An observation of the effects of microplastics on the reproductive system and intestine of *Caenorhabditis elegans*, a model organism

Jessica Bis*, Jhelaine Palo*, Alexis Hahn, Efraim Shkarofsky

Jean M. Parry Ph.D.

Department of Biology, Georgian Court University, Lakewood, NJ, USA 08701

GEORGIAN COURT UNIVERSITY

THE MERCY UNIVERSITY OF NEW JERSEY

Abstract

The earth has been in a state of tragedy due to the ever-increasing tons of plastic deposited into the environment each year. Microplastics (MPs), plastic particles of nano or micro sizes, build up in environments where they are consumed by organisms and have even been found in humans. With the ingestion of MPs, there is potential to alter gut function and health, which can impact human health (Berg et al. 2016). It is imperative to understand how MPs affect organismal health, which has yet to be thoroughly investigated. Using *Caenorhabditis elegans*, a model organism, samples were subjected to treated environments of MPs. Following this, reproductive systems of the nematodes were observed for egg division anomalies and the digestive tract was measured to detect changes in gut structure.

The gut microbiome consists of bacteria, archaea, viruses, and eukaryotic microbes that live in digestive tracts of organisms and interact with the host to influence disease, disorders, and overall health. Exposure to MPs can affect the gut microbiome, which may alter their structure and function within the host. To begin, *C. elegans* were bleached and hatched on a sterile media to ensure growth without any MP influence. The nematodes were inoculated into sterilized soil environments enriched with compost microbial extract to mimic their native environment. We carried out a series of microcosms to determine optimal conditions for the growth and collection of nematodes. In the future, MPs will be added to microcosms and high throughput 16S rRNA gene sequencing coupled with bioinformatics pipelines will be conducted on extracted DNA. The ultimate goal of this research is to establish the native gut microbiome of *C. elegans* within microcosms and expose the organisms to different concentrations of MP to determine changes within the microbial community structure.

Introduction

380 million tons of plastic is produced each year, with 10 million tons ending up in the oceans every year. 50% are single-used products, exacerbated by the recent COVID-19 pandemic. 10 million tons of plastic end up in the oceans each year. (Edwards et al. 2022). MPs, plastics less than 5 microns in diameter, are produced synthetically for personal care products but can also come from being broken down from erosion and corrosion (Jasińska et al. 2022). MPs can be consumed by smaller and larger organisms, entering the food chain. Microplastics have been detected in human blood, feces, lungs, and placenta and therefore have the potential to alter gut function and structure. This research focused on the observation of digestive and reproductive changes of *Caenorhabditis elegans* in environments that contained microplastics

C. *elegans* make for a strong model for digestive systems due to capabilities of sterilization via bleaching gravid adults for their eggs and exposing them to controlled environments where they can consume bacteria and MPs directly from their surroundings. (Berg et al 2016). Using this, C. *elegans* were exposed to MPs and studied for physical differences in digestive and reproductive systems. Separately, nematodes can be bleach washed and crushed to collect samples from their gut for sequencing and metagenomic analysis to analyze gut bacteria composition. Understanding interactions with the gut microbiota is essential because the microbiome dictates health in varying ways, including but not limited to immunity, development, and nutrient absorption and use (Berg et al 2016).

Procedures

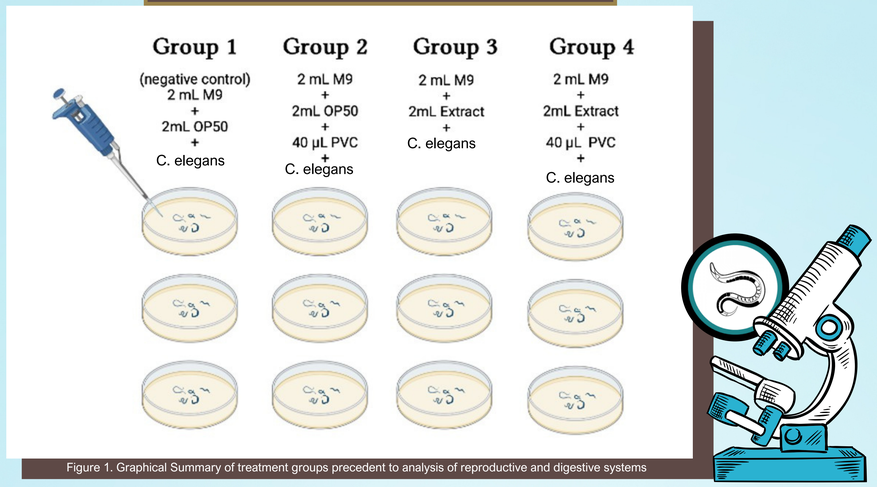

Figure 1. Graphical Summary of treatment groups precedent to analysis of reproductive and digestive systems

