on surrounding plant life. In this experiment, we assessed the effect of water containing different nitrogen levels on the height and number of mature flowers of the English daisy (*Bellis perennis*). We compared English daisy plants exposed to water with high levels of added nitrogen (representing high pollution), low levels of added nitrogen (representing low pollution), and tap water. The high pollution treatment caused plants to decrease in height over three weeks. Plants in the low pollution treatment increased in height the first week, but then decreased during weeks two and three. The plants treated with tap water stayed fairly constant in height during the experiment. There was a small increase in the number of mature flowers in the low pollution treatment, while the plants exposed to the high pollution treatment and tap water treatment both lost similar numbers of flowers. This indicates that there is a negative effect of pollution on plant height and an inconclusive effect on number of mature flowers. Further studies, including beginning plants from seedlings and investigating the effects of different chemical pollutants, are needed to determine the full effect of water pollution on the English daisy.

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Introduction

Chemicals, such as phosphorous and nitrogen, are often used in fertilizers and pesticides for agricultural practices. However, these chemicals can often end up in nearby rivers and bodies of water through runoff effects or by seeping through the soil to the river itself (1). These toxins can have many negative impacts on specific organisms, as well as the ecosystem as a whole. For example, increased nitrogen and phosphorous in water can lead to harmful algae blooms, which use up all the oxygen in the water, making it difficult for other organisms to survive (2-4). Some studies have found that increases in nitrogen can decrease the overall number of species in an area (5). Finally, although nitrogen can initially increase plant growth, levels of nitrogen that are too high can lead to decreased plant growth (6). As river pollution from fertilizers and pesticides is an even larger issue than it was in the past (7), we conducted an experiment to test the direct effect of increased nitrogen levels on plant growth.

In this experiment, we used the Thames River, located in southern England, as a model for investigating the impact of increased nitrogen levels in polluted rivers. The Thames River contains pollution from the runoff of nearby farms. As a result, this may affect the growth of plants that rely on the water of the Thames and upset the surrounding ecosystem. We used water that mimicked the chemical levels of nitrogen in the Thames and tested the effects of this polluted water on the English daisy, *Bellis perennis*. The English daisy is a small flowering plant native to the British Isles and parts of Europe and is abundant along some of Europe's major rivers, including the Thames River in the south of England (8). The plant blooms from early summer to early fall and thrives in moist soil and in cool, wet weather. Though the English daisy is not specifically at risk of being impacted by river pollution, we selected this plant for two reasons: 1) the English daisy has many uses including herbal medicines and ingestion (8,9), adding potential relevance to our results, and 2) the English daisy is common, and thus we had easy access to fully mature plants. We chose to measure the height and number of mature flowers on a plant to give us insight into the plants' health.

Our hypothesis is that the plants watered with regular tap water (no pollution treatment) would grow the tallest, plants watered with low levels of added nitrogen (low pollution treatment) would not grow very much, and plants watered with high levels of added nitrogen (high pollution treatment) would decrease in height. We also hypothesized that the number of mature flowers will increase the most on plants watered with regular tap water and decrease on plants watered with high levels of nitrogen, because we thought that larger plants would produce more flowers.

Results

The experiment consisted of five English daisy plants exposed to regular tap water, five English daisy plants exposed to low levels of nitrogen added to the water (low pollution treatment), and five English daisy plants exposed to high levels of nitrogen added to the water (high pollution treatment). Each plant was watered with one cup of water every other day.

The amount of nitrogen affected the average height of the plants in the high pollution, low pollution, and tap water treatments (**Figure 1**). In general, plants in the high pollution treatment decreased in height, plants in the low pollution treatment first increased, then decreased in height, while the height of the tap water treatment didn't change very much. During the first week, plants in the high pollution treatment decreased an average of 3.90 cm in height, and the plants in the low pollution treatment increased in height 4.60 cm. Plant height in the tap water treatment increased 1.66 cm. During the second week, the plants in the high pollution treatment decreased in height 2.12 cm, plants in the low pollution treatment decreased in height 5.52 cm, and plants in the tap water treatment decreased in height 1.1 cm. During the last week of the experiment, the plants in the high pollution treatment decreased in height 4.34 cm, the plants in the low pollution treatment decreased in height 3.04 cm, and the plants in the tap water treatment decreased in height 1.22 cm.

