Water is Water is Water!

The Effect of Greywater on Plant Growth, Soil Microbial Biomass Carbon, and Soil Fungi to Bacteria Ratio

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Abstract

This project was designed to find out if greywater and treated greywater can safely hydrate plants, and promote plant growth, just as well as tap water. I live in drought-prone California and it's important to find different ways of conserving water. My experiment tested the watering of grass pots with three different types of water (independent variable): Tap water, Greywater, and Greywater treated with Activated Charcoal. Over the course of 8 weeks, I measured plant growth, soil *Microbial Biomass Carbon (MBC)* levels and soil *Fungi to Bacteria (F:B) ratio* (dependent variables). Many controlled variables ensured a valid experiment. I hypothesized that each of the water types would result in the same growth rate, soil MBC and soil F:B ratio. My hypothesis, however, was incorrect. Greywater resulted in stunted growth and spiked the F:B soil ratio so high that the pot sprouted 13 fungi heads. Tap water and Treated Greywater, however, were equally good in terms of healthy plant growth and both pots had the two best average F:B ratios closest to 1:1 (which is the best ratio for grasses). Neither of these pots produced fungi. Soil carbon levels (MBC) fluctuated for all three plants, however each plant maintained an "Excellent" level[1]. This indicated that each water type was fine for watering grass if you don't mind stunted grass growth and some fungi in your lawn. My experiment also proved that Activated Charcoal effectively "adsorbs" chemicals in greywater that alter a soil's F:B ratio.

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[1] According to **microBIOMETER®** soil testing kit

Question/Problem of this scientific study

Given the serious drought situation in California (even after all the rain recently!) Can we use greywater from the shower instead of tap water to safely water our gardens? Does greywater negatively impact plant growth, the number of microbes in soil, and the fungi to bacteria ratio in soil?

Hypothesis

If two pots of grass are watered with, respectively, greywater and greywater treated with activated charcoal, *then* the plant growth, soil microbial biomass carbon, and soil fungi to bacteria ratio of those two plants will be the same as the grass plant watered with regular tap water.

Variables

Type of Variable	In this experiment "Water is Water is Water"
Independent	● Type of water that each grass plant is watered with. These are regular tap water, greywater and greywater treated with activated carbon.
Dependent	● Observation of the average percentage of brown grass stems of each plant at the end of the experiment. ● Maximum height and average height of the grass plants. ● ug C/g Microbial Biomass Carbon of the soil. ● Fungi to Bacteria Ratio of the soil.
Controlled	● Same potting soil and pot sizes used for each grass pot. ● Same type and weight of grass seeds used for each grass pot. ● All plants were grown in the same location with the same amount of sunlight. ● Each batch of greywater was collected on the same day from the same shower. ● Same brand of activated charcoal used for the whole experiment. ● Same shampoo, conditioner, and soap used to shower with. ● All plants watered on the same day at the same time. ● All plants tested on the same day in the same time frame. ● All soil testing materials were sterilized after each test.

Background Research

This project is called “Water is Water is Water " and it will test the effect of different types of water on plant growth, as well as on soil microbial biomass carbon levels and soil fungi to bacteria ratio.

California is experiencing a major drought right now, even though we’ve had a lot of rain recently. Governor Jerry Brown, in January 2022, declared a statewide drought emergency calling this “perhaps the worst drought California has ever seen since records began being kept about 100 years ago.” Because of this, Californians are under the tightest water restrictions ever experienced. From June 10, 2022, the California State Water Board banned the use of tap water on decorative or non-functional grass at commercial, industrial, and institutional properties. Even regular households are under restrictions and are only allowed to water their gardens twice a week.

Each year, according to the Los Angeles Department of Power and Water, Los Angeles County uses 100 billion gallons of tap water on gardens and landscapes, and 91 billion gallons on showering and bathing. The LA County of Public Works says that, on average, most families use about 500 gallons of water per day and over 30% of that is used to water their yards and gardens. What would happen if Californians used greywater from the showers and baths that they take, instead of using tap water to water gardens?

Research was carried out into the possible uses of greywater for watering plants. Of course, greywater is not drinkable as it contains many chemicals and contaminants that the human body cannot handle, such as sulfates, possibly E-coli, staphylococcus aureus, fecal contaminants, and - in the case of a swimmer showering - chlorine. Plants also can’t handle water that is contaminated with too much bacteria or chemicals but howstuffworks.com says that “some additives in greywater (like phosphorus and nitrogen) can actually help them [the plants] grow”. Other studies state that greywater from the bath or shower is the best for your plants and that all of the harmful properties in greywater are absorbed by the roots. From this, it can be concluded that greywater should not be used to water root vegetables that are eventually going to be eaten. However, greywater may be fine for regular garden watering. Another study completed by Alma Siggins says, “Greywater can contain plant macronutrients that may benefit plant growth.” On the other hand, studies conducted by M. Travis, N. Weisbrod, and A. Gross[2] show that greywater can be harmful to soil because oil and grease from greywater can accumulate in soils, influencing the soil’s ability to take in water, and what this does is practically makes the soil waterproof. This would affect plant growth and would show up in my experiment.

