

# ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

The longevity of *Caenorhabditis elegans* in soil

by

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Electronic appendices are refereed with the text; however, no attempt is made to impose a uniform editorial style on the electronic appendices.

**Supplemental Material:** Analysis of factors potentially responsible for increased mortality of *C. elegans* in soil and sand media and the affect of different *E. coli* bacterial strains on survivorship.

### **Extraction Method.**

The high soil/sand mortality rates could be caused by the soil/sand sample extraction method used, which could itself be killing the worms. However, a check of survivorship of a group of *C. elegans* placed in soil and then extracted 3 hrs later found that 95% of the extracted worms were alive. This indicates that the extraction method was not responsible for the high mortality rates observed.

### **Lack of food/ excess density of worms in soil.**

Both sand and soil samples were supplemented with *E. coli* bacteria normally eaten by lab populations of *C. elegans*. Additionally, laboratory populations of *C. elegans* were able to thrive on native bacteria isolated from the soil type used in the experiment. Finally, adult *C. elegans* can tolerate long period of starvation. To determine this worms were placed in a Ringers solution which contained no external nutrient supplies. After four days of starvation >96% of worms remain alive with over 50% surviving after 12 days of starvation.

The initial population density of *C. elegans* was 150 worms/ml of soil. This density is more than an order of magnitude less than the density of *C. elegans* grown on agar plates or in liquid media. In liquid media worms can commonly be grown to an estimated density of around 3000 worms/ml of liquid media (Sulston and Hodgkin p.602, in Wood, W.B. (ed.) *The*

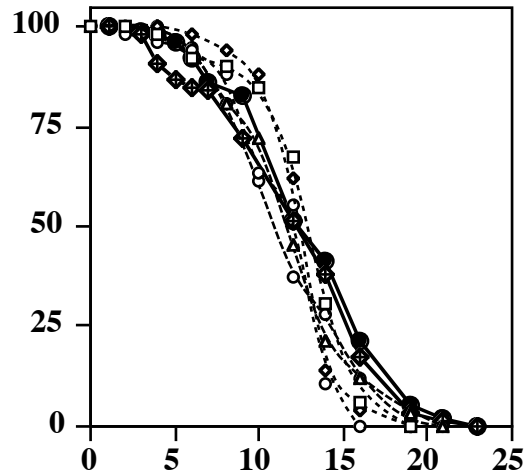
*Nematode Caenorhabditis elegans* (Cold Spring Harbor Laboratory Press, New York, 1988) and *C. elegans* can be grown at similar densities on agar plates.

**CO<sub>2</sub> or O<sub>2</sub> levels: in the soil or sand chambers were lethal to the worm populations.**

Measurements of both these gasses in soil and sand environments found that the levels of CO<sub>2</sub> and oxygen levels in either of these environments were approximately equivalent to that found in atmospheric air. The concentration of CO<sub>2</sub> in the sand and soil environments was < 500 ppmv while the concentration of oxygen was > 18 kPa.

**Extreme environmental pH.**

The pH of both the soil and sand samples used were measured and were near pH 7.0. This is well within the range for normal growth and development in *C. elegans*.



### Adult longevity (days)

Survivorship of wild-type *C. elegans* (*fer-1*) on nematode growth media fed on either *E. coli* strain OP50 (open symbols) or NA22 (closed symbol). Each line plots survivorship of a group of 50-100 worms. There is no significant difference in survivorship in worms reared on plates inoculated with either OP50 or NA22 as food sources (students 2 tailed t-test,  $p=0.13$ ).