UREAP Final Report:

Using compost to reduce cardboard waste and exploring the relationships microbes play

By

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Abstract:

This project aims to reduce cardboard waste through composting throughout the City of Kamloops. Partnering with the City of Kamloops, this project will investigate if cardboard has any impact on compost microbial diversity. In Canada, large quantities of cardboard end up in landfills and are responsible for an increase in greenhouse gas emissions. Recycling cardboard is also an energy and water-intensive process. Therefore, composting cardboard could be an alternative way to reuse cardboard. Composting also has beneficial agricultural impacts and the microbial community in the compost can aid in crop yield. The methods to compare the microbial diversity in compost containing cardboard and compost without, will be observed through microbiology culturing techniques like spread plating, plate counting, and Gram staining. Additionally, 16S rRNA amplicon sequencing will be done to determine the microbial diversity between all compost samples. It is found that cardboard increases diversity and the microbial community is different from compost without cardboard. A chemical analysis using ICP-MS will be done on cardboard and compost to determine if the elements found in them have an impact on the microbes present.

Introduction:

Composting is a natural process of recycling nutrients back into the soil that involves microbes like bacteria and fungi that decompose organic matter from food and plant waste and turn it into a usable fertilizer.

Composting improves soil nutrient retention, creating better soil conditions for agricultural purposes. Additionally, the soil microbiome can also increase soil conditions and fertility (4). Compost with an increased microbial community composition balances the soil ecosystem and improves the health of the soil and plants (10). Many microbes share a symbiotic relationship with plants and aid in plant growth and yield, nutrient uptake and nutrient cycling. Therefore, having more bacterial and fungal diversity within the compost results in a preferable compost for agricultural use (10).

There are three phases of composting, the mesophilic, thermophilic, and maturation phase. During the mesophilic phase, temperature and CO_2 increase and the degradation of sugars and proteins begins (8). In the second phase, the thermophilic phase, the temperature increases from about 45-70°C, and at about 50°C thermophilic bacteria take over (8). This high heat kills off most pathogens and is an optimal range for microbial activity, which is why it is also referred to as the ‘active phase’ (9). In the third phase, the maturation phase, also known as the ‘curing phase’, the temperature decreases to about 37°C and there is low oxygen consumption (9). In this phase, organic materials continue to decompose for some time until the compost is mature (9). A longer curing phase is especially important when toxic organic acids and other resistant compounds are present so they can be stabilized during this phase (9). Having a longer curing phase could be an important aspect in decreasing the effect of toxins found in cardboard.

The overarching goal of this project is to tackle the issue of cardboard recycling. In Canada, 85% of old corrugated boxes are recycled, however, this still results in a high percentage that ends up in Canadian landfills (11). The cardboard that is recycled goes through the recycling process which requires large amounts of water by using a hydrapulper to break down the material (12). Environment Canada stated that it takes about 324L of water to produce 1kg of paper (2). Using cardboard as a bulking agent in compost can help reduce the cardboard that ends up in landfills and reduce the water consumption required in the recycling process.

Additionally, cardboard that ends up in landfills contributes to the large amount of greenhouse gas emissions released into the atmosphere. In Canada, methane gas from landfill contributes to 20% of the national methane emissions (5). Greenhouse gas emissions are an important concern that must be regulated to reduce the impacts of climate change.

Cardboard is an important element to this project and there are two main types of cardboard. The first is corrugated cardboard which is thicker and has an additional wavy fiber. In households, corrugated cardboard is typically items like shipping or packing boxes. The second type is paperboard or chipboard cardboard, which is made up of thin short recycled fibers and are typically items like cereal boxes and pop cases (3). This project intends to test both corrugated and paperboard cardboard because they are both common types of cardboard found in most residential households.

Another focus for the type of cardboard will be looking to see if cardboard with heavy dyes, glue, and glossy finishes have any impact on the microbial community. The glue used for corrugated cardboard is starch glue which is biodegradable and environmentally friendly (13). However, little research has been done to see how microbes interact with other toxins found in cardboard like diisobutyl phthalate which is a common chemical found in cardboard printing ink

(1). Cardboard itself commonly has heavy metals like zinc, lead, cadmium, and chromium which are used in production (6). The common printing ink contains barium, copper, and zinc (14), as well as it has been found that high amounts of lead, cadmium, chromium, copper, nickel, and mercury are found in the ink used on cardboard (6). This project aims to study if the toxic heavy metals have a negative impact on microbial diversity or if they are beneficial to microbial metabolism.