Further research was carried out and showed that soil health has a lot to do with microbial levels of fungi and bacteria, as well as the amount of carbon in the soil.

Fungi to bacteria ratio

Interest in these soil microbes is actually fairly new, according to Laura Decker, owner of the MicroBiometer company that has developed a portable, on-site, soil-testing kit to test soil health. We now know that there are more microbes in a handful of soil than all the humans on the planet so it makes sense that we should be considering soil microbiology. A healthy balance of soil microbes means that nutrients can be ‘fixed’ to the soil, and that plants can use those nutrients to grow. Diverse populations of soil bacteria and fungi can also suppress root diseases and therefore help plants fight off disease, increasing plant immunity. In addition, healthy microbial levels in soil also improve soil structure and allow water to be stored more efficiently for plant use. In fact, fungi are some of the main microorganisms that tie the soil together preventing soil from leaching away. Finally, bacteria and fungi are the start of the soil food web that supports other microorganisms and the functions of a healthy soil; basically they keep the soil alive. The best fungi to bacteria ratio varies a lot according to different habitats and plants. Grasses, like the ones in this experiment, grow best with a 1:1 fungi to bacteria ratio, just like “Highly productive agricultural soils” according to experts at MicroBiometer and Dr. Elaine Ingham.

[2] Accumulation of oil and grease in soils irrigated with greywater and their potential role in soil water repellency". *Science of the Total Environment* 394 (2008) pp. 68-74

Soil Carbon

Carbon in soil is extremely important because it also leads to better plant health and growth through the carbon cycle. It's important to understand that soil already has lots of carbon in it. In fact, it's the world's largest carbon storage. All of the microbes living in soil, like fungi and bacteria, eat and thrive on this vast amount of carbon. This makes microbes reproduce and multiply which in turn leads to better and healthier soil. The plants then feed on the large amount of food and energy that these microbes produce which results in bigger, taller, and more robust plants. Carbon is also essential for soil structure and soil mineralization. It increases water storage and protects soil from erosion. Therefore, increasing soil carbon is highly desirable. However, carbon is also easily lost, so maintaining high carbon levels is extremely important. According to Rattan Lal, director of Ohio State University's Carbon Management and Sequestration Center, "the world's cultivated soils have lost between 50 and 70 percent of their original carbon stock, much of which has oxidized upon exposure to air to become CO_2." Deforestation, compaction of soil, farming, and urbanization are all causes of soil losing valuable carbon. So, carbon leaching out into the atmosphere not only makes our soil become less healthy but it also contributes to global warming.

Activated Charcoal

Activated charcoal is basically carbon in a solid form. It is made from any material that is burned that has lots of carbon in it, such as trees. As a result of this burning process, called oxidation, the charcoal has lots of pores in it. Consequently, it has a large surface area, and can trap impurities through a process called "adsorption". The research suggested that using activated charcoal to treat greywater might trap some of the chemicals and impurities in that water source..

Knowing all this information about microbial biomass carbon and fungi to bacteria ratio shows that it's important to treat soil properly so that carbon stays in the soil and plants get the right nutrients. Research has shown that the microbial biomass is affected by factors that change the water or carbon content of the soil.[3] So what would happen if greywater and greywater treated with activated carbon were used to water plants? It would be interesting to find out how the different waters used affect the microbiology of the grass soil as well as plant growth.

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[3] *https://soilquality.org.au/factsheets/microbial-biomass-qld*

The independent variable for this experiment will be the type of water used to water each plant. The dependent variables will be as follows: a) the average and maximum plant height of each grass pot, b) the microbial biomass carbon (uc C/g) of the soil in each plant, c) the fungi to bacteria ratio of the soil in each plant. There will be several controlled variables as follows: a) the same potting soil and pot sizes will be used for each grass pot, b) the same type and weight of grass seeds will be used for each grass pot, c) all plants will be grown in the same location with the same amount of sunlight, d) each batch of greywater will be collected on the same day from the same shower, e) the same brand of activated carbon will be used for the whole experiment, f) the same shampoo, conditioner, and soap will be used to shower with, g) all plants will be watered on the same day at the same time, h) all plants will be tested on the same day in the same time frame, i) all soil testing materials will be sterilized after each test.

