EFFECTS OF CONTAMINATED SOILS ON SURVIVAL, GROWTH AND REPRODUCTION OF *EISENIA ANDREI*

N.D.K.Saumyakumari[1] and P. M. C. S. De Silva[2]

[1]*Department of Zoology, Open University of Sri Lanka, Nawala, Nugegoda*
[2]*Department of Zoology, University of Ruhuna, Matara*

INTRODUCTION

Global agriculture has largely succeeded in satisfying world demand for food over the last several decades with intensive agricultural production, and it contributes to covering a large portion of the global food demand (Giller et al. 1997*)* and is *directly* linked with the heavy use of pesticides. It was estimated that 2.5 million tonnes of pesticides are applied to agricultural crops worldwide each year (Van der Werf 1996). As a consequence of the green revolution, agriculture in Sri Lanka has undergone significant intensification in the last decades. Pesticide management is a serious problem in the local context. Farmers neglect technical recommendations and apply according to their own experiences due a to lack of knowledge and guidance. (Thiruchelvam and Bandara 2008). Agricultural soil has been considered to be a primary recipient for agrochemicals and synthetic inorganic fertilizers (Pereira et al. 2009). It has been estimated that approximately 1% of pesticides applied to the agriculture reach their target pest and more than 99% of it adversely affects non-target and beneficial organisms (Atreya et al 2012) such as soil invertebrates. Earthworms are an important group that has been considered as a bio-indicator species in the terrestrial ecosystem and a good model organism to determine the effects of agrochemicals. *Eisenia andrei* (*Oligochaeta ;Lumbricidae*) is a standard earthworm species used in ecotoxicology (ISO, 1998b; OECD, 2010). *Eisenia andrei* is an epigeic earthworm that inhabit in surface litter in soil. Their relatively short generation time, high cocoon production, continuous breeding, wide temperature and moisture tolerance, cosmopolitan distribution and easy culturing under laboratory condition have led *E.andrei* to be selected as a standard species for soil ecotoxicology (De Silva et al. 2009). It is hypothesized that the heavy loading of agrochemicals persists in the soil ecosystem, which result in short- and long-term effects on soil fauna. Hence, in the present study, we study the effects of contaminated soils on survival, growth and reproduction of the tropical variant of earthworm *Eisenia andrei* to develop it as a potential bio-indicator in agro ecosystems.

METHODOLOGY

Six sites were selected randomly in the hill country, from Nuwaraeliya to Bandarawela, as possible contaminated sites based on information from farmers. Two uncontaminated sites were selected in Diganatenna area representing the same study area and another site near the Ruhuna University, Matara. Both control sites had no record of previous contamination due to agrochemicals. A random sampling design was used to obtain a composite soil sample per site in the 0-5cm soil surface layer. The organic matter content, maximum water-holding capacity and the pH were tested for each soil sample. Soil samples from the selected sites (n=5) were collected and sieved to get a homogeneous structure. A standard artificial was used as a reference soil that was developed according to OECD guidelines by mixing 70% quartz sand, 20% kaoline clay and 10% sphagnum peat with a small amount of $CaCO_3$. The epigeic earthworm *Eisenia Andrei*, cultured under laboratory conditions, was selected as the test species. Glass bottles (750ml) were filled with 500g (dry weight) of each test soil samples. It was moistened to 50% of their respective maximum water-holding capacities. Age synchronized adult earthworms (300mg-600mg) with well-developed clitellum were obtained

[1] Correspondence should be addressed to Ms.N.D.K.Saumyakumari, Department of Zoology, Open University of Sri Lanka, Nawala, Nugegoda. (email: ksaumya11@gmail.com)

from cultures at the Department of Zoology, University of Ruhuna, Matara and introduced into the containers with moistened soil. The initial mass of earthworms was determined before exposure to soil samples and the bottles were incubated at 26 ± 2 °C and 12:12 hour's photoperiod for 28 days. Five grams of moistened, finely-ground cow manure was added as the food. After 28 days, adults were removed by hand sorting, and the surviving earthworms were counted. The final biomass of the remaining adults of each container was also recorded. After this, the soil was returned to the test containers and incubated for another 28 days for cocoon development. After 56 days, juveniles were extracted from the test soil using a water bath kept at 60 ^{0}C and the numbers of juveniles were counted. The final endpoints were adult survival, change of biomass after 28 days, and the number of juveniles produced after 56 days. The results were tested for their normal distribution with the Komogorov-Smirnov test and homogeneity of variance with the Levene's test. Results were analyzed by one way ANOVA using SPSS software version 16.0.

