Reproductive parameters as assessment tools for arsenic and chromium induced toxicity in _Eudrilus eugeniae_

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ABSTRACT

The present study of 28 days was carried out to assess the effect of arsenic and chromium on biomass gain and reproduction parameters (cocoon and hatching production) of _E. eugeniae_. The worms were cultured in substrate having different concentrations of arsenic and chromium alongwith control in triplicates. Dose and time dependent decreased in number of adults, cocoon production and hatching emergence and reduction in growth was observed. In worms exposed to Cr (0.1 ppm), reduction in population (24.66%), body weight (2.44%), cocoon production (47.14%) and hatching emergence (39.99%) was observed. While As (0.1 ppm) exposure resulted in reduction of adult population (10.66%), body weight (0.24%), cocoon production (37.05%) and hatching emergence (28.53%) was observed. Cocoon production was observed to be the most sensitive parameter for heavy metal exposure in all treatments. Chromium was observed to be more detrimental to earthworms when compared with arsenic.

Key words: Cocoon, _E. eugeniae_, Growth, Heavy metal, Reproduction.

INTRODUCTION

Recent toxicological evaluations have advocated the presence of pesticidal residues (Shefali and Kataria, 2017) and traces of heavy metals as significant soil poisons (Sivakumar, 2015). Enhanced levels of heavy metals in soil degrade soil fertility, as well as poses threat to soil flora and fauna (Yadav _et al._, 2017a). Generally, heavy metals are the result of industrial procedures like metallurgy, mining, electroplating and metal finishing (Vandana and Keshav, 2015). However, agricultural practices add to significant levels of heavy metals through atmosphere which gets deposit on upper soil surfaces (Vidovic _et al._, 2005). Consistent introduction of waste water from metal finishing and tanning industries elevates chromium level in soil (Sivakumar _et al._, 2009), whereas arsenic containing compounds are created mechanically and have been utilized to make items with farming applications like herbicides, fungicides, algaecides, wood additives and color stuffs (Tchounwou _et al._, 2012). Because of longer persistence of heavy metals in soil as compared to other organic compounds, their effect on soil fauna stays for a longer time. Earthworms play essential part in enhancing soil ecology by increasing the levels of available nutrients and microflora (Yadav _et al._, 2017b) because of their tunneling movement. Previous studies have stamped worms’ utility in bioremediation as worms have the ability to uptake heavy metals from contaminated soil (Romero-Freire _et al._, 2015; Ekperusi and Aigbodion, 2015; Verma _et al._, 2016 and Wang _et al._, 2016). Accordingly, the role of worms’ as soil engineers can’t be ignored. Worms being widely distributed in soil biological communities are influenced because of heavy metal contamination (Bamidele _et al._, 2015). Heavy metals may cause changes in different physiological development, reproduction and survivability of night crawlers (Bernard _et al._, 2014). The greater potential of _E. eugeniae_ for vermicomposting process has previously been reported (Coulibaly and Bi _et al._, 2010 and Sonia _et al._, 2016). Higher fecundity and growth rate of _E. eugeniae_ in comparison with with _Eisenia fetida_ (Maboeta _et al._, 1999) has brought about extensive use of it in commercial vermiculture (Sonia _et al._, 2016). Due to their wide distribution, night crawlers are the most broadly acknowledged test life form for ecotoxicological assessment particularly for soil environments. As the skin of worm is thin and wet, it is more inclined to the cutaneous uptake of pollutants because of its regular contact with soil. Dutta and Dutta (2016) have reported the risk of that if worms survival is at risk, it will negatively affect the sustainability of environment. Although, OECD (1984) recommends _E. fetida_ as test worm species for carrying out ecotoxicological assessments, yet _E. eugeniae_ may also act as a potential test life form for such test. Growth and reproduction abilities of worms act as sensitive end points in ecotoxicology (Reinecke _et al._, 2001). Hence, the present study was done to investigate the effect of arsenic and chromium contamination as these are carcinogenic metals on growth, survivability, cocoon and hatching production of _E. eugeniae_.

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MATERIALS AND METHODS

Collection of test animal: Healthy and clitellated E. eugeniae (approx. 600-700 mg) were procured from the Vermicomposting unit, Department of Zoology, CCS Haryana Agricultural University, Hisar. Thereafter, they were cultured on substrate (cow dung) and third generation was used to avoid the incidences of pre exposure to heavy metals.