The results of this experiment might be useful to urban planners, farmers, forest rangers, environmentalists, and home gardeners. All of the research shows that soil is a very complex community. It will be interesting to see the results!

Materials

For the grass pots
- 3 planting pots (8“ diameter each)
- 1 bag of Kellogg Garden Organic Patio Soil from Home Depot
- 1 lb. California Native Grass Mix

Watering materials
- 1 cup measuring jug
- 3 glass jars with screw tops (to transport 3 different waters to the school lab)
- 10 - 12 sticks of activated charcoal (for the treated greywater)

Testing plant growth and soil microbial levels
- **microBIOMETER®** soil testing kit, or access to a lab that can test soil
- **microBIOMETER®** app downloaded on phone
- large plastic straws to remove soil from each grass pot
- 1 timer to time soil testing
- ruler
- notebook to log results

Safety items for watering and soil testing
- face masks
- goggles
- surgical gloves

Sterilizing equipment
- bottle of bleach
- bucket (to soak equipment in sterilizing solution).

Methods/Procedures

Procedures Diagram

Set Up 3 Grass Pot Plants from seed.
Label: "Tap Water", "Greywater", "Treated Greywater".
Grow grass to 1.5" - 2"

Week 1 of testing: Measure average height of each grass pot & maximum grass blade height of each grass pot. Test soil using microBiometer soil test kit. Record all results.

Twice a week, collect 2 jars of shower greywater.
In 1 jar, place activated charcoal stick.
Within 24 hours, water the plants with the type of water they are labelled with.

Repeat measuring, soil testing and recording on the same day of each week.

Procedures Table

Step	Procedure
1.	Using Kellogg Garden Organic Patio Soil from Home Depot, set up three pots of grass seed and grow the grass to 1.5 - 2 inches in average height.
2.	Label the pots according to the water that each plant will be watered with. Label one pot as ‘Tap water’, one as ‘Greywater’, and one as ‘Greywater treated with activated charcoal’
3.	On day 1 of the experiment, a) record average grass height and tallest grass blade height, and b) conduct a soil test on each pot of grass using the microBIOMETER® soil testing kit. Record results of Microbial Biomass Carbon and Fungi to Bacteria Ratio of all three separate soils for that day. (microBIOMETER® is a patented soil testing kit. The soil testing procedures are specific to this kit and are listed below this table)
4.	Discard all soil samples and soil test cards in a secured ziplock bag in the regular trash.
5.	Sterilize all testing materials in 10% bleach solution.
6.	Prepare three glass jars and label the jars the same as you did for the plants. Collect the three types of water in their own labeled jars. Tap water is collected from the tap. Two jars of greywater are collected from the shower and a stick of activated charcoal (prior-boiled for 10 minutes to activate) is placed in the jar labeled “Greywater treated with activated charcoal”. Note that greywater of any kind cannot be stored for more than 24 hours before use because it can become putrified.
7.	Twice a week, water each plant with one cup of the type of water shown on the pot label. Tap water is to be taken from the tap.
8.	On the same day each week for 8 weeks, repeat soil testing, data recording, and sterilization of soil testing equipment (steps 3 to 5 above).

microBIOMETER® *soil testing kit procedures*

Step	Procedure
a.	Prepare the extraction solution that will be mixed with the soil. In a plastic vial (provided with the kit) mix the Extraction Powder with 9.5 ml of water. Use the whisk provided to mix briefly.
b.	Obtain a composite sample of MOIST soil from the top 2 to 5 inches Using the included sifter, shake to remove debris and collect the sifted soil in the plastic bag provided in the test kit.
c.	Fill the test-kit soil sampler syringe to ~1ml with sifted soil. Compress against your finger to 0.5 ml, remove any excess from the end, and push into the extraction vial with the extraction solution.
d.	Break up any clumps of soil using the included metal spatula. Allow the vial to rest in the hole provided within the kit, insert the whisker, turn on, and allow to mix for 30 seconds.
e.	After mixing, allow the liquid to rest for 5 minutes. Tap the bottom of the vial on a hard surface to coax floating debris to settle. Allow to settle for an additional 15 minutes. Soil particles will settle to the bottom, creating a microbial suspension above.
f.	Use the small pipette provided in the kit to draw up liquid from about half an inch below the surface. Squeeze the pipette before entering to avoid blowing bubbles. Avoid any floating debris and foam at the edges.
g.	Carefully put 3 drops from the pipette onto the sample window of the test card provided in the kit. Allow each drop to soak in fully before applying the next.
h.	Analyze with the (previously downloaded) smartphone phone app within 2 minutes.

