Life Cycle Parameters of the Earthworm *Eisenia fetida* Exposed to Cr(III) and Cr(VI) Amended Organic Substrates

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ABSTRACT

The effects of Cr(III) and Cr(VI) on the growth and reproduction of the earthworm *Eisenia fetida* have been assessed in organic substrate media. Both Cr species caused dose dependent reduction in the growth and maturation rates in earthworms; however, Cr(VI) was more toxic than Cr(III). The difference between the EC_{50} (50% effect concentration) and NOEC (no observed effect concentration) values for both Cr species to induce changes in earthworm growth was more than fivefold. The reproductive parameters (cocoon production, hatching success and hatchlings produced per worm) showed a decreasing trend as the treatment concentration of Cr was increased. The EC_{50} values of Cr(III) for cocoon production and hatchling numbers were more than four times higher than the values recorded for Cr(VI). The NOEC values for Cr(III) and Cr(VI) for earthworm growth and reproduction show that growth was less sensitive to both Cr species, than was reproduction. The body concentration of Cr in worms generally increase with an increase in the treatment concentration of both species of Cr. Compared to control P generation worms, the life cycle parameters of the F_1 generation worms did not show significant changes when exposed to uncontaminated organic substrate.

INTRODUCTION

Chromate and dichromate are discharged frequently into the environment, which results in increasing local chromium concentration in the soil. Elevated levels of Cr are present in the soil consequent to its introduction through wastewater from the metal finishing and tanning industries and land disposal of sewage sludge (Bluskov *et al.* 2005). These increased Cr levels are expected to have toxic effects on soil organisms, as well as potentially impacting plants and the herbivores that feed on them. Although many studies have been performed on the toxic effects of Cr on terrestrial plants (Dube *et al.* 2003; Cohen *et al.* 2006; Montes-Holguin *et al.* 2006) and microorganisms (Turpeinen *et al.* 2004; Branco *et al.* 2005) little is known on the toxicity of Cr toward soil dwelling invertebrates. Arillo and Melodia (1991) studied Cr(VI) metabolism in the earthworm *Eisenia fetida*. Soni and Abbasi (1981) and Abbasi and Soni (1983) reported lethal concentrations of Cr(VI) to two species of earthworms (*Octochaetus pattoni* and *Pheretima posthuma*). The toxic effects of Cr(III) on survival, morphology and burrowing behaviour of the earthworm, *Megascolex konkanensis* have been investigated by Maleeka Begum (1996). Reduction in reproduction of the earthworm *Eisenia andrei* exposed for three weeks to soil concentrations of 100 mg/kg and higher has been reported by Van Gestel *et al.* (1993). Chronic toxicity of Cr(III) was assessed for *E. fetida* by Lock and Janssen (2002a). The toxicity of Cr(III) and Cr(VI) on the survival, behaviour, and morphology of the earthworm *E. fetida*, in water and in ten different soils and cow dung have been assessed by Sivakumar and Subbhuraam (2005).

Growth is the net result of many essential processes, such as consumption, excretion and respiration. Long term experimental studies have indicated growth to be quite sensitive to pollutant stress. Reproduction is the single most important function in the life cycle of an organism. It is often noted that there are life stage differences in sensitivity to pollutants. Therefore, various studies have been conducted in order to investigate the effect of various heavy metals on growth and reproduction (cocoon production, hatching success and number of hatchlings produced) of the earthworm (Bindesbol *et al.* 2007; Jones *et al.* 2009; Owojori *et al.* 2009). Further, such studies have reported 50% effect concentration (EC_{50}) and no observed effect concentration (NOEC) values for the effect of various metals on growth and reproduction (Lock and Janssen 2002a, 2002b; Owojori *et al.* 2008; Wilkea *et al.* 2008; Loureiro *et al.* 2009).

