

Biological Treatment of Monteverde Gray Water using Effective Microorganisms and Mountain Microorganisms

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ABSTRACT

As in many rural or less structurally developed areas, homeowners and businesses in Monteverde, Costa Rica filter grey water through grease traps or biojardineras or release it directly into their yards. This study evaluates the efficacy of two concentrations of Effective Microorganisms (EM) and Mountain Microorganisms (MM) in improving local grey water. These solutions contain biologically beneficial bacteria, yeast, and fungi. I collected grey water from six residential and commercial sources, treated each source with EM and MM in different concentrations, and gathered water quality metrics from each group for seven days. The metrics included surface cover, sedimentation, layering, smell, percent Total Dissolved Solids (%TDS), Dissolved Oxygen (%DO), and pH. After seven days, all treated containers had more surface cover than the control containers. All treatment groups reduced %TDS significantly more than the control group. Percent dissolved oxygen for all treatments decreased compared to the control, and continued decreasing until day seven, indicating that the microbes remained active until the last day of measurements. Both EM and MM solutions have a low pH, which may explain the higher acidity in treatment groups than control groups. EM concentrate performed best in the categories of sedimentation and smell. Therefore, addition of similar ratios of EM would best enhance grey water system efficacy and pleasantness, improving the quality of life of those who use them.

Tratamiento Biológico de Aguas Grises en Monteverde usando Microorganismos Efectivos y Microorganismos de Montaña

RESUMEN

Al igual que en muchas áreas rurales o menos desarrolladas estructuralmente, los propietarios de casas y negocios en Monteverde, Costa Rica filtran sus aguas grises a través de trampas de grasa o biojardineras o la liberan directamente en sus patios. Este estudio evalúa la eficacia de dos concentrados de Microorganismos Efectivos (EM) y Microorganismos de Montaña (MM) en la mejora del agua gris local. Estas soluciones contienen bacteria, levadura, y hongos beneficiosos. Recogí aguas grises de seis fuentes residenciales y comerciales, traté cada fuente con EM o MM en diferentes concentraciones y medí ciertos parámetros de calidad del agua de cada grupo durante siete días. Los parámetros incluyen cobertura de la superficie, sedimentación, estratificación, olor, porcentaje de sólidos totales disueltos (%TDS), oxígeno disuelto (%DO) y pH. Después de siete días, todos los recipientes tratados tenían más cobertura de superficie que los contenedores del control. Todos los tratamientos redujeron %TDS significativamente más que el grupo control. El porcentaje de oxígeno disuelto para todos los

tratamientos disminuyó en comparación con el control hasta el último día, lo que indica que los microorganismos todavía estaban activos el último día de mediciones. Las soluciones EM y MM tienen un pH bajo, lo cual pudo haber causado que los tratamientos fueran más ácidos que el control. El concentrado EM se obtuvo los mejores resultados en las categorías de sedimentación y olor. Por lo tanto, la adición de proporciones similares de EM mejoraría la eficacia de sistema de aguas grises, mejorando la calidad de vida de quienes lo aplican.

Grey water from businesses and houses includes wastewater from showers, sinks, and laundry systems, but does not include toilet water or water from animal enclosures. It contains contaminants and nutrients that can disrupt the balance of ecosystems and threaten the safety of waterways for human use. Contaminants include heavy metals that poison many aquatic species, while disruptions of nutrient balance (usually an excess of nitrogen and phosphorus) in aquatic ecosystems can facilitate excessive growth of some aquatic weed and algal species (Sheffield, 1968).

Natural processes deal with waste material in an efficient way. Biological treatment and bioremediation employ microorganisms to degrade or transform pollutants, while phytoremediation uses plants for the same purpose. Both methods offer environmentally sustainable and economically viable methods of cleaning water and soil. Other methods, such as chemical treatments, often harm the environment or prove expensive (Wenzel, 2009). Effective microorganisms (EM) may help to improve grey water quality via bioremediation. EM include fungi, bacteria, and yeasts grown from a simple mix of a commercially produced mother culture or whey (the source of the microbes) and molasses (a food source) (Zakaria et al., 2010). They are primarily used to fertilize crops or improve soil and water quality (Park et al., 2016). Microbes in EM enhance water quality by fixing nitrogen, decomposing organic wastes, reducing pathogen count, breaking down toxins, and/or recycling and solubilizing nutrients. Dr. Teruo Higa introduced EM to improve agricultural sustainability in Japan in 1980, and it has since improved many aspects of sanitation and organic farming. In comparison, mountain microorganisms (a type of EM) come from natural systems (with microbes sourced from leaf litter instead of whey), but will also cultivate in a mix of rice flour, molasses, leaf litter, brown charcoal, and water. Mountain microorganisms most commonly serve as compost accelerators (Campo-Martinez et al., 2016).