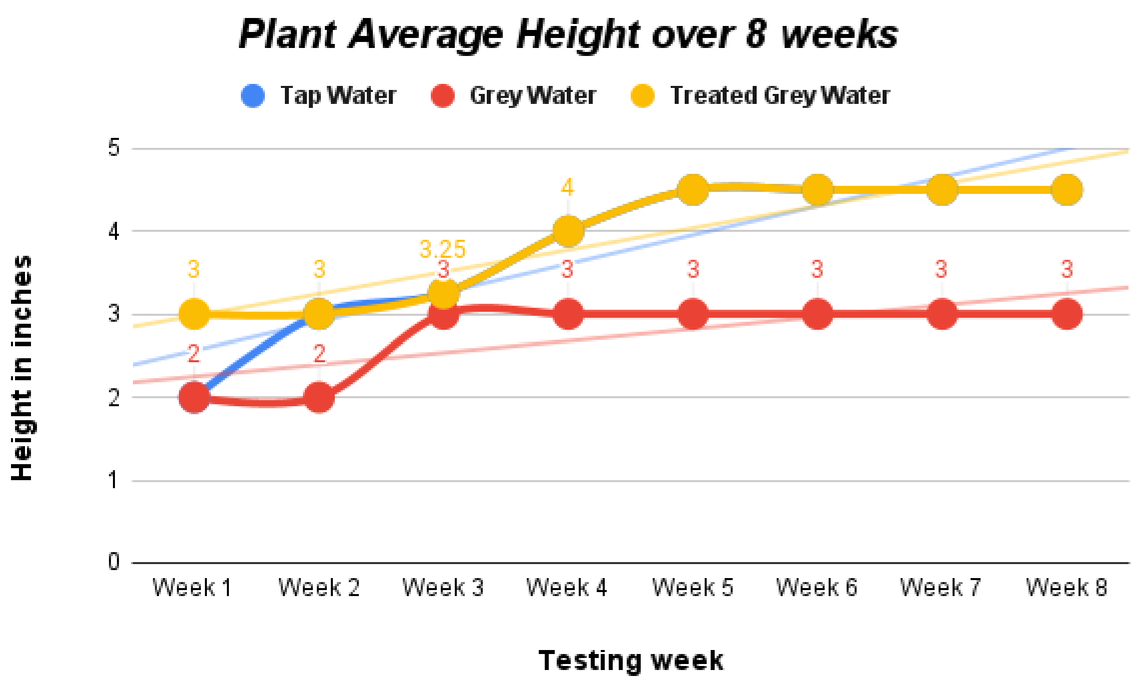
Safety Procedures for Los Angeles County Science Fair Requirements

1. All testing took place in the school science lab and under the supervision of the qualified supervisor (in my case, Ms. Christy Novak)
2. Face masks were worn during testing.
3. Gloves and goggles were worn during soil testing.
4. After testing, all soil samples and soil test cards were discarded in a secured ziplock bag into the regular trash.
5. After testing, all testing materials were sterilized in a 10% bleach solution.

Data & Graphs

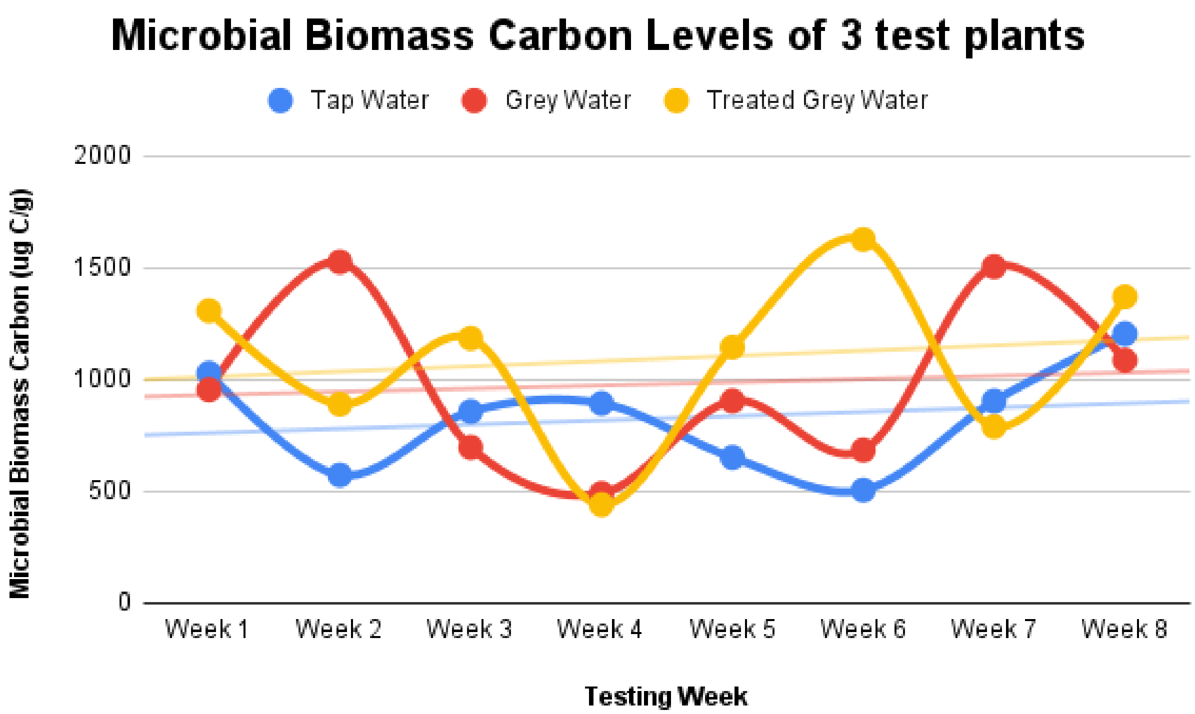
Data & Graph 1: Plant Average Height (in inches)