RESULTS AND DISCUSSION

The tested soil parameters were in optimal range for earthworms. Therefore, the soil parameters of each site did not contribute to the changes of the end points from site to site. There were no effects on the survival of adult earthworms in all control soils because they were free from agrochemical contamination. The performance of *Eisenia andrei* in OECD artificial soil (AS) and the Matara control soil (MCS) were the same. In the Diganatenna control soil (DCS), the performance of *Eisenia andrei* varied from the other two types of control soils. This may be due to the physical and chemical properties of soil. Significant biomass loss was recorded from AS and MCS, but a higher biomass gain was recorded in DCS. In earthworm growth test, effects of control soil on biomass loss were highly significant ($F= 100.354$, $p < 0.001$) and the mean percentage weight of adults in DCS was significantly different from the AS ($p < 0.001$) and MCS ($p < 0.001$).

In contaminated soil samples, no effects on survival were also recorded during the 28-day exposure period. Homogeneous distribution of earthworms in the experimental bottles was observed in each of the replicates and it may be due to the level of soil contamination and rather low or not lethal to *Eisenia andrei*. This may have resulted due to an agrochemical degradation paradigm in the natural environment and multiple degradation pathways that occurred at field conditions. It results rapid degradation, volatilization and photo decomposition (Van der Werf 1996). After 28 days of exposure, the mean weight was reduced in all tested contaminated soil samples. The highest mean biomass loss was recorded at the Diganatenna contaminated soil (CSD) and the lowest value was in the Pattipola contaminated soil (CSP). It was reported that the mean weight change of the earthworms was significantly different ($p < 0.05$, Figure 1 and Figure 2) from the comtrol sopils, AS and MCS. The mean weight loss of adults in CSP was significantly lower from the OECD artificial soil (AS) and MCS ($p < 0.05$). Compared to DCS, the overall effects of the different contaminated soil types on the mean weight change of the earthworms was significant ($F= 22.756$, $p < 0.05$, Figure 3). According to the Dunnetts test, the growth of adults in all contaminated sites has had a significantly higher mean weight loss when compared to CSD (CSD, $p < 0.05$) except CSP.

The highest mean number of juveniles was recorded at the Ambewela contaminated soil (CSA) (175.6±40.8) and CSP (163.8±24.5) and the lowest in CSD (16.6±7.0). The soil samples taken from CSP and ASP recorded the highest significant mean numbers of juveniles when compared to AS and MCS ($p < 0.05$, Figure 4 and Figure 5). A high number of juveniles in CSA and CSP could be linked to higher organic matter in the content. It was recorded that the use of cow dung as manure in the Pattipola and Ambewela farms, and it may positively affect the performance of earthworms. On the other hand, organic matter in the soil may reduce bioavailability of the agrochemicals resulting in a higher reproduction potential in the tested soils. However, the number of juveniles in the Nuwara Eliya (CSN), Boragas (CSB) and Keppetipola (CSK) contaminated soils were not significantly different from the mean number of juveniles in AS and MCS ($p > 0.05$). The mean number of juveniles produced after 56 days also varied in different test soils ($p < 0.05$, Figure 6) from DCS. A significant lower number of juveniles were recorded from CSA, CSN, CSK and CSD contaminated soils ($p < 0.05$) from DCS. It can be predicted that sub lethal endpoints, such as growth and reproduction, are more sensitive than survival, although the level of contamination was not lethal to the tested earthworms. However, in the long term, persisting soil contamination can still cause serious effects on growth and the reproduction of earthworms.

CONCLUSIONS/RECOMMENDATIONS

The degree of soil contamination was not lethal to *Eisenia andrei* for the tested six contaminated soils. In addition, long term endpoints, such as growth and reproduction, have also confirmed that the level of contamination in the studied contaminated soils (Pattipola, Ambewela, Nuwara Eliya, Boragas and Keppetipola) have no serious effects on growth and reproduction of *Eisenia andrei* except in the Diganatenna contaminated soil. This study is the first to establish survival, growth and reproduction data for six soil types in arable lands from Nuwara Eliya to Bandarawela. These generated values can be used as standard initial values for the development of a bio-indicator index. Further, the development of such indices can be accomplished with more sampling sites in the area. In addition, soil sampling should also be done in different periods of the crop cycles, such as just after spraying, a day before spraying and a day after spraying of agrochemicals and application of synthetic fertilizers.