In Monteverde, Costa Rica, and other similarly developed areas, many homeowners and businesses run their gray water through biojardineras or grease traps, which separate the grease and sediment layers (producing cleaner water), or release the water directly into their yards. A few already add EM to their tanks. EM may be able to aid the sanitation of these tanks by breaking down grease layers, increasing sedimentation, reducing smell, and decreasing percent Total Dissolved Solids, or TDS, which includes organic matter and inorganic matter (most notably salts). Decreasing TDS increases water quality because high levels of TDS cause some organisms to uptake harmful chemicals (Chapman and McPherson, 2015). EM also influences Dissolved Oxygen (DO), which affects waterways because water with higher %DO supports greater biodiversity and positively influences biogeochemical element cycling (Huang et al, 2017).

This study evaluated the efficacy of EM and MM biological treatment of grey water in Monteverde. To accomplish this, I tested the efficacy of concentrated and diluted EM and MM in

improving the quality of water from four household grey water sources and two commercial kitchen grey water sources. The study tracked changes in the water through the metrics of surface coverage (grease or colony formation), sedimentation, smell, and total dissolved solids. I assumed that most noteworthy changes in water metrics would occur within a one week evaluation period due to depletion of resources by the microbes. The efficacy of these methods is directly applicable to Monteverde residents and businesses as it may allow them to improve their own water filtering and sanitation systems. Improved gray water systems would also release less contaminants into streams, mitigating pollution related harm to ecosystem functions such as oxygen cycling.

MATERIALS AND METHODS

To compare the efficacy of EM and MM, I studied gray water from households and commercial buildings in Monteverde and Cerro Plano in Puntarenas Province, Costa Rica. I collected 3 L samples of water from four household grey water sources and two commercial grey water sources in Monteverde. I separated 200 mL samples into cups at the Instituto Monteverde, then added 5 mL of two concentrations of EM and MM (concentrate and 10x dilution), for a total volume of 205 mL in each cup. Concentrate solutions consisted of EM to water ratios of 1:40, while dilution cup ratios were 1:409. Each group of treatment and source had three replicates. Additionally, two control cups per source received 5 mL of distilled water in place of EM mix (Table 1 and Figures 1 and 2).

Table 1. Treatments and grey water source groups.

	Source 1	Source 2	Source 3	Source 4	Source 5	Source 6
EM						
Concentrate	3 cups	3 cups	3 cups	3 cups	3 cups	3 cups
EM Dilution	3 cups	3 cups	3 cups	3 cups	3 cups	3 cups
MM						
Concentrate	3 cups	3 cups	3 cups	3 cups	3 cups	3 cups
MM Dilution	3 cups	3 cups	3 cups	3 cups	3 cups	3 cups
Control	2 cups	2 cups	2 cups	2 cups	2 cups	2 cups

My ratio of EM to gray water (1:40) was much higher than that recommended by commercial EM producers (such as one part EM per 1,000 parts grey water per month (www.teraganix.com, 2017)) because the EM in this experiment (made locally by Justin Welch) was likely much less concentrated than industrially produced EM. Additionally, Monteverde residents and companies typically use ratios that are similar to mine in their grease traps (J. Welch, pers. comm.). The cups had limited access to oxygen or sunlight (removal of cup covers once daily to record water metrics inevitably introduced oxygen) because this mimicked the wastewater environments likely to benefit from EM (most notably grease traps).



Figures 1 and 2: Experimental setup in the Fox-Maple Room at the Instituto Monteverde.

After setting up the experiment, I recorded the metrics of percent water surface coverage, sedimentation surface area, sediment layering, smell, total dissolved solids (%TDS), dissolved oxygen (%DO), and pH. I found %TDS, %DO, and pH values using a YSI Water Sampling Device. I tested the changes in water metrics in the cups once daily for seven days (22 November 2017-28 November 2017). I visually approximated percent surface coverage (grease, colonies, or other material), sedimentation surface area, and layer formation of each cup. I defined a layer as sedimentation that covered 100% of the cup bottom and was at least 2 mm thick. A significant decrease in grease and %TDS, and increase in sedimentation/layering in comparison to the control cups would indicate that the EM had successfully facilitated removal of contaminants from the waste water. The extent to which EM can improve smell will help establish its worth as a water treatment, as people are more willing to adopt changes which increase the pleasantness of their grey water systems. I measured smell using a personalized index with four levels: (1) unbearable (prompted gag reflex), (2) unpleasant, (3) undetectable/neutral, and (4) pleasant.

I ran an ANOVA to evaluate the significance of the percent change in %TDS from day one to day seven within each treatment group. To evaluate whether surface coverage and sedimentation developed significantly from day one to day seven, I evaluated changes in these metrics with a paired t-test.

RESULTS

EM concentrate produced the best smell and highest sedimentation. All EM and MM treatments had significantly lower %TDS than the control, but the control had the lowest surface coverage. Percent DO and pH were not constructive metrics due to microbial use of DO and the high acidity of EM and MM solutions.

Surface Coverage

Almost all source/treatment groups showed increases in surface cover from day one to day seven, although this increase varied in uniformity and intensity by source and treatment. The control groups showed the least increase, and EM concentrate groups ended with the highest combined surface cover (Figure 3). This surface cover represented both grease and colony formation, as by day seven, 10 out of 30 groups contained visible white surface colonies. The control surface cover did not yield a significant difference from day one to day seven, but all four treatment groups produced highly significant differences. (Table 2).