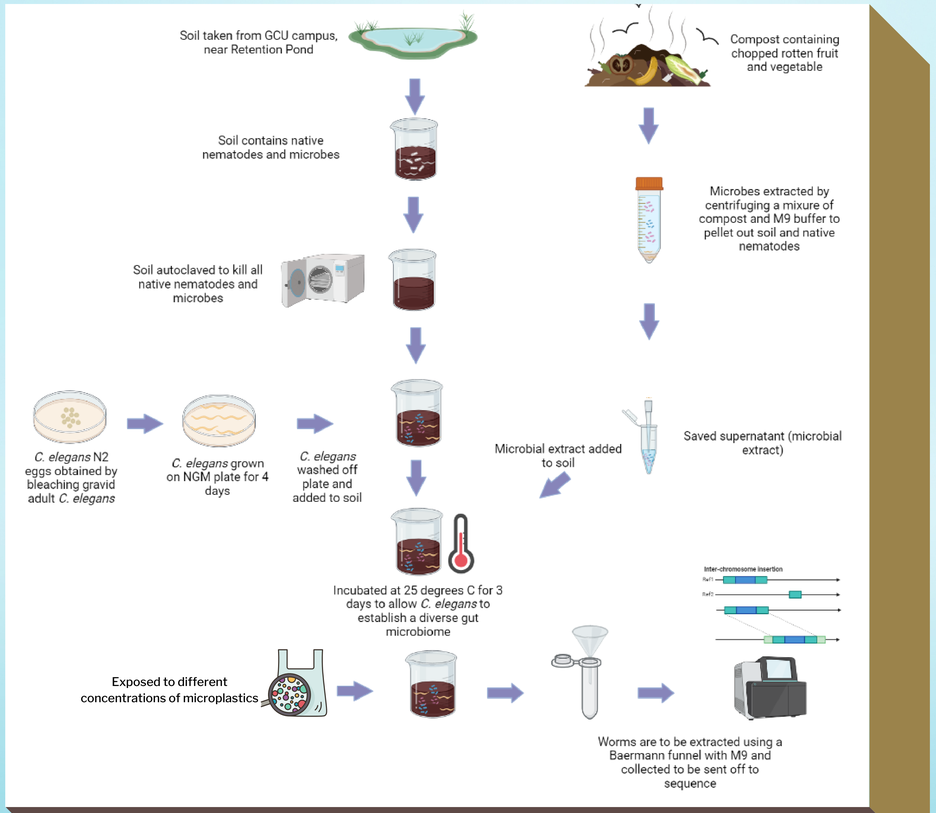

Figure 2. Graphical summary of procedure pertaining to gut microbiome analysis. Soil and nematodes were collected from the environment and sterilized, followed by adding mirobial extract obtained from compost. *C. elegan eggs* obtained from bleaching gravid adults were added and incubated. Worms were extracted using Baermann funnel with M9.

Results and Discussion

In the OP50 environment, all of the oocytes developed normally in each of the c. elegans worms. In the OP50 environment treated with PVC, 5 of the worms displayed a lateral egg development. 2 worms displayed bilateral egg development, while 2 worms displayed normal egg developments. No MPs were present in the intestine in the worms that displayed normal egg developments. The mean intestinal diameter of c. elegans in the OP50 environment was 5.3041 µm (± 0.177 S.E.).

The mean intestinal diameter of c. elegans in the OP50 environment treated with PVC was 8.0960 µm (± 1.091 S.E.). There is a significant difference between the intestinal diameter of c. elegans worms in the OP50 environment in comparison to the intestinal diameter of c. elegans worms in the OP50 environment treated with PVC ($p = 0.024$).

Consumption of MPs showed adverse effects in the reproductive development and intestine size of c. elegans. The lateral and bilateral arrangement of the reproductive system indicate that the eggs are endomitotic and they could not undergo cell division. A larger intestinal diameter of the c. elegans worms indicated that their intestines are obstructed with MPs which have been ingested from the environment. Future experiments can be done to determine the genes that are affected by the addition of MPs into the C. *elegans*' environment. The human consumption of MPs and its effects on the human microbiome is not yet fully understood. The growing abundance of MPs within the environment presents a concern on how it affects human health.

