Identifying Microbes and Plastic Properties in Purchased and Recycled Clay: An Integrative Approach to STEAM-ing up STEM

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Introduction

The world of microbial species in ceramic clay is one that remains a final frontier and is shrouded in mystery and questions. We researched the microbial community of new and recycled clay and explored if clays with different microbial communities had different plasticity's. Additionally, building a relationship between the science and art community, specifically, a relationship between Laramie High School art students and undergraduate microbiologists at the University of Wyoming. The relationship that we built will encourage the transfer of skills and knowledge, and ultimately allow for an educational system rich with an array of multi-disciplines.

Past studies have explored this notion of the impact that microbes have on the plasticity of clay. This was explored by V. Uza, H. Ozdag, S. Ilhanc, A. Ceylana and I. Isika, in a study where they investigated the impact that microorganisms had on the plasticity of "roof-tile making clay"(2010)[1]. In an additional study, The Effect of Microbial Contamination upon the Plasticity of Ceramic Clays, the idea of how microbial species impact the plasticity of clay was further propagated, but this study delved into ceramic clay. The results, however, were similar: clay samples that were aged with bacteria were found to be more pliable and have a higher rate of plasticity (Henrikson and Fleming, 2011)[2].
Although these studies shed light on the world of microbial species in clay, many questions still remain.

After reviewing relevant literature we visited the 3D art studio at Laramie High School where the students informed us that they noticed a difference in plasticity between their recycled clay and the newly purchased clay. Their questions and the questions raised by the literature drove us to research the possible relationship between microbial communities in the clay and the clay's plasticity.

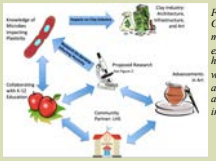

Figure 1. Conceptual map explaining how research will impact art, education, and the clay industry.

Objectives

- To further our understanding of microbial species inhabiting clay and determining their contributions to the plasticity of clay.
- To understand how recycled clay differs from purchased clay and its plastic properties.
- To learn about the impact that microbial species found on students' hands contribute to the microbial community found in the recycled clay.
- To integrate art into the STEM community. By involving our project with art students at Laramie High School.

Hypotheses

- We hypothesize that microbial diversity is greater in recycled clay than in new clay.
- We hypothesize that if a bacterium is isolated from recycled clay, this same microbe will also be isolated from the tools of the students.
- We hypothesize the plasticity of the recycled clay will be greater than that of the new clay

Methods

We swabbed tools from the Laramie High School art classroom and collected the clay samples that they provided us for bacterial identification and titer calculation.

The tool swabs and clay were both put in to PBS to allow for dilutions up to 10^{-5} and 10^{-6}. The colonies on the clay samples were enumerated to look for differences in titers of recycled* clay and purchased clay samples.

Colonies from both the tool and clay samples were grown on TSA and incubated for 24 hours at 37 °C, and then identified using MALDI-ToF with the Bruker Method.

Clay was also taken and rolled into 30g balls of each the recycled and purchased* clay. Some clay was autoclaved* and also rolled in to 30g balls as a control for our drop test for plasticity testing. The drop test was a novel idea that involved dropping the balls from a meter and measuring the depth of the indent created.

To test plasticity qualitatively we let the students play with three pieces of clay blindly. One piece autoclaved, one recycled, and one purchased. We had them rank the clay from most to least plastic.

After all of the data was collected statistical analysis was done using JMP statistical analysis software

*__purchased__ clay was purchased from Continental Clay © in Denver Colorado

*__Recycled__ clay was made by the LHS art professionals by taking scraps of used clay and letting it soak in buckets of water for a few days to rehydrate, and added to a pugmill in order to de-aerate and return to its original consistency.

*__Autoclaved__ clay was prepared by taking recycled clay and autoclaving the sample at 210°C and 21psi for 20 minutes.

*__Bruker Method__ involves using molecular grade water, 100% ethanol, 100% acetonitrile, and 70% formic acid to effectively extract proteins needed to identify bacteria.

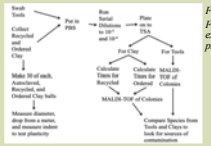

Figure 2 Flow chart for experimental procedure

Results

To qualitatively analyze clay plasticity, we set up a blind experiment, shown in figure 3, with the Laramie High School art students where 72% ranked recycled clay as being more plastic than the purchased clay. Since plasticity is an obscure property, it is important to acknowledge the ability of professionals to determine plasticity as they have the most hands-on experience.

Trial	Clay Ranking	What is More Plastic
1	1,3,2	Recycled More Plastic
2	1,3,2	Recycled More Plastic
3	2,3,1	Ordered More Plastic
4	3,2,1	Recycled More Plastic
5	3,2,1	Recycled More Plastic
6	3,2,1	Recycled More Plastic
7	3,2,1	Recycled More Plastic
8	3,2,1	Recycled More Plastic
9	3,2,1	Recycled More Plastic
10	1,2,3	Ordered More Plastic
11	2,3,1	Ordered More Plastic

Figure 3. Qualitative data from Laramie High School students and how they ranked the three different clays from most to least plastic.