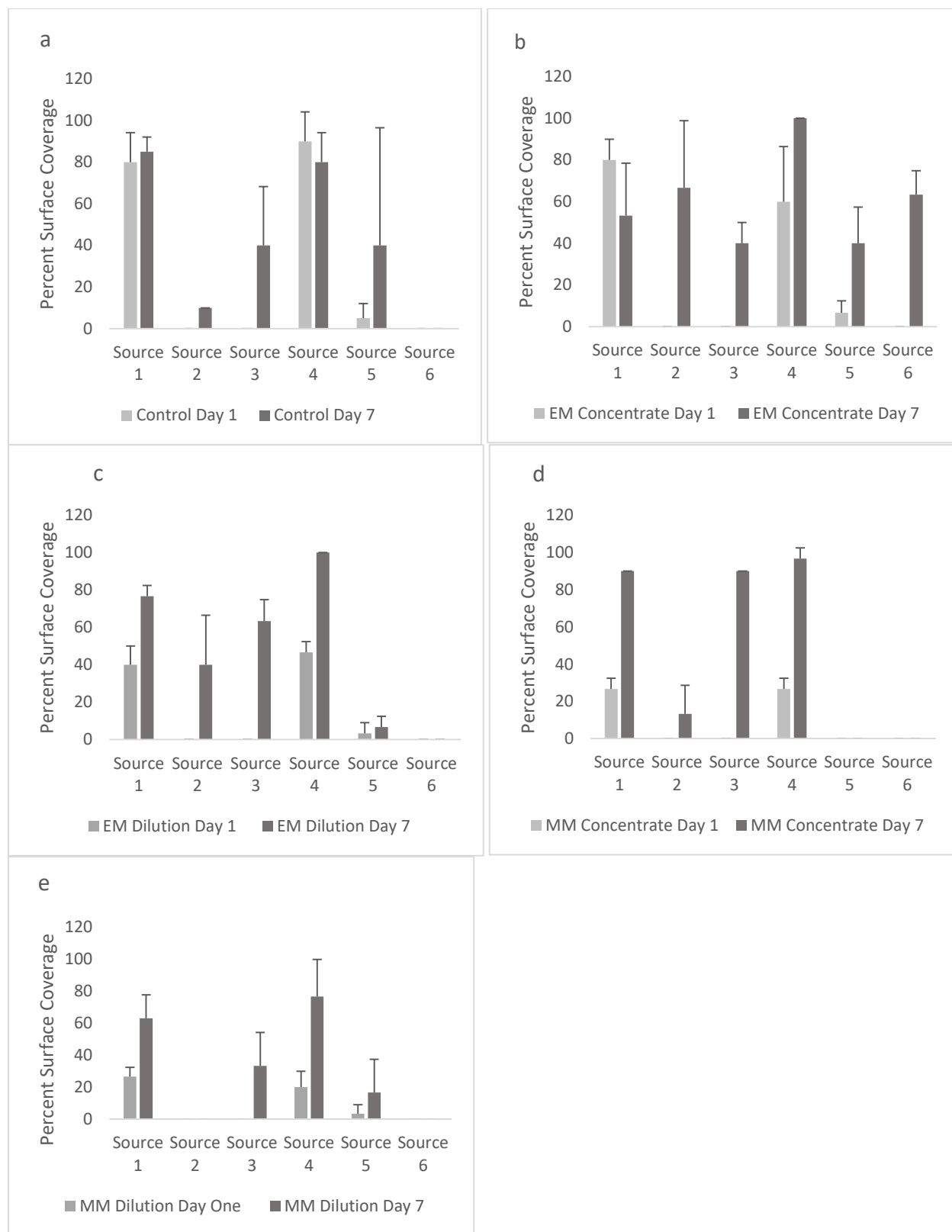


Figure 3. Differences in percent surface cover from day 1 to day 7 for all treatments a. *Control*. b. *EM Concentrate*. c. *EM Dilution*. d. *MM Concentrate*. e. *MM Dilution*.