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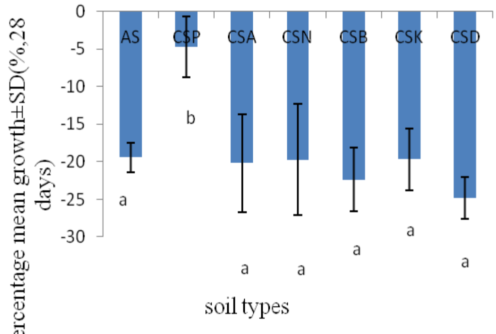

Figure 1: Percentage mean growth of *Eisenia andrei* (mean±SD, n=5) after 28 days of incubation in OECD artificial soil (AS) and different contaminated soil.

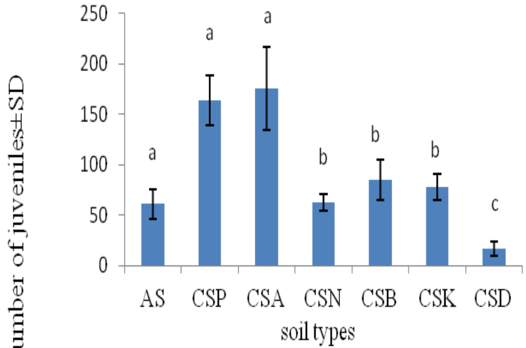

Figure 4: The number of juveniles produced (mean±SD, n=5) after 56 days of incubation in OECD artificial soil(AS) and different contaminated soils.

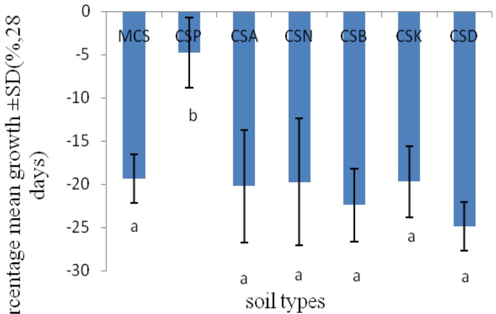

Figure 2: Percentage mean growth of *Eisenia andrei* (mean±SD, n=5) after 28 days of incubation in *Matara* control soil (MCS) and different contaminated soils

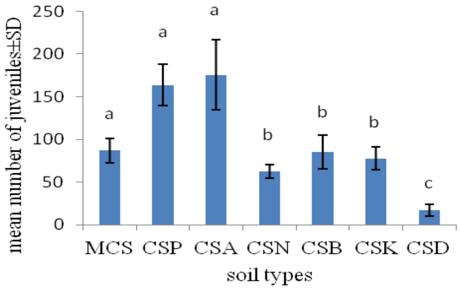

Figure 5: The number of juveniles produced (mean± SD, n=5) after 56 days of incubation in *Matara* control soil (MCS) and different contaminated soils.

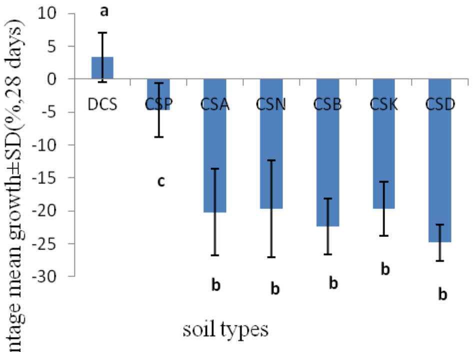

Figure 3: Percentage mean growth of *Eisenia andrei* (mean±SD, n=5) after 28 days of incbation in *Diganatenna* control soil(DCS) and different contaminated soils.

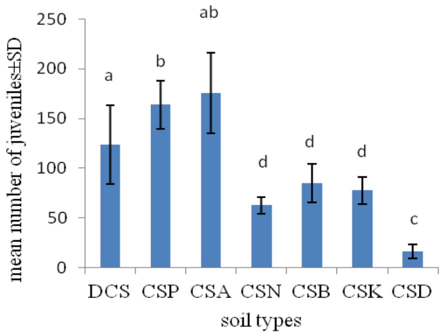

Figure 6: The number of juveniles produced (mean±SD, n=5) after 56 days of incub ation in *Diganatenna* control soil(DCS) and different contaminated soils.