Experimental set-up: 50 mature E. eugeniae were added to the tubs having predecomposed cattle dung as substrate that was procured from Biogas plant of Department of Microbiology, CCS HAU, Hisar. For exposure of E. eugeniae to various concentrations of arsenic and chromium, sodium arsenate (Na₃AsO₃·7H₂O) and potassium dichromate (K₂Cr₂O₇) was used. The details of treatment given are mentioned in Table 1. Three replicates per dose were maintained by spraying heavy metals in corresponding tubs and control was maintained by sprinkling distilled water. The distinctive life stages (cocoon, juvenile, adult) of E. eugeniae were counted and earthworms were weighed utilizing an electronic balance after washing and drying them with tap water and paper towels respectively, every week for 28 days.

Table 1: Description of treatments given to earthworms.

| Treatment | Description |
|-----------|-------------|
| Control   | Control     |
| T1        | Arsenic (0.06 ppm) |
| T2        | Arsenic (0.08 ppm) |
| T3        | Arsenic (0.1 ppm) |
| T4        | Chromium (0.06 ppm) |
| T5        | Chromium (0.08 ppm) |
| T6        | Chromium (0.1 ppm) |
| T7        | Arsenic (0.03 ppm) + (Chromium 0.03 ppm) |
| T8        | Arsenic (0.04 ppm) + (Chromium 0.04 ppm) |
| T9        | Arsenic (0.05 ppm) + (Chromium 0.05 ppm) |

Results and Discussion

Effect of heavy metals on body weight of E. eugeniae:

Time and dose dependent gradual decrease in body weight of worms was observed which corresponds to the findings of Malecki et al. (1982) and Neuhauser et al. (1985). Retarded growth of worms due to heavy metal exposure has also previously been observed by Lapinski and Rosciszewska (2008); Miguel et al. (2012) and Bilialis et al. (2013) which may be attributed to the avoidance behavior and low feeding activity on metal contaminated substrate. Body weight loss also reflects the reduced feeding of worms as reported by Gomez-Eyles et al. (2009). However, biomass reduction may also be attributed to certain physiological changes induced by heavy metal exposure, as worm feeding in treated and control soils was reported to be similar by Kreutzweiser et al., (2008). It is also worth noticing that up to 14th day after treatment non significant change in body weight was observed in worms exposed to various treatments. But, significant difference in body weight of treated worms as compared to control was observed even after 14th day of treatment. After 28th day, maximum decrease i.e. 2.44% and 1.84% in body weight was observed in worms treated with T6 and T5, respectively. Increased energy demands due to heavy metal induced stress may also be the most probable reason for biomass reduction in worms exposed to heavy metals (Yadav et al., 2017a).

Effect of heavy metals on survivability of E. eugeniae:

Even after 21st day of treatment, non significant difference in survivability was observed in worms exposed to T1 as Statistical analysis: Data was analyzed by completely randomized outline (CRD) with three replicates. The critical difference was worked out at 5% (0.05) probability levels utilizing Software ‘OPSTAT’, created at the Computer Center, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar.

**Table 2: Effect of Arsenic and Chromium on the growth (body weight) of earthworm, E. eugeniae.**

| Treatment | Weight/ worm (in mg) |
|-----------|----------------------|
|           | 1 DAT | 7 DAT | 14DAT | 21 DAT | 28 DAT | Mean  |
| Control   | 957.67±2.87 | 988.33±2.73* | 1,137.00±4.16* | 1,182.00±5.69 | 1,207.00±3.79 | 1094.40 |
| T1        | 956.33±3.18 | 966.33±3.48* | 972.00±3.6* | 964.00±4.58* | 956.48±3.52* | 963.03 |
| T2        | 955.33±2.99 | 967.33±2.73* | 969.33±4.63* | 963.68±2.58* | 953.90±2.47* | 961.91 |
| T3        | 955.00±3.21 | 962.67±2.02* | 968.00±4.93* | 960.33±2.73* | 952.67±2.84* | 959.73 |
| T4        | 953.67±3.28 | 967.00±4.04* | 966.33±5.29* | 961.68±3.17* | 951.33±1.77* | 960.00 |
| T5        | 955.67±3.47 | 966.00±5.51* | 965.67±3.38* | 952.68±3.17* | 938.00±4.16* | 955.60 |
| T6        | 954.00±2.81 | 965.00±2.65* | 968.33±5.55* | 944.00±3.60* | 930.67±3.48* | 952.40 |
| T7        | 956.00±3.78 | 966.33±5.76* | 960.33±4.91* | 953.68±2.58* | 948.00±4.16* | 956.87 |
| T8        | 954.67±3.52 | 961.67±2.33* | 959.00±5.86* | 951.68±2.03* | 946.67±3.52* | 954.74 |
| T9        | 954.33±3.18 | 965.00±3.05* | 959.33±2.19* | 947.00±4.16* | 942.00±3.46* | 953.53 |
| Mean      | 955.27 | 967.57 | 982.53 | 978.07 | 972.67 | 972.67 |