1: Autoclaved
2: purchased
3: Recycled

73% of the Laramie High School art students blindly ranked the recycled clay as being more plastic than the purchased clay.

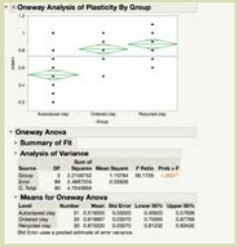

Figure 5. ANOVA test on clay drop test showed that while recycled clay was slightly more plastic, it was not a statistically significant difference when compared to purchased clay

Ordered clay	Species found
Ordered clay 2	*Propionibacterium avidum*
Ordered clay 4	*Staphylococcus epidermidis*
Ordered clay 3	*Microbacterium spp.*
Recycled clay	**Species found**
Recycled clay 5	*Staphylococcus capitis*
Recycled clay 1	*Enterobacter cloacae*
Recycled clay 2	*Staphylococcus hominis*
Recycled clay 5 colony 2	*Enterobacter cloacae*
Recycled clay 4	*Escherichia coli*
Recycled clay 1 colony 1	*Enterobacter cloacae*
Recycled clay 1 colony 2	*Actinobacter baumlli*
Recycled clay 4 colony 2	*Enterobacter cloacae*
Recycled clay 2 colony 2	*Enterobacter cloacae*

Tools	*Species found*
Paintbrush	*Propionibacterium avidum*
Needle 2	*Propionibacterium avidum*
Tool 1	*Bacillus amyloliquefaciens*
Work board 1	*Microbacterium spp*
Work board 2	*Arthrobacter creatinolyticus*
Tool 2	*Bacillus amyloliquefaciens*
Cup 1	*Propionibacterium avidum*
Sponge 1	*Propionibacterium avidum*
Bucket 2	*Microbacterium spp*
Needle 3	*Staphylococcus capitis*
Knife 1	*Microbacterium spp*
Bucket 1	*Microbacterium spp*
Sponge 2	*Staphylococcus capitis*
Tool 3	*Propionibacterium avidum*

Figure 4. purchased clay shared many of the bacteria isolated from the tools that the students used. As shown in green. While the recycled clay had a wider variety of biodiversity but shared less with the bacteria isolated form the tools. As shown in red.

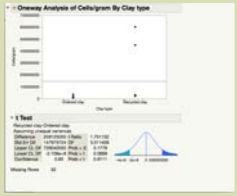

Figure 6. The titers of the recycled clay and purchased clay were not statistically different in their cells/gram counts.

We also wanted to look at water content in clay, as we know that water affects clay plasticity. We took a 30g ball sample from each type of clay and left it out for a week, exposed to air, with the same starting weight. At the end of the week, we measured each sample again and found that they had all lost the same amount of mass.

Discussion

Our quantitative plasticity test showed that there was no statistically significant difference between the recycled and purchased clay, however, the autoclaved clay was significantly less plastic than the other two clays. Perhaps this test could discern smaller but significant differences by increasing the weight of the clay balls and dropping them from various heights to potentially discern differences in plasticity between the recycled and purchased clay. Additionally, the autoclaved clay served as a control for microbes in clay. However, the high heat of the autoclave may have baked the clay which would have impacted the water content and ultimately the plasticity. This issue could be addressed in future studies by exploring alternative methods to sterilize clay. These adjustments to the quantitative test would potentially better address our third hypothesis stating that the recycled clay will be more plastic than the purchased clay.

With the use of MALDI-ToF, we were able to identify three different species found in the purchased clay, five species in the recycled clay and five species in the 14 tools we swabbed. This data explored our second hypothesis, which was examining the relationship between bacteria in the recycled clay and bacteria found on the tools. The various species identified from these samples are found on normal skin flora such as: *Staphylococcus epidermidis, Microbacterium spp.*, and *Propionibacterium spp.* Although the recycled clay did not share as many species with the tools as the purchased clay, it did possess more species in general which could lead to the variance in plasticity. Altogether, our data on species diversity between the two types of clays was not statistically supported, but there were more species present in the recycled clay than the purchased clay which does support our first hypothesis which explores this relationship.

Our exploration of plasticity and bacterial differences between the two types of clay addresses the questions raised by our community partner by the following: most of the students were able to qualitatively identify recycled clay as being more plastic than the other two clays, and that there were more bacterial species found in the recycled clay than the purchased clay. Overall, we infer that the differences in plasticity may be due to bacterial diversity.

Conclusion

- The species diversity in recycled clay was not statistically more significant between the purchased clay, however, there were more species in the recycled clay that we could identify.
- Species found on the tools were more commonly found on the purchased clay than the recycled clay.
- Quantitatively we did not find a difference in plasticity between the recycled and purchased clay but qualitatively we found the recycled clay to be more plastic.
- LHS students visited our lab and learned to worked with microbes. We anticipate that this collaboration continues in the future.

Acknowledgements

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