Plant Average Height (in.)	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Tap Water	2	3	3.25	4	4.5	4.5	4.5	4.5
Grey Water	2	2	3	3	3	3	3	3
Treated Grey Water	3	3	3.25	4	4.5	4.5	4.5	4.5



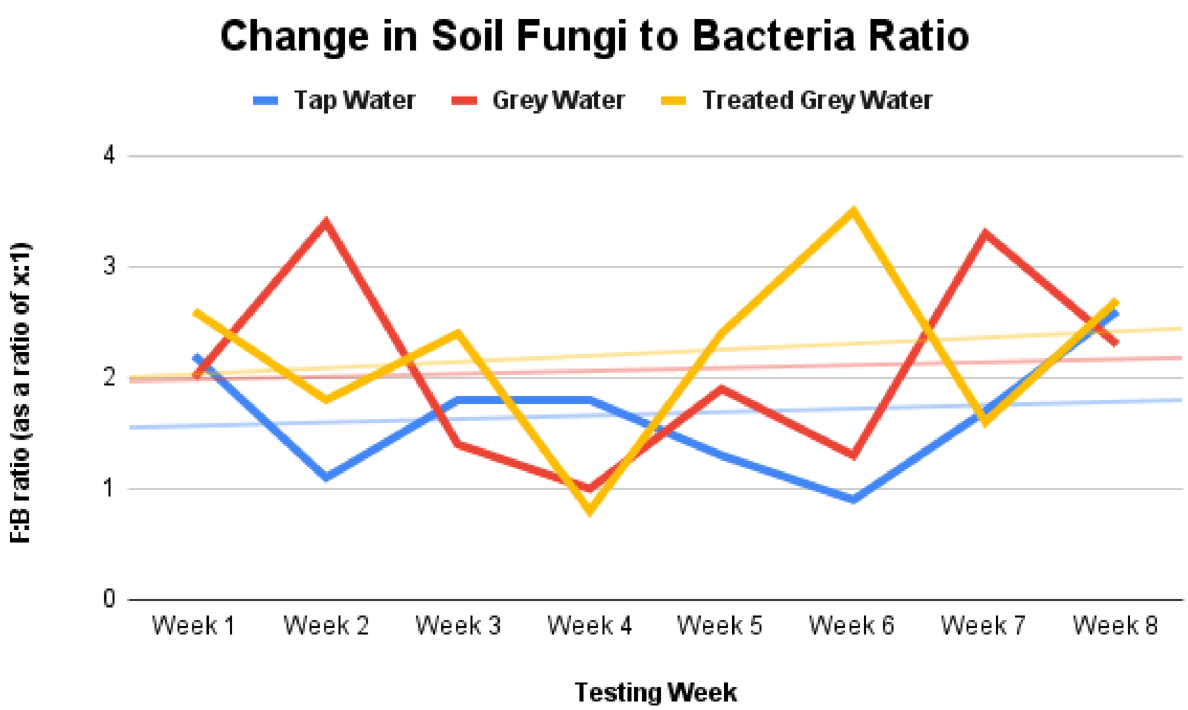
Data & Graph 2: Microbial Biomass Carbon Levels of 3 Test Plants

MBC (ug C/g)	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
Tap Water	1029	573	858	894	652	506	904	1205
Grey Water	955	1526	697	491	905	685	1505	1087
Treated Grey Water	1308	890	1185	441	1145	1627	790	1371