C.D. at 5% 10.02 13.03 10.82 10.09

Mean ± S.E  
*DAT: Days after treatment  
Values with the same superscript in same column do not differ significantly
### Table 3: Effect of Arsenic and Chromium on the survivability of adult earthworm, *E. eugeniae*.

| Treatment | 1 DAT | 7 DAT | 14DAT | 21 DAT | 28 DAT | Mean | C.D. at 5% |
|-----------|-------|-------|-------|--------|--------|------|------------|
| Control   | 50.00±0.00 | 50.00±0.00 | 49.33±0.33 | 48.67±0.33 | 48.33±0.33 | 49.27 | 2.12 |
| T1        | 50.00±0.00 | 49.67±0.33 | 48.67±0.33 | 47.67±0.33 | 46.00±1.00 | 48.40 | 2.12 |
| T2        | 50.00±0.00 | 48.67±0.33 | 47.67±0.33 | 47.00±0.57 | 45.67±0.88 | 47.80 | 2.12 |
| T3        | 50.00±0.00 | 47.33±0.33 | 47.33±0.33 | 46.00±1.00 | 44.67±0.33 | 47.27 | 2.12 |
| T4        | 50.00±0.00 | 49.00±0.57 | 45.67±1.20e | 46.33±0.67 | 44.00±0.57 | 47.00 | 2.12 |
| T5        | 50.00±0.00 | 47.33±0.33 | 44.33±1.20ab | 42.67±1.45 | 39.00±0.57 | 44.67 | 2.12 |
| T6        | 50.00±0.00 | 46.00±1.00 | 43.00±0.57 | 41.33±0.88 | 37.67±0.67 | 43.60 | 2.12 |
| T7        | 50.00±0.00 | 48.33±0.33 | 48.00±0.57 | 47.33±0.33 | 46.00±0.57 | 47.93 | 2.12 |
| T8        | 50.00±0.00 | 47.67±0.33 | 47.00±0.57 | 46.00±0.57 | 45.67±0.33 | 47.27 | 2.12 |
| T9        | 50.00±0.00 | 46.33±0.66 | 45.67±0.88 | 44.33±1.20 | 43.67±0.88 | 46.00 | 2.12 |
| Mean      | 50.00 | 48.13 | 46.67 | 45.73 | 44.07 | 44.07 | 1.96 |

Mean ± S.E
*DAT: Days after treatment.
Values with the same superscript in same column do not differ significantly

### Table 4: Effect of Arsenic and Chromium on the cocoons production by earthworm, *E. eugeniae*.

| Treatment | 1 DAT | 7 DAT | 14DAT | 21 DAT | 28 DAT | Mean | C.D. at 5% |
|-----------|-------|-------|-------|--------|--------|------|------------|
| Control   | 00.00±0.00 | 78.33±4.41 | 82.33±5.36 | 93.33±4.41 | 102.33±4.33 | 71.26 | 16.47 |
| T1        | 00.00±0.00 | 60.33±2.90 | 54.67±3.33 | 44.33±4.70 | 43.67±6.89 | 40.60 | 15.21 |
| T2        | 00.00±0.00 | 58.33±5.55 | 52.00±2.08 | 38.67±4.33 | 40.00±5.77 | 37.80 | 15.21 |
| T3        | 00.00±0.00 | 56.67±4.41 | 49.00±5.51 | 34.00±4.93 | 35.67±7.88c | 35.07 | 15.21 |
| T4        | 00.00±0.00 | 58.67±3.37 | 49.33±2.91 | 40.67±2.85c | 37.67±6.39a | 37.27 | 15.21 |
| T5        | 00.00±0.00 | 47.67±4.84 | 43.67±4.18 | 36.67±6.00 | 29.00±4.62 | 31.40 | 15.21 |
| T6        | 00.00±0.00 | 41.00±3.79 | 36.00±6.00 | 32.00±3.60 | 21.67±6.39c | 26.13 | 15.21 |
| T7        | 00.00±0.00 | 54.00±4.58 | 48.67±5.46 | 41.33±8.76 | 36.67±6.39b | 36.13 | 15.21 |
| T8        | 00.00±0.00 | 53.67±6.88 | 45.00±7.64 | 38.33±5.17 | 34.33±7.45 | 34.27 | 15.21 |
| T9        | 00.00±0.00 | 49.33±7.22 | 43.33±6.00 | 36.33±6.12a | 29.67±5.04a | 31.73 | 15.21 |
| Mean      | 00.00 | 55.80 | 50.40 | 43.57 | 41.07 | 41.07 | 18.46 |

Mean ± S.E
*DAT: Days after treatment.
Values with the same superscript in same column do not differ significantly