In collaboration with the City of Kamloops, our goal of reducing cardboard waste and creating a compost product that is beneficial for agricultural use can be achieved during this project. The City of Kamloops is funding this project through the 2024 Climate Action Grant and the TRU Sustainability Research Grant. Through the City of Kamloops Residential Waste Collection Program, they are trying to shift the behavior of local residents and encourage them to compost their food waste but also their cardboard. This partnership with the community allows this research to be funded for laboratory supplies, and future DNA and RNA metagenomic sequencing and ICP-MS analysis, as well as, an opportunity to spread the word about the benefits of composting.

Methods:

Composting is done on the TRU campus up on the roof of the science building in five separate 18.5-gallon compost tumbler bins. Each bin has a single chamber where the pre-compost material is placed. A starter batch from Grassland Organics new composting facility in Kamloops was used to help mimic the microbial community already present on site. The stater batch contains a 1:1 ratio of municipality city food waste and bulking agent. The bulking agent

consists of soft wood chips to help with structure, and moisture and be used as a primary carbon source.

Figure 1. Grasslands Organics starter batch used as a base to set up the cardboard and control experiments.

Figure 2. Compost bins set up on the roof of TRU science building.

Six compost bins can be seen in Figure 2. One of the bins is being used for a different experiment, while the other five bins are exclusively for the current report.

Experimental Design

Five different experiments were set up in their own compost bin. The control groups consist of only the starter mixture provided by Grassland Organics. The other four experiments are looking at different types of cardboard. All cardboard types were cut into pieces about 1 cm wide and about 10-15 cm long, as seen in Figure 3. In one bin glossy pieces of cardboard were added to the mixture. In a separate compost bin, cardboard that contained ink/dye was added. In the fourth bin corrugated cardboard was added and in the fifth bin, compostable "Back to Earth Bags" were cut and added to its own compost bin. The four compost bins that contain the additional cardboard need more nitrogen to satisfy the preferred 25-35:1 carbon-nitrogen ratio for compost, therefore, more food waste was added to the four bins. The food waste was collected from the Culinary Arts program at TRU and consisted mainly of wet food waste. The recipe to make the compost is arranged in Table 1. and follows that C:N ratio percentage as seen in Table 2.

Figure 3. Four different types of cut and prepared cardboard for each separate compost experiment.

Table 1. Weight in pounds for the recipe to make the compost for each different cardboard type.

Cardboard type	Cardboard weight (lbs)	Compost starter batch weight (lbs)	Food waste weight (lbs)
Control (no cardboard)	0	0.46	0.29
Glossy	0.130	0.48	0.28
Ink/Dye	0.132	0.47	0.29
Corrugated	0.133	0.45	0.30
Back to Earth Bags	0.132	0.46	0.29

Table 2. "Carbon and Nitrogen Content of Common Compost Ingredients" table from Organic Gardening article to determine carbon and nitrogen percentage of compost materials.

Materials	% C	% N
Wood chips (compost starter)	40	0.1
Municipal city food waste (compost starter)	10	1.0
Cardboard	40	0.1
Additional food waste	10	1.0

To maintain composting, all five bins are rotated every three-four days to allow the contents to aerate and remain as an aerobic system. Additionally, sterilized water is sprayed on the compost if the mixture starts to become dry.

Cultural approach

Initial compost samples were collected for culturing techniques to observe the bacteria present in each compost trial. The samples were allowed to mature for about two weeks before

being collected. To analyses the bacteria present in the initial trials, serial dilutions were made and plated on LB agar. To create the serial dilutions, about 1 g of the compost sample is mixed with 99 mL of sterile water to create a slurry. 100µl of the stock slurry solution was transferred to an Eppendorf tube containing 900µl of sterile water. From this, 100µl of the diluted samples are added to the next Eppendorf tube and is repeated up until a $x10^{-5}$ dilution is obtained. Dilutions $x10^{-1}$, $x10^{-3}$ and $x10^{-5}$, are plated on the LB agar to create dilution of $x10^{-2}$, $x10^{-4}$ and $x10^{-6.}$ The hockey stick method is used to spread plate the bacteria and is then allowed to incubate at 25°C for 72 hours.