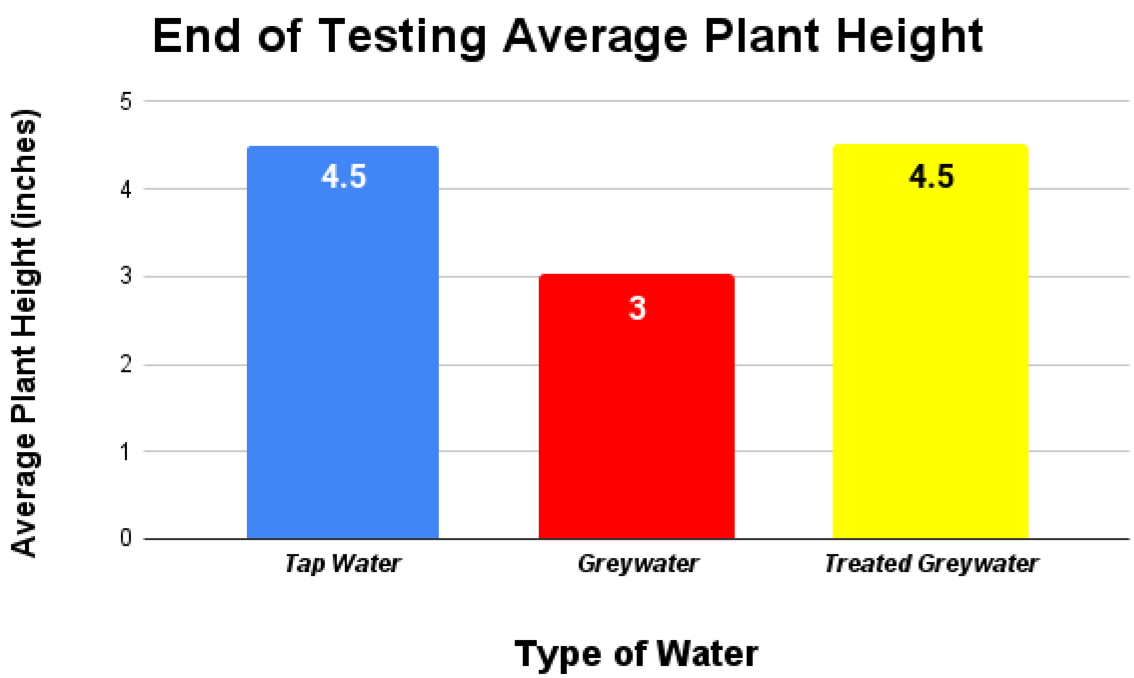


Data & Graph 3: Change in Fungi to Bacteria Ratio

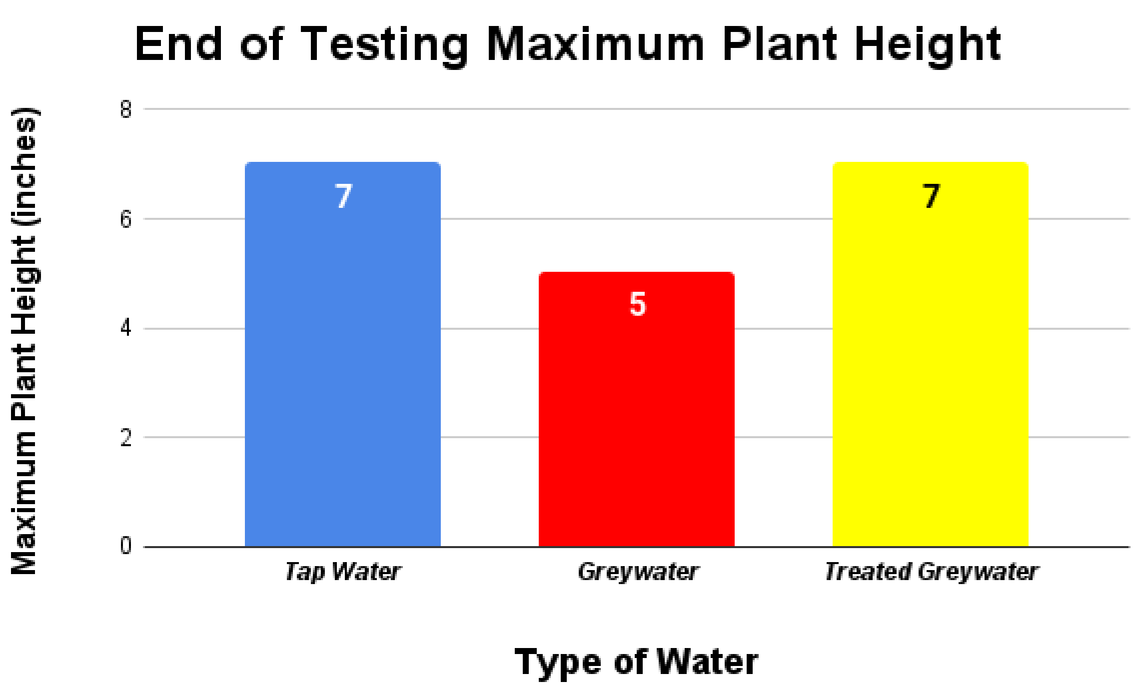
Fungal to Bacterial Ratio (#:1)	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Average**
Tap Water	2.2	1.1	1.8	1.8	1.3	0.9	1.7	2.6	1.95
Grey Water	2	3.4	1.4	1	1.9	1.3	3.3	2.3	2.65
Treated Grey Water	2.6	1.8	2.4	0.8	2.4	3.5	1.6	2.7	2.1