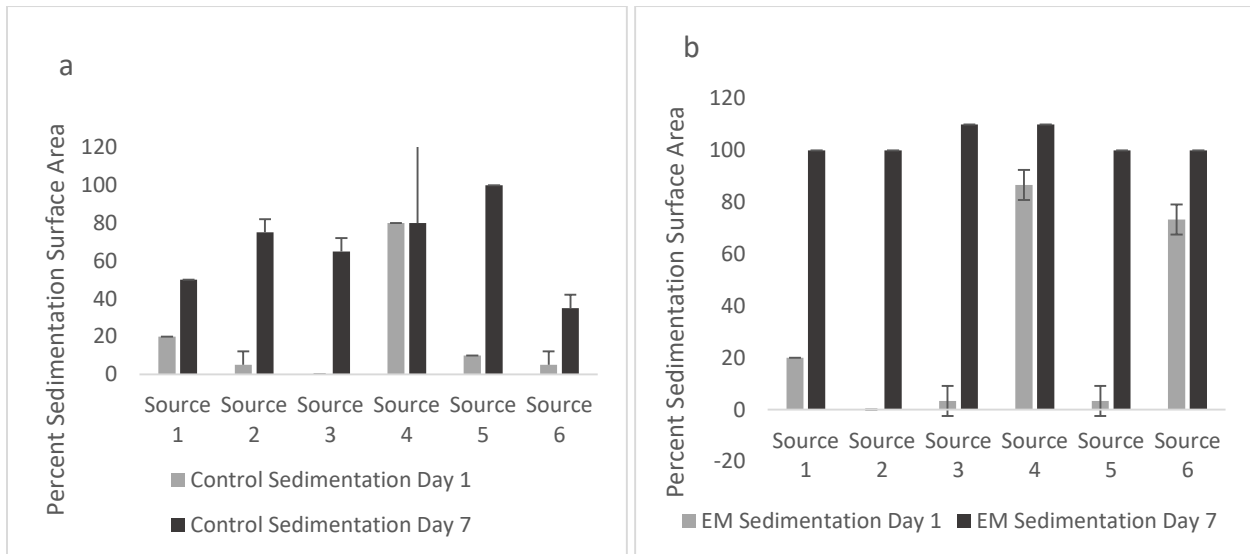
In Figure 3, “Source” refers to the house or business from which I sourced the grey water. Grey bars represent percentages on day one, while black bars represent percentages on day seven. On average, each group increased in surface cover by day seven.

Table 2. Treatment, t-value, and p-value for significance of surface cover change per treatment from day one to day seven. The change in surface cover in control groups was not significant.

Treatment	t_{17}	p
EM Concentrate	4.23	< 0.0001
EM Dilution	5.24	< 0.0001
MM Concentrate	4.43	0.0004
MM Dilution	3.82	0.0014

Sedimentation and Layering

Similarly to surface coverage, sedimentation increased in most (25 of 30) groups. Control groups produced the least sedimentation, followed by MM dilution groups. EM concentrate showed the greatest and most consistent sedimentation (followed by MM concentrate), and also led to the most layer formation (Figure 4). All treatments and control cups accumulated significant amounts of sediment by day seven as compared to day one (Table 3).