From the plates, bacterial colonies are observed in regards to the colour, size, shape, margins, and elevation to determine their macroscopic morphology. Based on these observations, different "species" of bacteria were found in each compost trial. The colonies were then isolated and subjected to Gram staining to determine the microscopic morphology of each isolated colony.

The results of the macroscopic and microscopic morphology observations were used for statistical tests, Simpson's diversity index, Shannon diversity index, and Bray Curtis dissimilarity to determine the microbial diversity between the two composting types.

Calculations for Carbon-Nitrogen Ratio

Compost Starter (0.44 lbs total)

- 0.22 lbs of wood chips
- 0.22 lbs of Municipal city food waste

Wood chips:

(0.22 lbs wood chips) x (40% C) = 0.088 lbs C

(0.22 lbs wood chips) x (0.1% N) = 0.00022 lbs N

Municipal city food waste:

(0.22 lbs municipal city food waste) x (10% C) = 0.022 lbs C

(0.22 lbs municipal city food waste) x (1.0% N) = 0.0022 lbs N

Cardboard (0.133 lbs)

(0.133 lbs cardboard) x (40% C) = 0.0532 lbs C

(0.133 lbs cardboard) x (0.1% N) = 0.000133 lbs N

Food waste (0.2866 lbs)

(0.2866 lbs food waste) x (10% C) = 0.02866 lbs C

(0.2866 lbs food waste) x (1.0% N) = 0.002866 lbs N

Total carbon value

0.088(wood chips) + 0.0022(municipal city food waste) + 0.0532(cardboard) + 0.002866(food waste) = **0.19186** lbs of carbon

Total nitrogen Value

0.00022(wood chips) + 0.022(municipal city food waste) + 0.000133(cardboard) + 0.02866(food waste) = **0.005419** lbs of nitrogen

Carbon-Nitrogen ratio

0.19186 lbs of carbon / 0.005419 lbs of nitrogen = 35

= **35:1** C:N ratio

Results:

After about a month of the compost maturing it is noticed that the cardboard is beginning to break down. The breakdown of the cardboard is a slow process however, you can see that the sides of the cardboard pieces are starting to fray. The Back-to-Earth bags show the most signs of degradation, the pieces are misshaped and wrinkly. The glossy cardboard shows the least amount of degradation compared to the other four cardboard types. Little fraying and wrinkling of the cardboard has occurred after one month of composting.

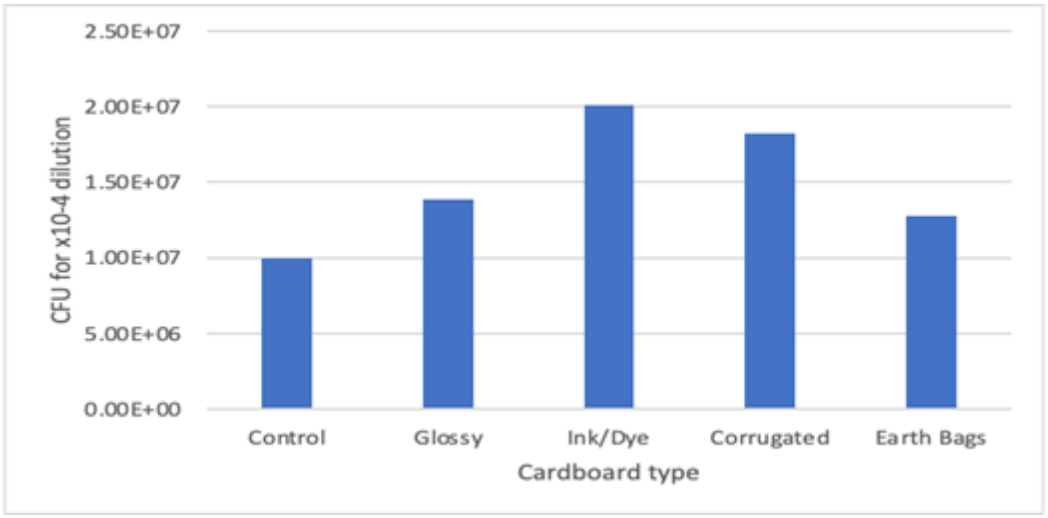

Figure 3. CFU for each initial compost trial to determine bacterial abundance.