### Table 5: Effect of Arsenic and Chromium on the hatchling production of earthworm, *E. eugeniae*.

| Treatment | 1 DAT | 7 DAT | 14DAT | 21 DAT | 28 DAT | Mean | C.D. at 5% |
|-----------|-------|-------|-------|--------|--------|------|------------|
| Control   | 00.00±0.00 | 00.00±0.00 | 155.67±4.70 | 158.00±4.36 | 160.67±3.48 | 94.87 | 17.04 |
| T1        | 00.00±0.00 | 00.00±0.00 | 138.33±4.41 | 123.67±4.41 | 107.00±5.69 | 73.80 | 17.04 |
| T2        | 00.00±0.00 | 00.00±0.00 | 130.33±8.66 | 111.67±6.33 | 97.67±5.81 | 67.93 | 17.04 |
| T3        | 00.00±0.00 | 00.00±0.00 | 127.33±4.41 | 109.00±5.51 | 91.00±3.46 | 65.47 | 17.04 |
| T4        | 00.00±0.00 | 00.00±0.00 | 116.67±4.09 | 105.67±3.84 | 79.00±5.86 | 60.27 | 17.04 |
| T5        | 00.00±0.00 | 00.00±0.00 | 101.00±2.08 | 98.00±4.73 | 68.67±3.76 | 53.53 | 17.04 |
| T6        | 00.00±0.00 | 00.00±0.00 | 98.33±6.01 | 94.67±4.84 | 59.00±5.77 | 50.40 | 17.04 |
| T7        | 00.00±0.00 | 00.00±0.00 | 134.67±5.48 | 118.33±6.00 | 102.33±7.84 | 71.07 | 17.04 |
| T8        | 00.00±0.00 | 00.00±0.00 | 126.33±8.82 | 113.33±4.11 | 95.67±2.33 | 67.47 | 17.04 |
| T9        | 00.00±0.00 | 00.00±0.00 | 123.00±5.29 | 98.33±4.05 | 87.67±4.33 | 61.80 | 17.04 |
| Mean      | 00.00 | 00.00 | 125.37 | 113.07 | 94.87 | 15.08 | 17.04 |

Mean ± S.E
*DAT: Days after treatment.
Values with the same superscript in same column do not differ significantly
compared to control. Significant decrease in the number of adult worms was noticed when exposed to heavy metals in comparison with control on the 28th day of study. Increased mortality of worms due to long term exposure signifies heavy metals as toxicant (Khalil et al., 1996 and Khalil, 2013). Maximum reduction (24.66%) in survivability was seen in worms exposed to T6 trailed by worms exposed to T5 in which there is 22% reduction in survivability of earthworms was seen when compared with control. While 10.66% decrement in survivability has been seen at T3. Cr (VI) was seen to be more toxic when compared with As (V).

**Effect of heavy metals on reproductive rate of *E. eugeniae***: Reproduction rate in terms of cocoon production and hatching production is considered as sensitive parameter for ecotoxicological assessments. Therefore, the reproduction rate of earthworms was assessed in the terms of rate of cocoon production and hatching production. Non significant alterations in cocoon production in worms exposed to various treatments as compared to control after 21st day of treatment was also worth noticeable. After 28th day of treatment maximum decrease (47.14%) in number. of cocoon production was seen in T6 i.e. trailed by T5 Cr in which there is 39.16% decrease in no. of cocoons happens and the minimum hatchability of cocoons was seen in the T6 i.e. 39.99%. Arsenic had likewise an adverse impact on the production of cocoons and hatchlings as there is 37.05% and 28.53% decrease occurs in case of T3 when compared with control. Significant decrease in cocoon production was observed in worms exposed to various treatments as compared to control. Lower reproduction rates in heavy metal exposed worms may be due to constraints in copulation (Novais et al. 2011), faulty gamete development (Rongguan and Canyang, 2009), abnormal embryo development (Kumar et al., 2008), lesser hatching success and early growth due to stress (Sivakumar et al., 2009). Cocoon production was observed to be the most sensitive and effected parameter of heavy metal toxicity which may also be due to delayed maturation of worms and contaminated substrate as stated by Garg et al. (2009). Reduction in cocoon production due to heavy metal exposure has been previously reported by Maleri et al. (2007) and Kaur and Sangha (2014).

**CONCLUSION**

It can be concluded that both heavy metals As (V) and Cr (VI) were detrimental for the growth, survival and reproduction of the worm *E. eugeniae*. Chromium is more harmful to the worms as compared to arsenic and thus effective management strategies have to be developed in order to implement the proper disposal of pollutants to enhance worms’ survivability, thereby improving the sustainability of agroecosystems.

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