The number of mature flowers was also affected by the amount of pollution (**Figure 2**). During the first week, the number of flowers on the plants in the high pollution treatment increased by an average of 3.4 mature flowers, flower number on plants in the low pollution treatment increased by an average of 5.8 flowers, and there was no change in the number of flowers in the tap water treatment. During week 2, the number of flowers on the plants in the high pollution treatment decreased by an average of 7 flowers, the number of flowers in the low pollution treatment decreased by an average of 2.6 flowers, and the number of flowers in the tap water treatment decreased by an average of 4 flowers. During the final week, there was no significant change in the number of flowers in the high pollution treatment and the tap water treatment. The number of flowers in the low pollution treatment decreased by an average of 2.8 flowers.

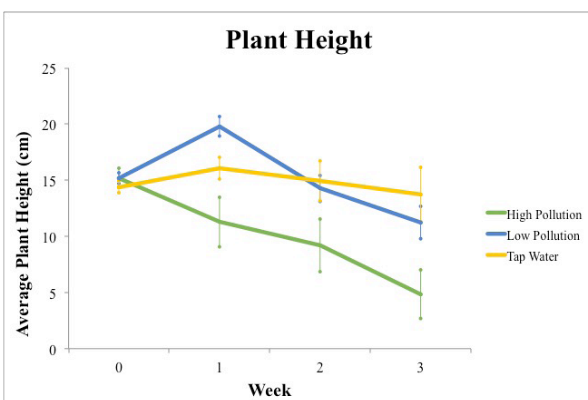

Figure 1: Effect of nitrogen pollution on plant height. Data represents plant height averaged across all 5 plants in the high pollution treatment (green line), low pollution treatment (blue line), and tap water treatment (yellow line). Week 0 represents the start date, and error bars are the standard error.

Discussion

Plants in the high pollution treatment decreased in height over the three weeks. Plants in the low pollution treatment increased in height the first week, but then decreased in height during weeks two and three. Heights of plants in the tap water treatment stayed fairly constant during the experiment. Overall, there was a small increase in the number of mature flowers in the low pollution treatment, while plants in the high pollution treatment and tap water treatment both lost similar numbers of flowers. This indicates that there is a negative effect of pollution on plant height and an inconclusive effect on number of mature flowers. In general, the plants did not show any obvious signs of being unhealthy; they had minimal dead leaves and flowers.

The initial increase in height in the low pollution treatment and the initial increase in flower number in the low and high pollution treatment could be due to the increase in the available nitrogen. This chemical is necessary for plants to grow and is often found in plant fertilizers. However, as more and more nitrogen is added to the system, the chemical levels exceed what the plant can handle and the chemical becomes toxic (6), leading to the observed decrease in plant height in the low and high pollution treatment, and the loss of flowers in the high pollution treatment. In the tap water treatment, where the smallest amount of growth was observed, the plant was likely not receiving enough nitrogen (7). It is unclear why the number of flowers in the tap water treatment decreased. Due to a small sample size, there was a lot of variation in the number of mature flowers between weeks. Therefore, this result is inconclusive, and more work needs to be done to determine if pollution has a positive or negative effect on the number of flowers of *Bellis perennis*.

There are numerous reasons why we should be concerned that increases in nitrogen could potentially lead to changes in plant size and flower number. If plants decrease in size, other animals that rely upon these plants as food might not have enough to eat. Decreases in the number of mature flowers might lead to less reproduction due to the lower number of seeds, which could impact the survival of the population. Finally, changes in plants size or flower number can potentially throw off the balance of an ecosystem, as interconnected species may not be able to adapt in time to match changes to the plants' flowering. Insect pollinators rely on flower nectar for survival, while flowering plants often rely on insect pollinators for successful reproduction. If this relationship is changed, due to flower loss or differences in when flowers are present, both species could suffer (10).