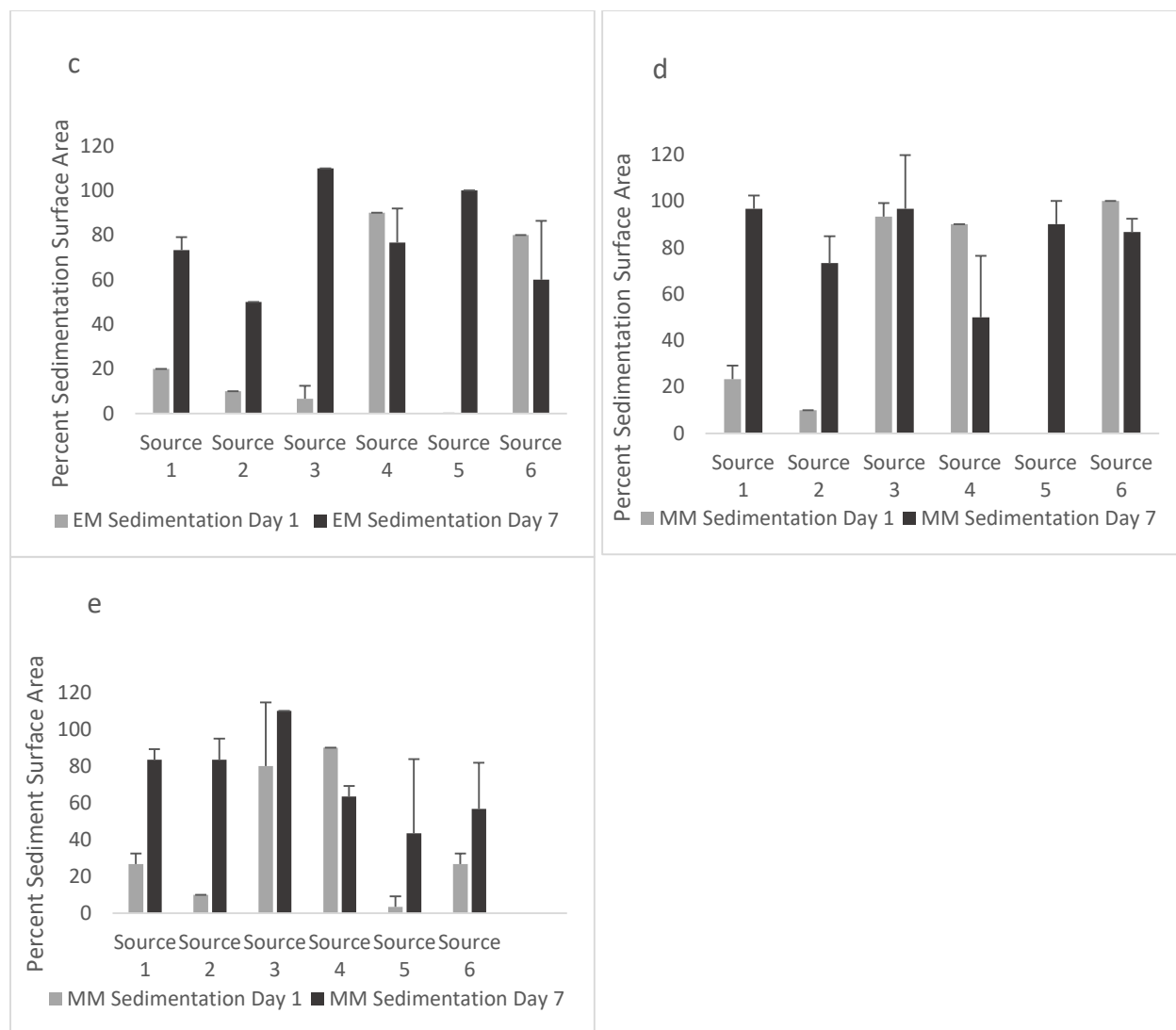


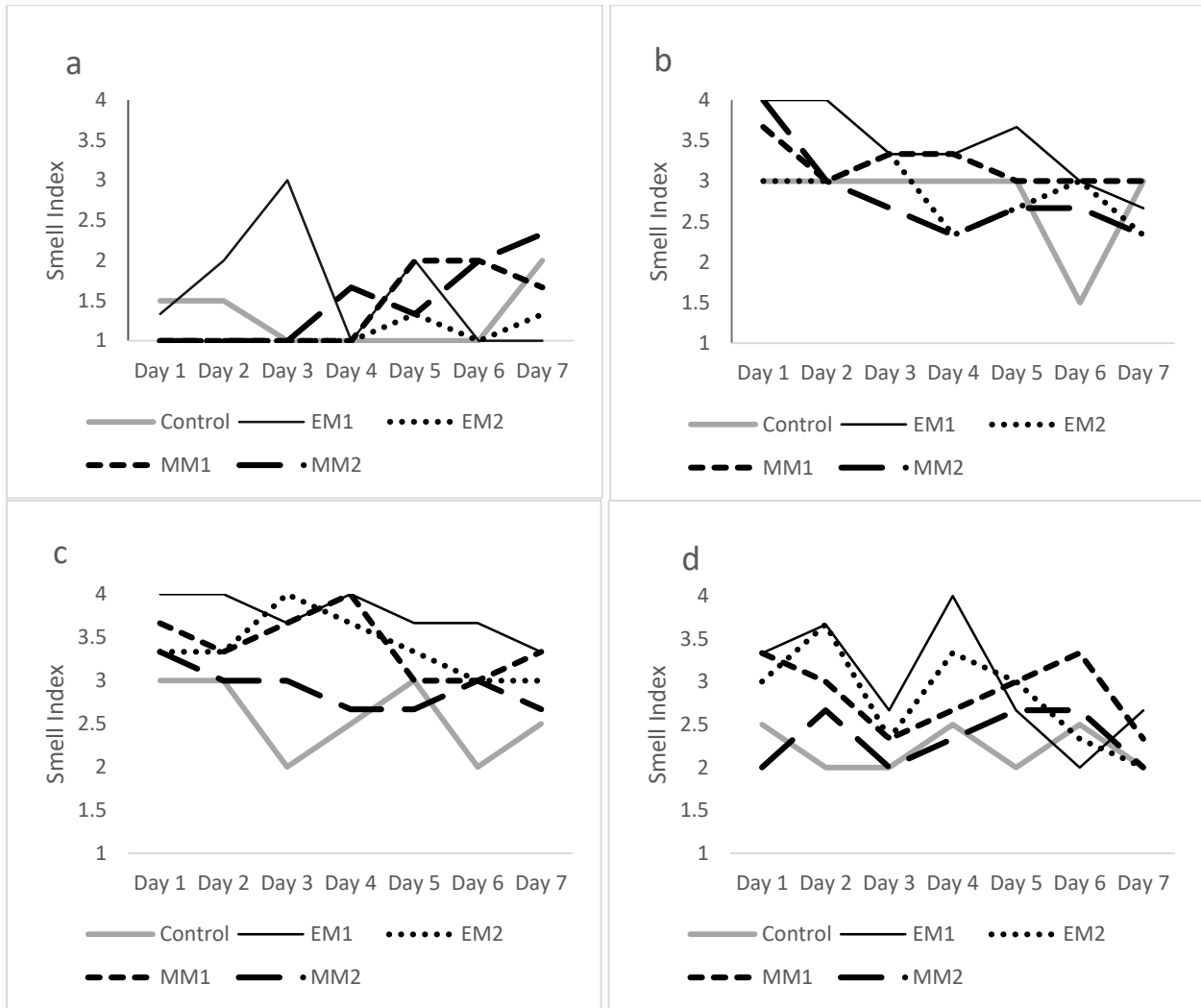
Figure 4. Differences in sedimentation surface area for each treatment from day 1 to day 7 for all treatments. a. *Control*. b. *EM Concentrate*. c. *EM Dilution*. d. *MM Concentrate*. e. *MM Dilution*. “Source” refers to the house or business from which I sourced the grey water. Grey bars represent percentages on day one, while black bars represent percentages on day seven. Sedimentation that formed a layer (> 2 mm) is represented as 110% sedimentation. On average, each group increased in sedimentation by day seven.