From Figure 3., it is notable that the Ink/Dye trial has the most CFU (2.01x10+7), while the control has the least (1.00x10+7). This suggests that the Ink/Dye as well as all the other cardboard trials has a greater abundance of bacteria than the control trial without cardboard.

Table 3. Species richness and similarity of isolated colonies for each compost trial.

Compost trial	Number of different colonies	Number of the same colonies
Control	5	6
Glossy	4	4
Ink/Dye	3	4
Corrugates	3	4
Back-to-Earth bags	4	1

Table 4. Individual representation of Gram-positive vs Gram-negative bacteria for all compost trials.

Compost trial	Gram-positive percentage (%)	Gram negative percentage (%)
Control	45	55
Glossy	71	29
Ink/Dye	67	33
Corrugated	75	25
Back-to-Earth Bags	50	50

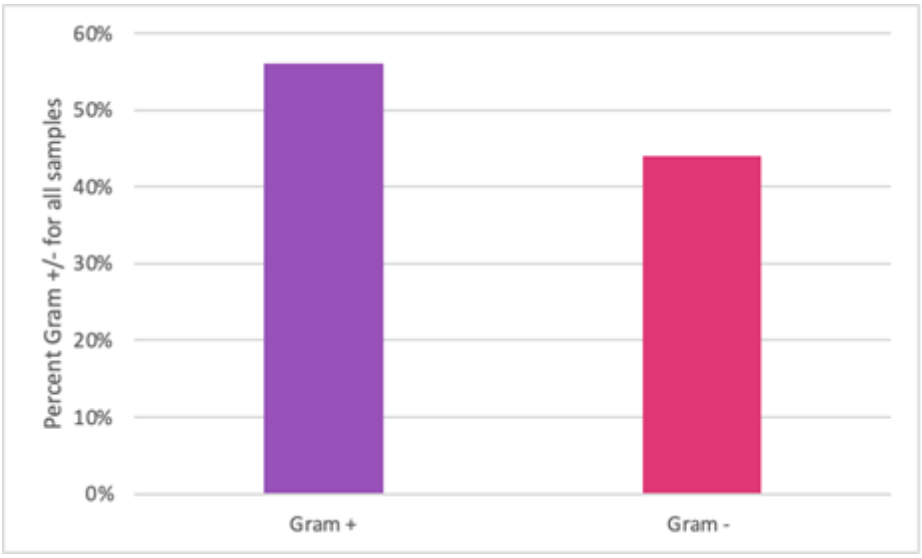

Figure 4. Combination of Gram-positive and Gram-negative bacteria in all initial (after two weeks of maturation) compost trials.

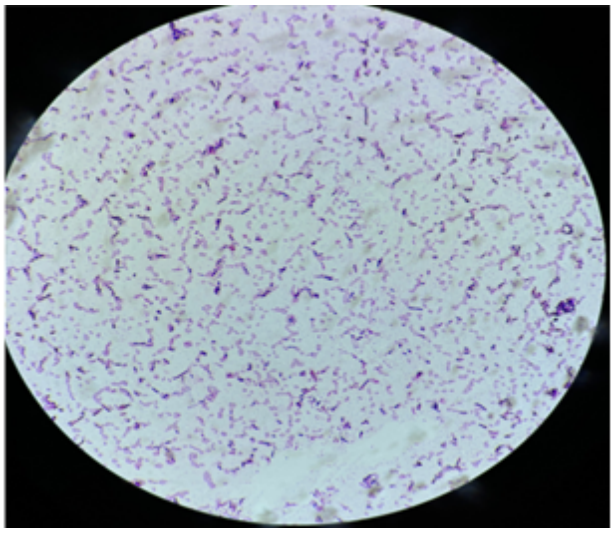

Figure 5. Gram-positive rod-shaped bacteria from control x10^{-4} dilution plate.