This experiment only looked at the effect of nitrogen on plant height and flower number of the English daisy. There are several caveats that should be mentioned. First, the English daisy is a very hardy plant and can grow in many locations, not just along the river of the Thames. Future work could look at plant species that only grow along the riverbank of the Thames, as well as plant species that grow submerged within the water of the Thames. Doing this would increase knowledge about how plants are adapting to changing river waters, if at all. It could also show how plants are affected, depending upon how much of their overall water they get from a polluted source (all for submerged plants, versus some for plants along the riverbank).

Second, there are three primary nutrients that are important for plant growth and flowering, all of which are included in plant fertilizers and potting soil: nitrogen, which has a large effect on plant growth (6, 11); phosphorous, which impacts root development and flowering (12); and potassium, which is important for root growth (13). All three chemicals are important, and at high doses, all will have different effects on plant health. However, given the resources we had available, we were not able to change the concentration of all three chemicals independently. As such, we chose to focus solely on the concentration of nitrogen, which can affect many different parts of plant health (6). It is possible that the patterns we observed were due to the increased levels of phosphorous and/or potassium, or the joint effect of all three nutrients, though it is likely that the levels of phosphorous and potassium added in each treatment are lower than those found in polluted water. However, future work that is able to independently add each type of chemical is needed to tease apart the relative influence of each nutrient.

Along the same lines, there are many aspects of plant growth and health that could be impacted by the addition of pollutants to the water that we did not investigate. For example, we did not distinguish between flowers lost due to the setting of seed, and those lost due to poor plant health. We also did not investigate the effect of pollution on leaf size, area, or quality (instead choosing to focus only on overall plant height). As nitrogen and other chemical nutrients affect different aspects of plant growth in different ways, future work could investigate this further to better understand the specific impact of pollutants.

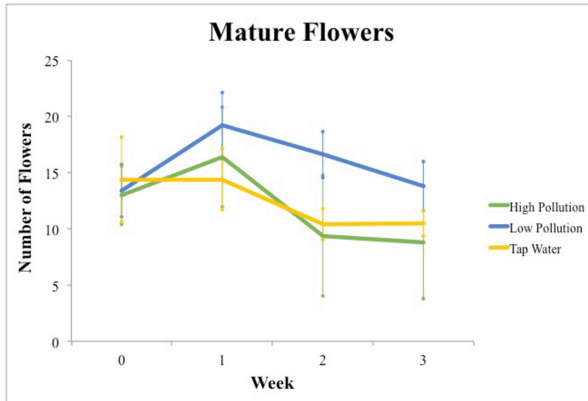

Figure 2: Effect of nitrogen pollution on plant flower number. Data represents the number of mature flowers (defined as flowers with a diameter ≥ 1cm) averaged across all 5 plants in the high pollution treatment (green bar), low pollution treatment (blue bar), and the tap water treatment (yellow bar). Week 0 represents the start date, and error bars are the standard error.

Finally, there were several sources of experimental error that are worth mentioning. This experiment took place in a highly controlled environment in order to remove confounding factors and test only the effects of water pollution. Though we tried to mimic the natural environment as closely as possible, we were limited in the amount of change we could make to the temperature and humidity in the classroom. In future experiments, it would be good to have more control over these factors. Time restrictions also prevented us from growing plants from seeds. Future work could investigate this issue and examine how pollution affects plant growth throughout life, rather than during a few short months. Finally, we were limited by our budget and so could not use more than five plants per treatment. In order to gain greater confidence in our results and test for more precise statistical differences between treatments, future work could include more replicates for each treatment. In the future, it would also be good to run the experiment over a longer time frame. However, we expect that the outcome we obtained with the current project is similar to what would be expected given a longer experimental time frame (but still starting with adult plants) because any initial increase in growth was during the first week, and then the plants continued to decrease in height for the rest of the experiment.

A final possible source of error could occur because many students had access to the plants when a teacher was not present. Watering was regulated carefully by the group, but there were times when some plants were more wet than others, which implies that water could have been added without notice. Another source of error could have occurred because of plant placement. At times, one or more plants would be found pushed away from the window, placing it in the shade more than the others. Readjustments were made as quickly as possible, but we were not able to judge the effect that plant movement might have had on growth. Weather conditions ranging from bright sunny days to rain most likely had an effect on some of the plants. If it had been possible to conduct our research outside in full sun, the results might be more conclusive.