Table 3. Treatment, t-value, and p-value for significance of sedimentation change per group from day one to day seven. Changes in all groups were significant.

Treatment	t_{17}	p
Control	4.78	0.0006
EM Concentrate	8.61	< 0.0001
EM Dilution	3.64	0.0020
MM Concentrate	2.40	0.0279
MM Dilution	3.76	0.0016

Smell

For most sources, groups with EM concentrate ranked highest on the index for the most days, followed by MM concentrate. Control ranked lowest on the most days (Figure 5).



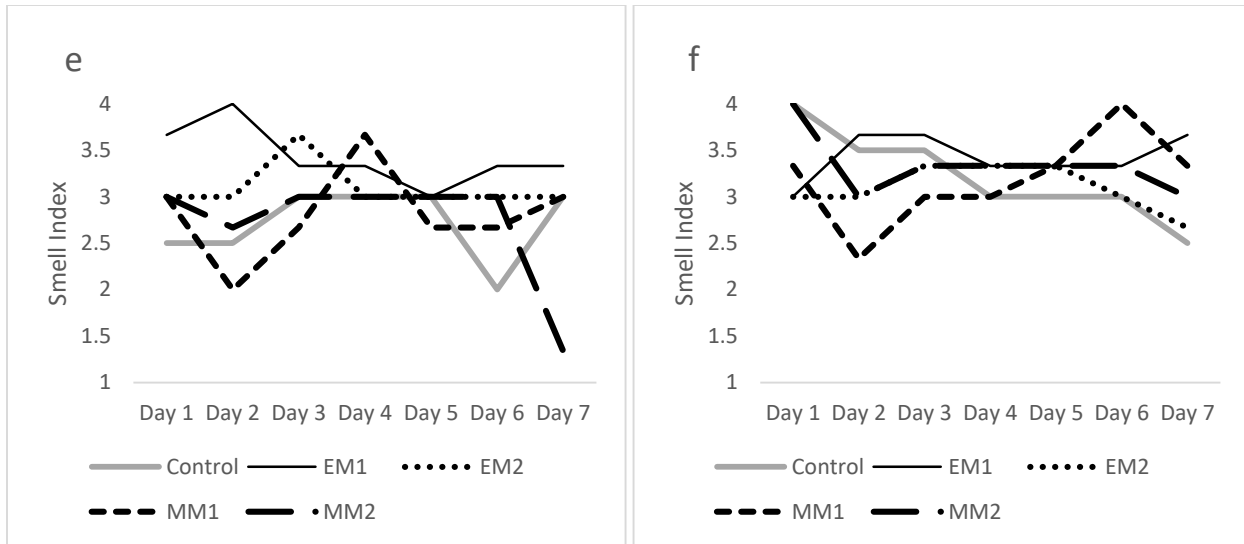


Figure 5. Graphs of source smell over time. a. **Source 1** b. **Source 2** c. **Source 3** d. **Source 4** e. **Source 5.** f. **Source 6.** The y-axis values are from a personalized smell index: (1) unbearable (prompted gag reflex), (2) unpleasant, (3) undetectable/neutral, and (4) pleasant.

Total Dissolved Solids

All four treatments prompted a significant reduction in %TDS compared to the control. Differences between treatment groups were not significant.