A large volume of data on metal concentrations in earthworms in relation to soil pollution and comparatively limited data on the effects of metals on the growth and reproduction of earthworms are available. Studies on the effects of Cr in particular on the growth and reproduction of earthworms in organic substrate have not been carried out. The increasing presence of heavy metals, which may accumulate in earthworms, in organic wastes could have an adverse effect on earthworm life cycle parameters like growth and reproduction. Therefore, in ecotoxicological assessment, apart from survival, growth and reproduction are of particular importance because of their influence on population dynamics. Hence, in the present investigation the growth, maturation rate, cocoon production, hatching success of cocoons and number of offsprings produced per worm have been assessed in Cr [Cr(III) and Cr(VI)] amended and unamended organic substrates. Further, accumulation of Cr [Cr(III) and Cr(VI)] in the earthworm has been assessed. Few studies are currently available that report on multigeneration experiments with terrestrial invertebrates (Reinecke *et al.* 1999; Spurgeon and Hopkin 2000; Lock and Janssen 2002b). Therefore, the present study also encompasses studies on the effect of parental Cr [Cr(III) and Cr(VI)] exposure on the growth and reproductive status of the F_1 generation.

Original Research Paper

MATERIALS AND METHODS

General

A culture of the earthworm *E. fetida* was maintained in the Bharathiar University laboratory over a period of two years, using cow dung as the substrate and food. Earthworms were free to feed *ad libitum*. Analytical grades of chromium chloride [Cr(III) (CrCl$_3$.6H$_2$O)] and potassium dichromate [Cr(VI) (K$_2$Cr$_2$O$_7$)] purchased from Hi Media Laboratories Ltd, Mumbai, were used in the present study.

Experimental design

The substrate used in this study was sun-dried, urine-free cow dung that was ground and sieved (particle size 500 to <100 μm). 200 g of substrate were wetted with different concentrations of Cr(III) (50, 100, 200, 400, 800 and 1600 mg/kg) and Cr(VI) (10, 20, 40, 80, 160 and 320 mg/kg) solutions to a moisture content of 60%. The concentration of Cr III and Cr VI was selected based on preliminary studies conducted with a wide range of concentrations with both test metals (Range fixing test). The substrate was placed in a plastic container of dimensions 200 × 150 × 100 mm with a perforated lid covered with fine gauze. Each concentration received ten previously weighed preclitellate worms (20-days old). Earthworms that did not burrow into the medium by the following day were removed and new ones were introduced. The containers were maintained under laboratory conditions (28 ± 2°C) with a natural day light cycle. Cow dung mixed with the same concentration of Cr as that of the medium was provided as food once in 14 days and was supplied in a shallow depression made in the medium. Once a week, moisture loss was compensated with the addition of distilled water. Control was maintained in distilled water without addition of metal solutions. Three replicates were used for all exposures.

Experimental measurements

The worms were removed and hand sorted every 14th day over a period of 56 days (until the worms were 76 days old) and the development of clitella was noted [Maturation (%) = (Number of worms matured/total number of worms) × 100]. The weight of 10 worms was taken together and calculated as an average single worm weight (Single worm weight in mg = total worm weight in mg/total number of worms used in the experiment). Cocoons were collected at weekly intervals throughout the experimental period. At the end of the 56th day, three worms from each treatment were removed and kept on moist filter paper in Petri dishes for 24 h at room temperature (28 ± 2°C) to allow depuration of their gut contents. Subsequently they were washed with distilled water, dried by leaving on paper towels, and weighed and frozen in sample vials to analyse their Cr content. Total chromium content of each earthworm was analysed using AAS (Perkin Elmer, Model 2280) after digesting the samples with HNO$_3$ at 180°C for 16 h (Van Gestel *et al.* 1993). Biota-to-soil accumulation factor (BSAF) were calculated from measured Cr content in earthworm and total Cr content in soil (Cortet *et al.* 1999).

Weekly harvested cocoons were transferred to separate Petri dishes and incubated in distilled water. Distilled water was added until the cocoons were almost immersed and incubated in the dark at room temperature. Distilled water was changed every second day. Hatchlings and empty shells were counted and removed every day for a maximum of 30 days after transfer. The number of cocoons laid per worm, percentage of hatching success and number of hatchlings produced per worm were calculated using the following formulae:

Number of cocoons per worm = total number of cocoons/total number of worms

Hatching success (%) = (number of cocoons hatched/total number of cocoons) × 100

Number of hatchlings produced per worm = total number of hatchlings/total number of worms.