From the Gram-staining results, it is found that for all trials except for the control, Gram-positive bacteria are the most abundant for the first two weeks of maturation. These

Gram-positive bacteria are rod shaped and many of them contain endospores and some have even been released from the capsule. In Figure 5, the bacteria are labeled “F” and are found in all the compost trials and are the most abundant “species” overall. It is a Gram-positive rod-shaped bacterium, and the capsule can be seen in the background.

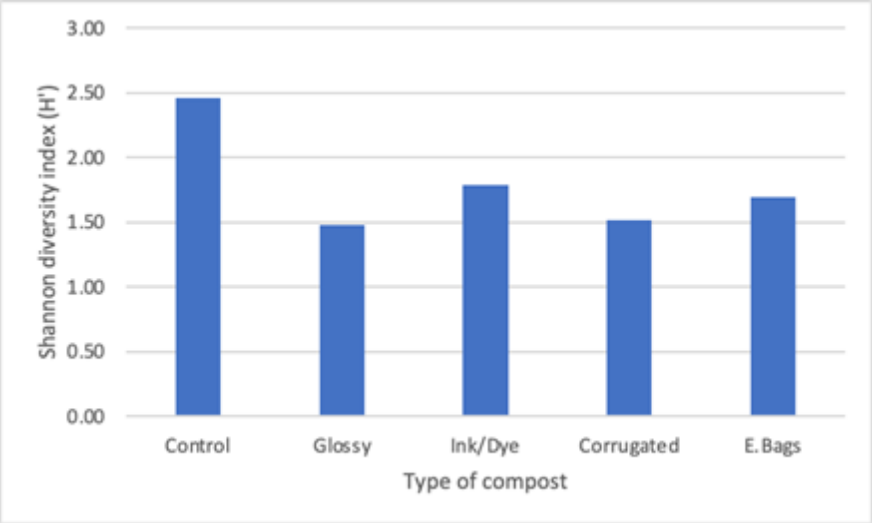

Figure 6. Shannon diversity index shows the diversity for all five compost trials.

Shannon diversity (H’) is a statistical analysis constant used to estimate species diversity within a potential microbial community. Shannon diversity takes into account the relative abundances of each population as well as community evenness. The lower the H’ value the more diversity there is within that community, while the higher the H’ value is the less diversity there is. Therefore, we can see in Figure 6., that the compost trial that contains the glossy cardboard has the most diversity compared to the other trials (H’=1.48) because of the lower H’ value. The control trial is the highest on the graph in Figure 6, with its H’=2.46, meaning that the control group has the least microbial diversity.

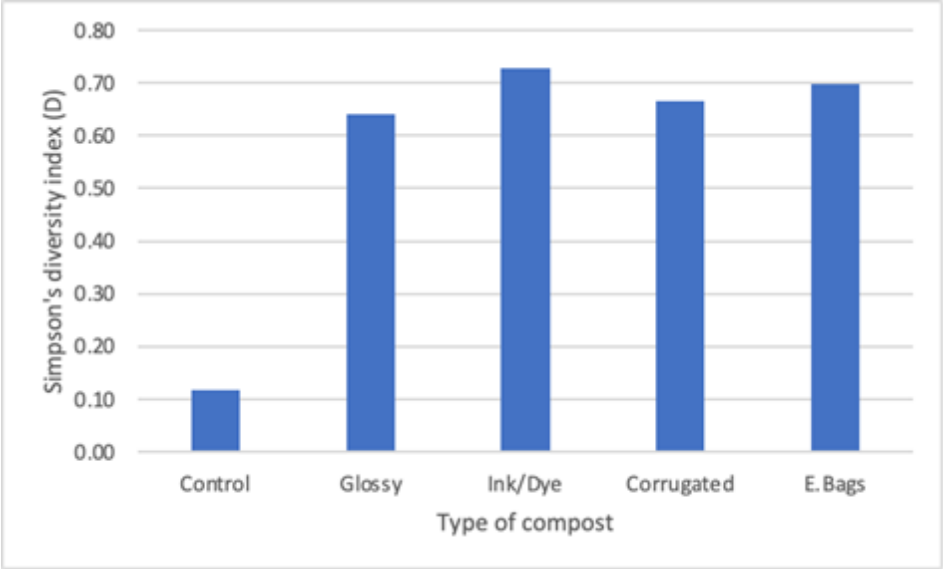

Figure 7. Simpson's diversity index showing the discovery for all five compost trials.