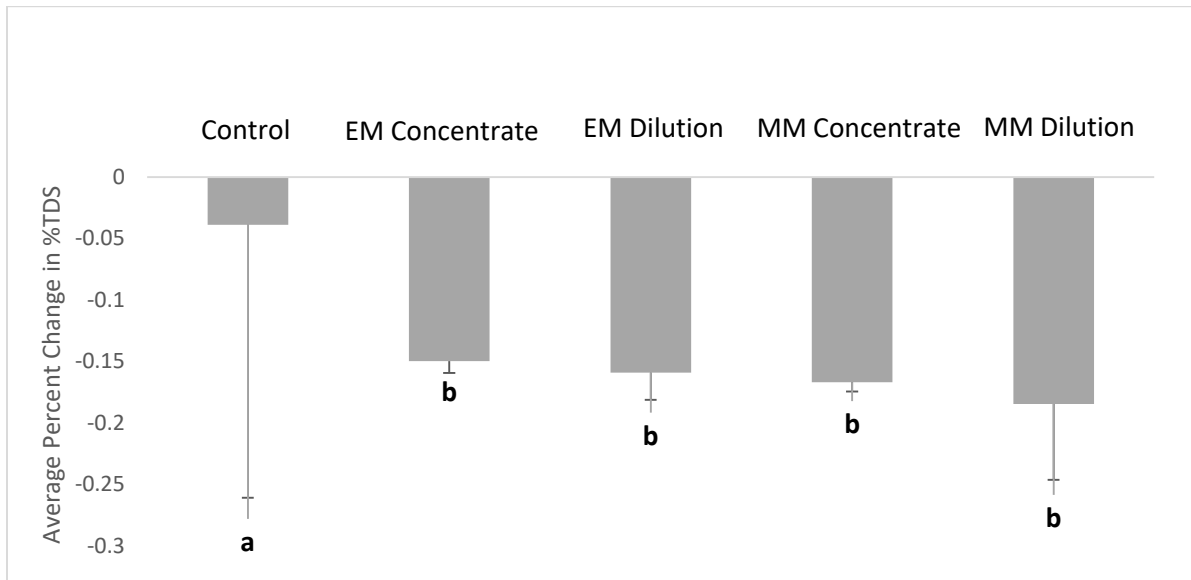


Figure 6. Average percent decrease from day one to day seven in %TDS by treatment or control group. Different letters (**a** and **b**) denote significant differences (Figure 17, $F_{(4, 79)} = 5.6, p < .0005$).

pH

The changes in pH over time were not significant. Values ranged from 4.5-6, with the control group having the highest average reading and the MM concentrate group typically producing the lowest reading. EM concentrate solutions changed the most, moving from 5.5 to 4.5.

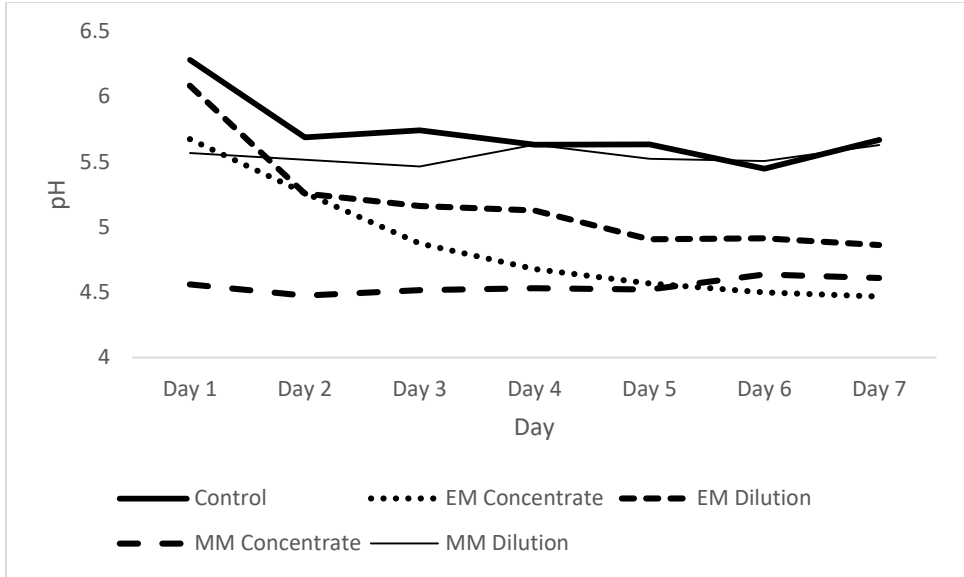


Figure 7. Average pH of control and treatment groups over time. Each value is an average of pH values for a given day within a treatment or control group containing grey water from each source.

Percent Dissolved Oxygen

Average %DO decreased gradually in each treatment group. MM dilution groups decreased the most, falling an average of 17%, while the control group decreased the least, falling five percent. The control group also maintained the highest %DO for five of seven days.

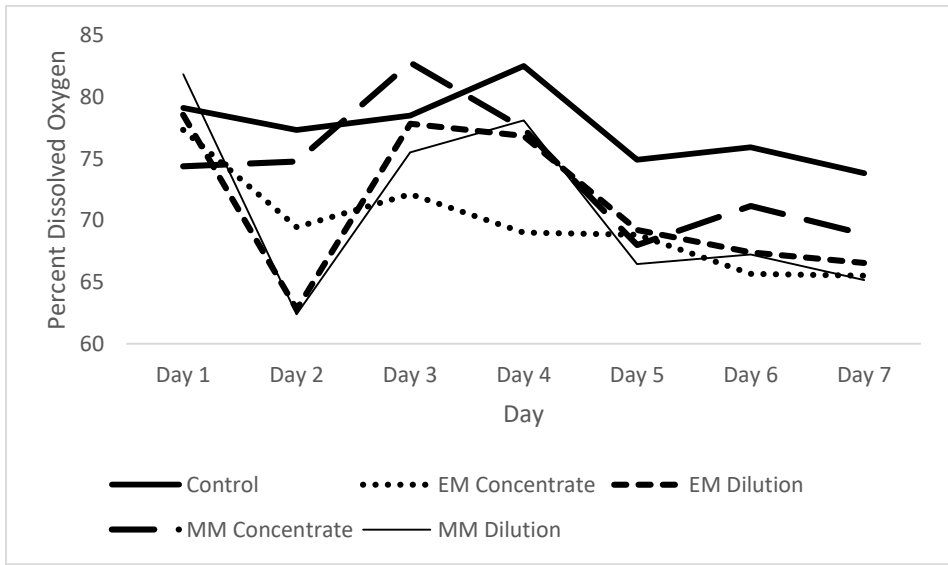


Figure 8. Average %DO of all grey water sources in a treatment group over time. Each value is an average of %DO values for a given day within a treatment or control group containing water from each grey water source.