Eight to ten healthy hatchlings from each of the treatments were carefully removed on the same day and grown in separate Petri dishes containing urine-free, dried cow dung (500 to ≤ 100 μm) until they were 20-days old. Five preclitellate (20-days old) F$_1$ generation worms were removed from each concentration and grown in uncontaminated cow dung medium, and their growth in terms of biomass and reproduction were observed over a period of 56 days (until the worms were 76-days old).

Statistical analysis

Fifty percent effect concentration (EC$_{50}$) values were estimated by probit analysis. One-way analysis of variance (ANOVA) was performed to evaluate the significance of the observed differences in the biomass, maturation, number of cocoons laid per worm and number of hatchlings produced per worm and to establish the no observed effect concentration (NOEC). The significance of the observed differences in the data pertaining to the effect of concentration as well as period of growth on the growth of the worm recorded periodically was statistically analysed using two-way ANOVA.

RESULTS AND DISCUSSION

During the entire study period, all earthworms survived in the control medium, but all worms died in 800 mg Cr(III)/kg and 160 mg Cr(VI)/kg exposure. In both Cr exposures, a clear concentration-response relationship was observed (**Figs. 1, 2**). Two-way ANOVA revealed that there were significant differences in growth of worms both among Cr(III) and Cr(VI) concentrations and among durations. The dose-dependent growth reduction in this study is in agreement with the earlier reports on the sensitivity of growth and growth reduction to exposure to toxic chemicals like Cd and Cu (Van Gestel *et al.* 1991; Spurgeon *et al.* 2004), Cd, Cu, Pb and Zn (Spurgeon and Hopkin 1995), Cd, Mn, Cu, Pb and Zn (Reinecke and Reinecke 1996), Cu and Zn (Reinecke *et al.* 1997), Ag, Cd, Pb and Zn (Nahmani *et al.* 2007), Cd (Rozen 2006; Nakashima *et al.* 2008,) and Zn (Owojori *et al.* 2009) (**Table 1**). Unlike the present study, wherein significant growth reduction was observed at even as low as 100 mg of Cr(III) and 20 mg of Cr(VI)/kg of substrate (**Figs. 1, 2**), respectively, significant growth reduction was reported only at and above 1000 mg Cr(III)/kg *E. andrei* (Van Gestel *et al.* 1993), and no report has been made so far on the growth effect of Cr(VI) in any species of earthworm.

Maturation is normally associated with attainment of a certain body size; therefore, it is more related to the growth of the earthworm. In this experiment, all control worms and worms exposed to ≤ 100 mg Cr(III)/kg and ≤ 20 mg Cr(VI)/kg reached maturity within 56 days (**Figs. 3, 4**). Above these concentrations, growth was slower and not all worms matured at the end of the experiment (**Figs. 3, 4**). Worms exposed to Cr showed a dose-dependent decrease in the maturation rate. This maturation rate did increase over time, during the growth period. This increase was slower in worms exposed to higher concentrations of Cr.

Cocoon production was significantly (P<0.01) affected by both Cr exposures. The mean number of cocoons produced per control worm was 7 ± 0.3 over the observation period of 76 days, while that of the Cr exposed worms was <5.3 (**Table 2**). The observed reduction in cocoon production with increasing concentration of both species of Cr is proof of their reproductive toxicity. The requirement of a higher range of Cr(III) concentration (five times higher than that of Cr(VI)) to cause such an effect is yet another proof of the lower toxic potential of Cr(III), compared with Cr(VI). Furthermore, the complete inhibition of cocoon production at the highest treatment concentrations of Cr(III) and Cr(VI) in which earthworms survived with signs of limited growth and maturation rates shows their greater impact on reproduction than on growth and maturity. Van Gestel *et al.* (1993) reported a significant reduction in reproduction of earthworms (*E. andrei*) at a concentration of 150 mg

Table 1 Studies investigating growth reduction by metal exposure to earthworm.