Similar to Shannon's diversity, Simpson's diversity index also measures microbial diversity in a community. Simpson's diversity takes into account richness (number of species present) and relative abundance. However, for Simpson's diversity, the higher the index, the more diverse the community is. In Figure 7., the Ink/Dye trial has the highest Simpson's diversity index (D=0.73) while the control trial has the lowest (D=0.12). These results indicate that based off the Simpson's diversity index, the Ink/Dye trial has the most diversity, while the control trial has the least.

Table. 5 Site by species matrix showing the Bray Curtis Dissimilarity between all five compost trials.

	Control	Glossy	Ink/Dye	Corrugated	Back-to-Earth bags
Control	X	0.54	0.59	0.60	0.65
Glossy	0.54	X	0.49	0.59	0.46
Ink/Dye	0.59	0.49	X	0.39	0.38
Corrugated	0.60	0.59	0.39	X	0.26
Back-to-Earth bags	0.65	0.46	0.38	0.26	X

The site-by-species matrix compares each compost trial to one another to determine if the Bray Curtis Dissimilarity (BCd) value is close to 1, meaning that the two sites are different. The Bray Curtis Dissimilarity range values range from 0.26-0.65. When comparing the control trial to the Back-to-Earth bags, the BCd highest value is the highest compared to all the others. Also, along the control column, all the BCd values are higher than the other comparisons.

Discussion:

The microbial compositions found in compost are quite abundant and diverse. It is found that cardboard does affect microbial diversity. The abundance of bacteria (CFU) and diversity (H', D and BCd) differ depending on the type of cardboard used. When looking at the Shannon diversity index (H') the Glossy cardboard is the most diverse group, however, Simpson's diversity index (D) shows that the Ink/Dye cardboard trial.

The discrepancies between the most microbial diverse trials does cause some concern with the overall results of the diversity experiments. However, these results do not need to be neglective. The two diversity indexes, both Shannon and Simpson, show that the control group,

without any cardboard, has the lowest microbial diversity compared to all groups that contain some type of cardboard. This implies that the addition of cardboard to compost, no matter the type of cardboard, is beneficial to increasing microbial diversity. In addition, the Bray Curtis dissimilarity value shows that the control group is far more dissimilar to the cardboard trials in regard to microbial community composition The cardboard trials have lower BCd values meaning they are more similar to each other regarding their microbial community. The similarity between cardboard trials is a logical reasoning behind the discrepancies between the most microbial diverse trials based on Shannon and Simpson's diversity indexes.

Having compost that contains cardboard is not detrimental to the microbial community, instead it increases microbial diversity, which in turn, creates a better compost. With this knowledge, the City of Kamloops can continue their initiative of recommending residents to add cardboard to their compost bins. The addition of cardboard to compost helps reduce cardboard waste in local landfills and conserve water that is needed to recycle it. If composting cardboard can be implemented in Kamloops it will be a more economic and sustainable choice for the city.

Future Work:

To continue this experiment additional work still needs to be done to analyze if the variation in the different types of cardboard is significant enough. The significance between cardboard type microbial diversity will determine if the toxic inks, dyes, and finishes have an impact on microbial diversity in compost. More data will be collected as the compost continues to mature. Not only will macroscopic and microscopic morphologies be studied but so will metagenomics and 16S rRNA sequencing identification of each compost trial. 16S rRNA and metagenomic sequencing will be done by UBC to get a more accurate understanding of the

community relationships. ICP-MS will also be done to determine the elements found in the cardboard as well as in the compost. The results of the ICP-MS can help clarify the types of heavy metals present and allow for a better understanding if the heavy metals present are decreasing microbial diversity or potentially increasing the abundance of certain species.

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