Although this experiment was staged on a small scale, its design can be used on a larger scale by scientists to help determine the effects of pollution on various types of plants throughout the world. Although there is some evidence that animals can adapt to changing climates (14), in general, human-caused climate change could lead to lots of negative effects. Pollution levels are on the rise as more vehicles are out on the roads, more energy production plants are being created, industrial agriculture is becoming more widespread, and chemical wastes often find their way into rivers, streams, and oceans. It is thus becoming increasingly important for scientists to determine the effects of these pollutants on the wildlife around them. Along with the English daisy, many other plants face danger through polluted sources of water such as the Thames River (2). Plant life still faces the dangers of pollutants such as pesticides and fertilizers. Through various experiments similar to the experiment that we performed, scientists can determine the level of tolerance that certain plants have to different pollution levels, giving us insight into the health of the ecosystem and the health of our world.

Methods

Mature *Bellis perennis* plants were purchased from the local Home Depot garden department (Santa Rosa, CA) in April 2014. Plants were carefully selected to be roughly equal in size and quality. Due to the time limitations of the experiment, we were not able to start plants from seeds. New potting soil was purchased at the same time to ensure quality and continuity in the experiment. After the plants were purchased, they were stored in a suitable environment until the experiment began. At the beginning of the experiment, plants were moved into identical pots. The pots were small, 6-inch round containers with 5 half-inch matching holes drilled into each bottom. They were placed onto foil-lined plates to collect excess moisture. Each plant was carefully removed from the small store container and placed in the center of the 6-inch pot. Five cups of potting soil were measured into each of the pots with the plants. Approximately equal pressure was applied to the soil to secure each plant. One cup of tap water was applied to each plant. The plants were then stored in a greenhouse for one night until they could be transported to the classroom the next day. For the duration of the experiment, plants were kept indoors on a southwest-facing windowsill. All plants were monitored for a total of 3 weeks, with the experiment beginning on April 4, 2014 and ending on April 25, 2014.

In order to increase sample size, a second round of the experiment began on February 1, 2015 and ended on February 28, 2015. The first round of the experiment included two plants per treatment, while the second round of plants included three plants per treatment. Plants in the second round were handled exactly the same as the first round, but were kept at the author's home, rather than the classroom. We kept the temperature and humidity as close to that of the classroom as possible. Following both rounds of plant growth, for each treatment we combined the measurements from 2014 with the measurements from 2015.

Plants were divided into three treatments, with five replicate plants per treatment. The plants in Treatment 1 simulated a no pollution environment, while the plants in Treatment 2 and 3 simulated a low pollution environment and a high pollution environment, respectively. For each treatment, plants were watered with one cup of treatment-specific water every other day. Since we did not have access to natural river water or polluted water, we added Super Soil fertilizer to tap water to model the effects of chemical pollutants, using the water quality of the river Thames as a benchmark. Currently, the average yearly concentration of NH_4 in the Thames is approximately 126.75 μg/L, with an estimated winter concentration of up to 250 μg/L by 2080 (15). As such, for the high pollution environment, we added 0.0045 g of fertilizer to half a gallon of tap water, which lead to a concentration of 285.31 μg/L of ammoniacal nitrogen. For the low pollution treatment, we added 0.001125 g of fertilizer to a half a gallon of tap water, which lead to a concentration of 71.33 μg/L of ammoniacal nitrogen. We could not precisely measure the available nitrogen in the potting soil, and as such, these concentrations are likely underestimates of the actual nitrogen available to the plants in this experiment.

We measured the height of the plant (in centimeters) by placing a standard ruler perpendicular to the soil and locating the tallest portion of the plant. By measuring plant height in this way, an increase in plant height will be due to the plant stem growing or becoming more rigid, while a decrease in plant height will be due to the plant stem shrinking or becoming less rigid. This doesn't take into account any changes in leaf size or area. Additionally, we measured the number of mature flowers at each time point. A mature flower was defined as being at least 1 centimeter in diameter, measured using a standard ruler. When determining if a plant had decreased in flower number between time points, we didn't distinguish between flowers lost due to setting seed, and flowers lost due to poor plant health.

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