References	Species name	Metals	Substrate used
Van Gestel *et al.* 1991	*Eisenia andrei*	Cd and Cu	Artificial soil
Spurgeon and Hopkin 1995	*Eisenia fetida*	Cd, Cu, Pb and Zn	Artificial soil
Reinecke and Reinecke 1996	*Eisenia fetida*	Cd, Mn, Cu, Pb and Zn	Organic substrate
Spurgeon *et al.* 2004	*Lumbricus rubellus*	Cu and Cd	Soil
Rozen 2006	*Dendrobaena octaedra*	Cd	Soil
Nahmani *et al.* 2007	*Eisenia fetida*	Ag, Cd, Pb and Zn	Soil
Nakashima *et al.* 2008	*Eisenia fetida*	Cd	Soil
Owojori *et al.* 2009	*Eisenia fetida*	Zn	Artificial soil

Fig. 1 The effect of different concentrations of Cr(III) (mg/kg) on the growth rate (weight/worm in mg) of the earthworm, *E. fetida*. Error bars represent standard deviation (n=30). * $P < 0.05$, ** $P < 0.01$.
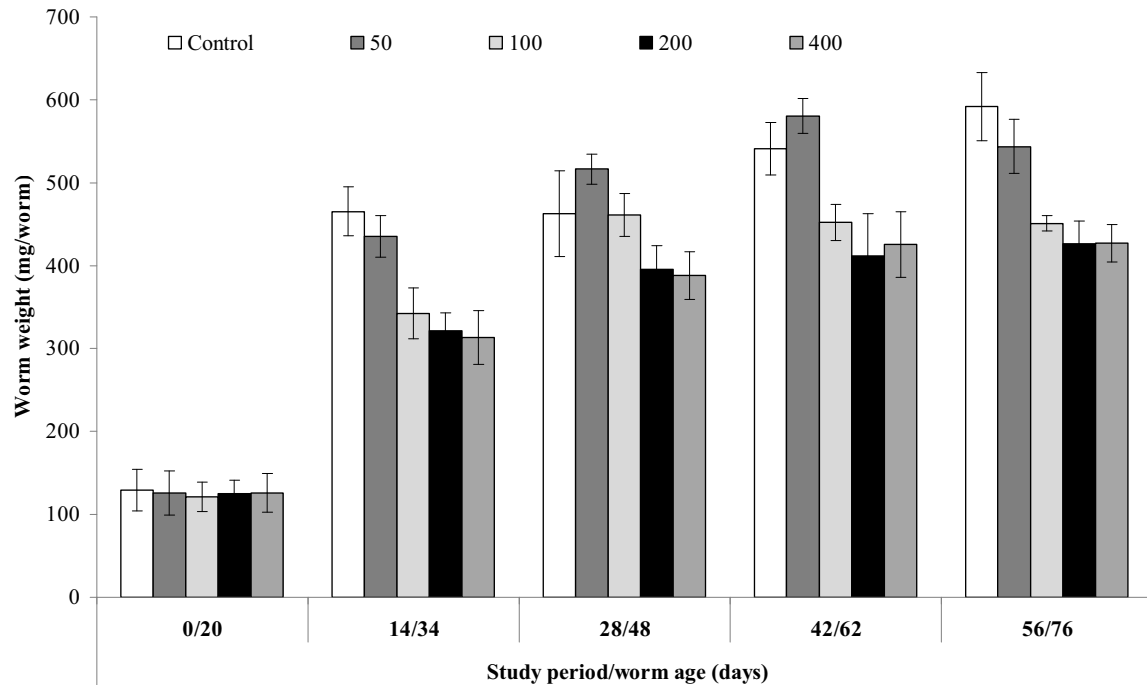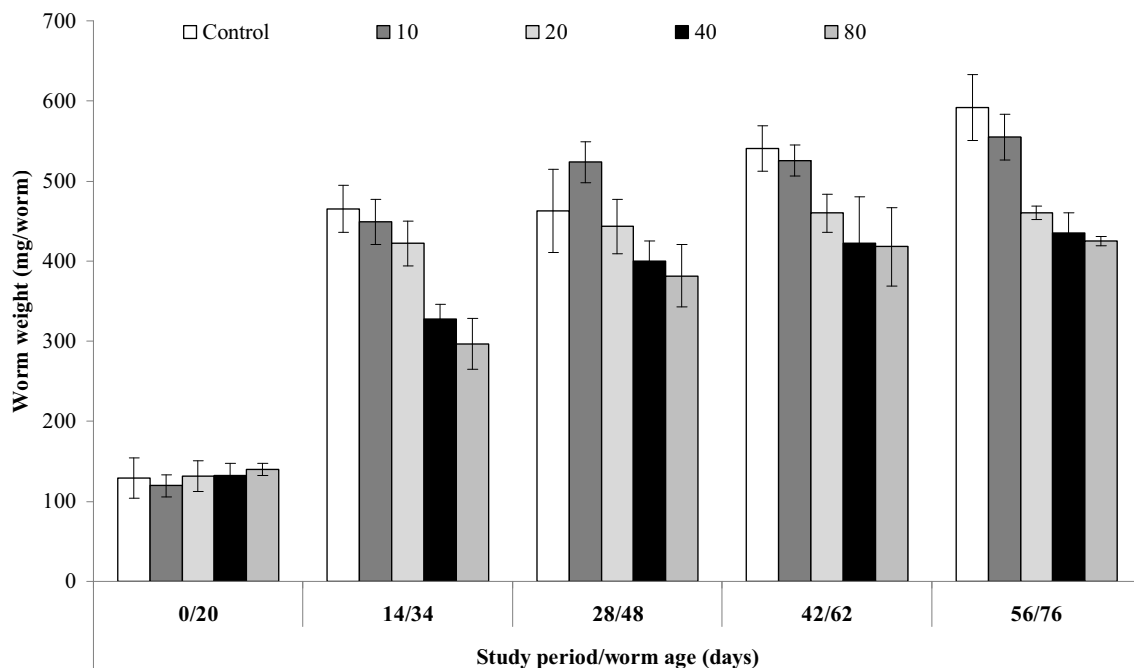