Graph 4: Average Plant Height at End of Testing



Graph 5: Maximum Plant Height at End of Testing



Discussion

END OF TESTING OBSERVATIONS

Type of Grass Pot	Observations at Week 8 final testing
Tap Water Grass Pot	● Average height: 4.5 inches ● Maximum height: 7 inches ● Overall healthy, full green grass blades with approximately 5% brown grass blades.
Greywater Grass Pot	● Average height: 3 inches ● Maximum height: 5 inches ● Stunted growth, less lush green grass blades with approximately 10% brown grass blades. ● 13 Hare's Foot Inkcap fungi appeared between week 7 and a week after week 8.
Treated Greywater Grass Pot	● Average height: 4.5 inches ● Maximum height: 7 inches ● Overall healthy, full green grass blades with approximately 5% brown grass blades.

At the end of the eight week testing period, I recorded my observations of the grass pots. The Tap Water grass pot and Treated Greywater grass pot looked equally healthy with lots of green blades and minimum brown grass blades (approximately 5%). On the other hand, the Greywater grass pot showed less growth, less green grass blades, and the most brown grass blades out of the three (approximately 10%). This pot had obvious stunted growth compared to the other pots.

One of the major observations that I made on the last day of testing was that the Greywater grass pot had sprouted fungi. It sprouted 13 fungi heads between week 7 and one week after week 8. I used the PictureMushroom app to identify the fungi and found out that it was the Hare's Foot Inkcap (Coprinopsis Lagopus, which comes from the Greek words "hare's foot living on dung"). This fungi can be found in forests of all types, and occasionally in urban settings. It is very common in North America and has been known to grow in grass. It feeds on wooden debris and my soil definitely had lots of wood chips in it but this might not have been to do with the soil because all of my grass pots were set up from the same bag of potting soil on the same day. Maybe it just happened that the Hare's Foot Inkcap spores were in the handful of soil that I used for the Greywater grass pot. Or maybe the Greywater pot sprouted fungi because of the untreated

Greywater. I think this is more likely because, on the previous week's test (week 7), the Greywater pot had tested very high for fungi to bacteria ratio so I think this is what caused this pot to sprout fungi.

<u>DATA ANALYSIS</u>

I have discussed my observations of the three grass pots. Now I will talk about what my data results showed.

The average plant height for each grass pot plateaued during my experiment. The Tap Water grass pot and the Treated Greywater grass pot plateaued from week 5 to week 8 at the same height of 4.5 inches. This shows that the water I used for *those* plants did not affect growth. The pure Greywater grass pot, however, plateaued earlier at week 3 to week 8 at an average height of 3 inches, which means that this plant struggled earlier to grow and it did not reach the height the other plants did. This tells me that pure Greywater does affect plant growth. In fact, the Greywater grass pot performed the worst with an average height of 3 inches and a maximum of 5 inches, compared to the Treated Greywater and Tap Water grass pots which performed equally as well with an average height of 4.5 inches and a maximum of 7 inches. That's a whole 2 inch difference in terms of average growth between Greywater and the other two types of water!
In terms of *maximum* grass height, the Tap Water pot and Treated Greywater pot both plateaued at 7 inches while the Greywater pot plateaued at 5 inches. That's another 2 inches difference in growth potential. Untreated Greywater definitely affects the plant growth of grasses.

Now let's talk about Microbial Biomass Carbon. Microbial Biomass Carbon (MBC) is the after-effect of microbes taking in oxygen and breathing out carbon dioxide, just like humans, so therefore it measures the amount of microbes in soil by measuring the carbon they leave behind during respiration.

Even though the Microbial Biomass Carbon (MBC) of all of the grass pots turned out to be "Excellent" on every test, the Greywater and Treated Greywater pots had the most fluctuating MBC based on my data. The Treated Greywater pot had a high of 1627 ug C/g and a low of 441 ug C/g, which is a minimum to maximum difference of 1186 ug C/g. The Greywayer pot had a high of 1526 ug C/g and a low of 491ug C/g, which is a minimum to maximum difference of 1035 ug C/g. The Tap water pot was the most consistent over the 8 weeks with a high of 1205 ug C/g and a low of 506 ug C/g, which is a minimum to maximum difference of only 699 ug C/g. This clearly shows that tap water nurtured the plant soil in a more stable way.