M. Berg and others' (2016) procedure for soil and microbiota establishment was followed but resulted in growth of fungi and mold and led to high mortality of *C. elegans*. Mylonakis and others (2002) reported that strains of *Cryptococcus neoformans*, a common fungi, increased mortality of *C. elegans*, which corresponds to observations seen since nematodes were initially undetectable in microcosms. Moisture content was reduced by using compost instead of rotten fruit-soil mixture. The addition of M9 was also eliminated. It was determined that lower soil moisture content provided more suitable conditions as it reduced fungal growth and therefore increased nematode population.

Microbial extract addition, incubation periods, and nematode addition were additional variables explored. Microcosms that had microbial extract added and incubated for 24 hours prior to adding *C. elegans* had notably more nematodes than microcosms with 48 hour incubation and simultaneous addition of nematodes with microbial extract. The 24 hour period enabled for microbial growth but not overpopulation, and acted as a nutritional resource for the nematodes. The nematodes added to this microcosm were bleached 4 days prior as opposed to 3, which also allowed a longer duration for repopulation and notably more nematodes. These aspects allowed for decreased fungal growth, increased nematode quantity and survival, and enabled us to explore recovery methodology.

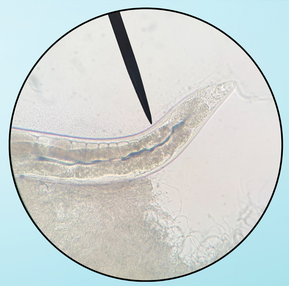

Figure 3. Intestinal lumen of PVC treated C. *elegan*

Figure 4. Intestinal Lumen of PVC treated C. *elegan* exhibiting distension due to blockage by PVC

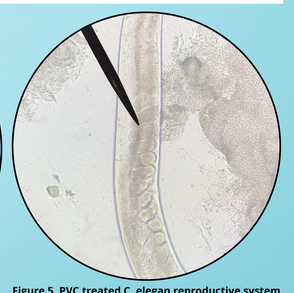

Figure 5. PVC treated C. elegan reproductive system revealing a mixture of normal and endomitotic eggs

References

Barrière A, Félix MA. 2014. Isolation of C. elegans and related nematodes. WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.115.2, http://www.wormbook.org.

Berg M, Stenuit B, Ho J. Wang A, Parke C, Knight M, Alvarez-Cohen L, Shapira M. 2016. Assembly of the Caenorhabditis elegans gut microbiota from diverse soil microbial environments. ISME J 10: 1998–2009. https://doi.org/10.1038/ismej.2015.253

Edwards S, León-Zayas R, Ditter R, Laster H, Sheehan G, Anderson O, Beattie T, Mellies JL. 2022. Microbial consortia and mixed plastic waste: Pangenomic analysis reveals potential for degradation of multiple plastic types via previously identified PET degrading bacteria. International Journal of Molecular Sciences. 23(10):5612.

•Jasińska A, Różalska S, Rusetskaya V, Staba M, Bernat P. 2022. Microplastic-induced oxidative stress in metolachlor-degrading filamentous fungus 0RW1S34RfeSDcfkexd09rT2trichoderma harzianum1RW1S34RfeSDcfkexd09rT2. International Journal of Molecular Sciences. 23(21):12978.

Mylonakis E, Ausubel FM, Perfect JR, Heitman J, Calderwood SB. 2002. Killing of Caenorhabditis elegans by Cryptococcus neoformans as a model of yeast pathogenesis. Proc Natl Acad Sci USA. 99(24): 15675–15680. https://doi.org/10.1073/pnas.232568599

Nassiri-Koopaei N, Abdollahi M. 2017. Health risks associated with the pharmaceuticals in wastewater. DARU J. Pharm. Sci. 25(1), 9. https://doi.org/10.1186/s40199-017-0176-y

Fay D. 2006. Genetic mapping and manipulation: Chapter 1- Introduction and basics. University of Whyoming Laramie. 1-12.

Singaravelu G, Rahimi S, Krauchunas A, Rizvi A, Dharia S, Shakes D, Smith H, Golden A, Singson A. 2015. Forward genetics identifies a requirement for the Izumo-like immunoglobin superfamily spe-45 gene in Ceanorhabditis elegans fertilization. Curr Biol. 25(24): 3220-3224. doi:10.1016/j.cub.2015.10.055.

Yook K. 2005. Complementation: A simple test for assigning a mutation to a genetic locus. Oxford. 1-2

Acknowledgements:

We would like to acknowledge Jennifer Ballina for her valued assistance with the Buchner vacuum filtration method.