DISCUSSION

Surface Coverage

Surface cover increased in the vast majority of study groups, decreasing in only 6.6%, or two of 30 source/treatment groups, which I did not predict. Control groups showed the least increase (Figure 3). Some surface material was grease, although 10 out of 30 groups eventually showed visible evidence of bacterial colony formation. Microbes in EM (around 80 species) effectively break down organic matter (Zakaria et al., 2010). Therefore, slight increases in treatment group surface cover (compared to control groups) likely stem more from microbial growth than grease accumulation, and do not represent a substantial drawback to adding EM and MM in grey water systems. Future studies could classify surface microbes in similar systems or analyze the chemical makeup of surface accumulate in order to determine what percent is attributable to grease.

Error in data collection for this metric may have stemmed from surface accumulate remaining on the YSI probe during its removal, causing artificial decrease in surface cover. I visually estimated the surface cover, which may have caused additional error. Although my visual approximation was not quantitative in absolute terms and may have introduced some systematic error, I believe that the relative scale is useful.

Sedimentation and Layering

Sedimentation increased in 25 of 30 groups after seven days, with control groups producing the least average sedimentation and EM producing the most (Figure 4). All treatments and the control accumulated significantly higher averages of sediment after seven days (Table 3). EM encourages sedimentation due to lactic acid bacteria (including *Lactobacillus plantarum*, *Lactobacillus casei* and *Streptococcus lactis*), which secrete organic acids and other antioxidants. These substances aid in solid-liquid separation (Zakaria et al., 2010). Additionally, EM influences the cycling of nitrogen and phosphorus, which contribute significant mass to sediment (Wididana).

As in surface cover evaluation, visual approximation of sedimentation and layering may have caused slight error.

Smell

Groups with EM concentrate ranked highest (best smelling) on the smell index for the most days as compared to other treatments for a given grey water source. MM concentrate ranked second best, and control groups smelled the worst (Figure 5). Odor reduction was one of EM concentrate's original purposes (management of odors caused by ammonia, hydrogen sulfide, and methane) (Zakaria et al., 2010). However, both EM and MM contain molasses, which smells sweet, and may have contributed to better smelling solutions more than microbes did. Therefore, the dilution treatment groups likely smelled worse than the concentration treatment groups partly because they contained less molasses. Likewise, absence of molasses in the control groups may have caused their less pleasant smell.

Total Dissolved Solids

All four treatment groups lowered %TDS significantly, as compared to the control group, although none of the treatment groups differed significantly from each other (Figure 6). Decreasing TDS increases water quality because high levels of TDS cause some organisms to uptake harmful chemicals (Chapman and McPherson, 2015). EM is especially effective at reducing dissolved nitrogen and phosphorus content (reducing %TDS and increasing water quality), possibly due to microbial uptake of these elements (Okuda and Higa). Some EM microbe species can even detoxify and/or precipitate metals (Monica et al., 2010). MM may have performed equally well as EM under this metric because MM microbes are selected largely for their ability to decompose organic matter (making them better suited for agricultural purposes such as compost acceleration) (Castellano et al., 2015). Furthermore, MM also fix nitrogen (Garnett, 2015), and therefore help lower levels of ammonium, nitrates and nitrites in water (Campo-Martinez et al., 2014).

pH

The pH measurements did not exhibit significant variation over time (Figure 7). In most natural systems, neutral pH correlates with higher water quality and better ecosystem function (Mosley et al., 2014). However, due to the acidity of the EM solutions, changes in pH do not accurately reflect EM efficacy. Concentrate EM mixtures have low pH values (around 3.5-4 (Zakaria et al., 2010)), so the initial acidification caused by addition of EM may have caused low pH for the remainder of the experiment. EM can still prove effective in initially basic systems by lowering pH to more neutral conditions (Okuda and Higa).

Percent Dissolved Oxygen

Average %DO decreased gradually in each treatment group (Figure 8). Percent DO did not serve as a quality metric of EM efficacy, as microbial metabolisms uptake oxygen, so EM would reduce %DO in a short-term closed environment (Park, et al., 2016). Instead, DO flux monitored the relative activity of the EM over time, and suggested that the microbes were active until the last day.

Conclusion

As the EM concentrate treatment group lowered %TDS and scored highest in the sedimentation and smell metrics, I would recommend addition of similar ratios of EM to grease traps, biojardineras and other grey water filtration systems. EM has the potential to improve grey water sedimentation, smell, and %TDS in these systems, making them more sanitary and pleasant and improving the quality of life of those who use them.

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