Since the MBC data was so erratic on my graph, I used trendlines to find the pattern and direction of my data. The trendlines showed that Tap Water and Treated Greywater both had the sharpest incline in MBC. Untreated Greywater, however, had a less sharp incline. This makes me think that, while each type of water is healthy for soil carbon levels, Tap Water and Treated Greywater are slightly better in terms of soil MBC health because they overall hold slightly more microorganisms.

Soil fungi to bacteria ratio (F:B) is also a very important factor to soil and grass health. It is basically the amount of fungi to the amount of bacteria in the soil. I learned through my background research that the best ratio of fungi to bacteria for grass is around a 1:1 ratio. In this experiment, because I was testing grass, I was looking for the closest to a 1:1 ratio. I think this might be the reason that the Greywater pot sprouted fungi because it had a notably higher average F:B ratio of 2.65:1. On the other hand, Treated Greywater and the Tap Water plant had a significantly lower F:B ratio: the Treated Greywater pot F:B ratio was 2.1:1 and the Tap water was 1.95:1. The Tap Water grass pot soil was the closest to a 1:1 F:B ratio and maybe this is why it grew as well as it did and why it looked as full and green as it did.

Conclusion

In conclusion, my hypothesis was incorrect because pure Greywater clearly affects the growth of plants and the Fungi to Bacteria ratio of soil.

Effects of Greywater

The grass pot watered with Greywater had noticeable stunted growth so it was clear that this water had a direct effect on its growth. This plant did not reach its potential height, like the grass pots watered with Tap Water and Treated Greywater, both of which grew to the same average and maximum height. This pot also sprouted a total of 13 fungi heads between week 7 and one week after the final testing. It was interesting that fungi sprouted soon after the week 7 soil test results which showed a much higher fungi to bacteria ratio for the Greywater grass pot. This did not happen in either of the other two pots. Consequently, I can directly relate this production of fungi to the different water that I used for this plant. Untreated Greywater produced a higher F:B ratio in soil, which was not an ideal ratio for the growth of grasses. My research told me that grasses like a 1:1 F:B ratio. I can conclude the fungi growth of the Greywater pot was due to the extra, untreated chemicals in pure Greywater.

From this, I can conclude that:

a) Greywater stunts the growth of grass.
b) Greywater alters fungi to bacteria ratio, raising the potential of fungi growth in soil.
c) Grasses prefer a 1:1 F:B ratio so you can safely use Greywater on your lawn but you can also expect a lot of fungi to appear.
d) Most fungi are not harmful and, in fact, can be good for your soil, so I concluded that, if people don't mind having fungi sprouting in their lawns and gardens, pure greywater can actually be a great substitute for tap water.

Comparing Treated Greywater to Tap Water

Tap Water and Treated Greywater promote the growth of grass in an equally healthy way. Both of these plants matched each other in average and maximum growth. Both the Tap Water and

Treated Greywater plants had similar average F:B ratios. The Tap Water grass pot achieved the closest 1:1 F:B ratio while the Treated Greywater was only 0.15 higher in F:B ratio..

From this, I can conclude that:

a) Tap Water and Greywater Treated with Activated Charcoal are equally healthy solutions for watering grass.
b) Activated charcoal played a significant role in making Greywater more like regular Tap Water, by adsorbing harmful impurities and stabilizing the F:B ratio of the soil.
c) Charcoal filters could therefore be useful in filtering greywater to water people's gardens. This could be expensive but it could also be useful in the future since water is so scarce here in California.

In terms of Microbial Biomass Carbon (MBC), all of the plants maintained "Excellent" MBC levels over the 8 week period. The Tap Water plant soil had the most constant MBC levels. The Greywater and Treated Greywater pot soil had similar MBC fluctuations to each other. Tap Water and Treated Greywater are slightly better in terms of soil MBC health because they overall hold slightly more microorganisms. In conclusion, however, all three types of water showed that they are capable of maintaining healthy and high MBC levels.

I found my experiment very interesting and understand that pure Greywater contains too many impurities and chemicals for healthy plant growth and soil microbiology.

In my next experimentation of the effects of Greywater, I might test different succulents, young trees, or cacti, instead of grass, because these plants need different fungi to bacteria ratios to grow well. Maybe these plants would act differently compared to the grass when watered with greywater and treated greywater. I could see how the MBC and F:B ratio levels in their soil change and how that affects their growth. I would also be interested to see how grasses grow over a longer test period like 16 weeks. Maybe fungi would eventually sprout from all three of the pots due to the fact that they all had a higher F:B ratio than 1:1!

Acknowledgements

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