

## Consequences of Cadmium exposure on growth and reproduction across three generations of earthworm

Panchamee Dharmadasa<sup>1\*</sup>, Nick Kim<sup>1</sup>, Yinsheng Li<sup>3</sup>, Michelle Thunders<sup>2</sup>

1. School of Health Sciences, Massey University, Wellington, Wellington, NEW ZEALAND

2. Department of Pathology and Molecular Medicine, University of Otago, Wellington, Wellington, NEW ZEALAND

3. School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, Shanghai, CHINA

\*Corresponding Author E-mail: L.Dharmadasa@massey.ac.nz

Heavy metal pollution disturbs the soil ecosystem by negatively affecting soil fauna and flora. In term of biomass and activity Annelids are a very important part of the soil invertebrate community. They are one of the first organisms affected by heavy metal contamination in soil and as such are good model organisms for assessing soil contamination.

The aim of this research is to observe how Cd impacts on health and reproduction in three consecutive generations of *E. fetida*.

The methods used to determine the effect of Cd on reproduction have been taken from the OECD guideline for the testing of chemicals with slight modifications. *Eisenia fetida* were obtained from local vermi-culturist. All the worms selected were of similar age with well-developed clitella. Artificial soil was spiked with three different Cd solutions; low (30mg/kg), Medium (90 mg/kg) and high concentration (270 mg/kg). Clean artificial soil was used for control. Four replicates from each concentration and from the control were maintained at  $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for one week prior to experimentation to establish equilibrium between Cd and soil particles. After a one week pre-incubation period in the clean artificial soil, the worms were introduced to the Cd spiked soils (10 worms per beaker). The beakers were covered by fabric net and incubated at  $23 \pm 1^{\circ}\text{C}$  under minimal light conditions. During the test period, the worms were fed oats once a week (5 g per each beaker with 10 worms) and water loss from the beakers through evaporation was replenished every 2 days.

At the end of days 28 and 56, cocoon production and morphological changes were assessed. Once hatchlings had become well-developed adults they were used for F2 experiments. Hatchlings from F2 were used for F3 experiments. At the start and end of each test period, the body weight of the earthworms was measured. Before measurements were taken the worms were washed with distilled water and excess water removed using a paper towel. The growth during each test phase was calculated using the weight measurements.

Cd concentration was negatively correlated with cocoon production in worms. In F1 the average cocoon production after 28 days was 8.3, 8.5 and 2 for worms in control soil, 30 mg/kg and 90 mg/kg respectively. There was no significant difference

in 30 mg/kg ( $p=0.555$ ) and 90 mg/kg ( $p=0.209$ ) test concentrations. However, there was a significant difference after 56 days with the average number of cocoons per beaker 41, 23 and 7 in control, 30 mg/kg ( $p= 0.047$ ) and 90 mg/kg ( $p=0.008$ ) respectively. There was no cocoon production by worms that were in high Cd concentration. F2 worms in Cd spiked soil showed a decrement of cocoons produced compared to F1. The worms that were in 30 mg/kg produced an average of 2 and 5 cocoons after 28 days and 56 days respectively. When compared with the control there was no significant difference after day 28 ( $p=0.129$ ) and after 56 days showed a significant difference ( $p= 0.008$ ). Cocoon production in F3 is similar to F2 but after 28 days the difference ( $p=0.051$ ) is considerably higher than F2. In F2 worms that were in 90mg/kg showed no cocoon production within 56 days.

During the first 28 days the F1 worms that were in 90 mg/kg and 270 mg/kg showed weight loss while those in 30mg/kg and control soils showed weight gain. The highest growth percentage was found in worms in 30mg/kg (27.9%). However, by day 56 the worms that were in 90mg/kg showed the highest growth percentage (28.67%). Worms that were in 270 mg/kg consistently showed significant weight loss throughout the 56 day period. In F2 worms in control and 30 mg/kg soil showed weight gain whereas worms in 90mg/kg showed weight loss during first 28 days. Nevertheless, by day 56 these worms also showed weight gain. There were only hatchlings from control and 30mg/kg for F3 which showed positive growth percentage during the experiment. The worms in 90 mg/kg showed significant growth difference during first 28 days in both F1 and F2. However, this difference did not persist at 56 days.

The results of this study revealed that Cd has a significant effect on the growth and reproduction of *E. fetida* and also suggested that Cd exposure weakens the worms' ability to respond and this is exacerbated in successive generations. The effect is more intense with increased dose of Cd.

**Keywords:** *E. fetida*, Growth, Multiple generations, Reproduction