Fig. 2 The effect of different concentrations of Cr(VI) (mg/kg) on the growth rate (weight/worm in mg) of the earthworm, *E. fetida*. Error bars represent standard deviation (n=30). * $P < 0.05$, ** $P < 0.01$.

Cr(III)/kg dry soil. However, in the present study a significant reduction was recorded in cocoon production at a much lower concentration of 50 mg Cr(III)/kg and, in the case of Cr(VI), at 10 mg/kg. Therefore, *E. fetida* appears to be more sensitive than *E. andrei* to Cr(III).

Reduced hatching success is considered as the most common index of stress in organisms (Birge *et al.* 1979; Peakall 1983; Nahmani *et al.* 2007). In the present study, hatching success was higher in control worms (64%) and lower in worms exposed to both species of Cr (<64%)
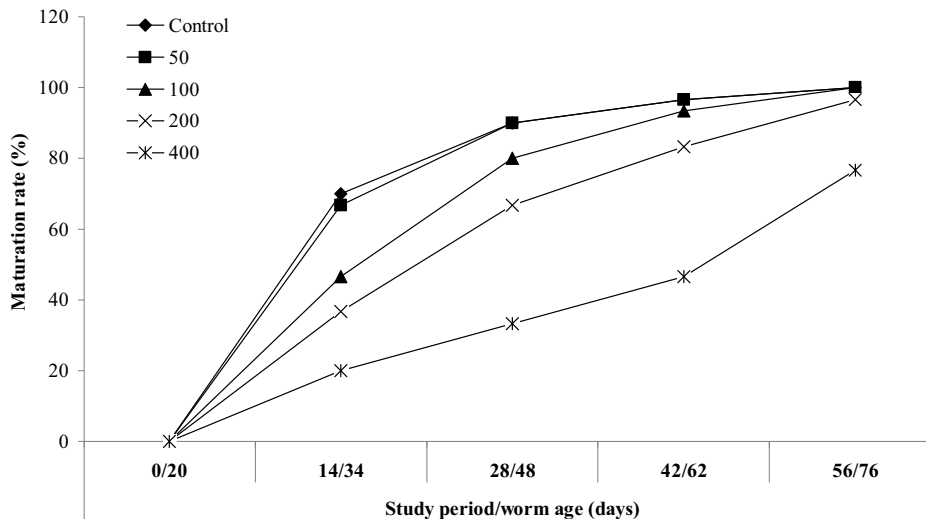
Fig. 3 Percentage of maturation rate of the earthworm *E. fetida* (*n*=30) exposed to different concentrations of Cr(III) (mg/kg).
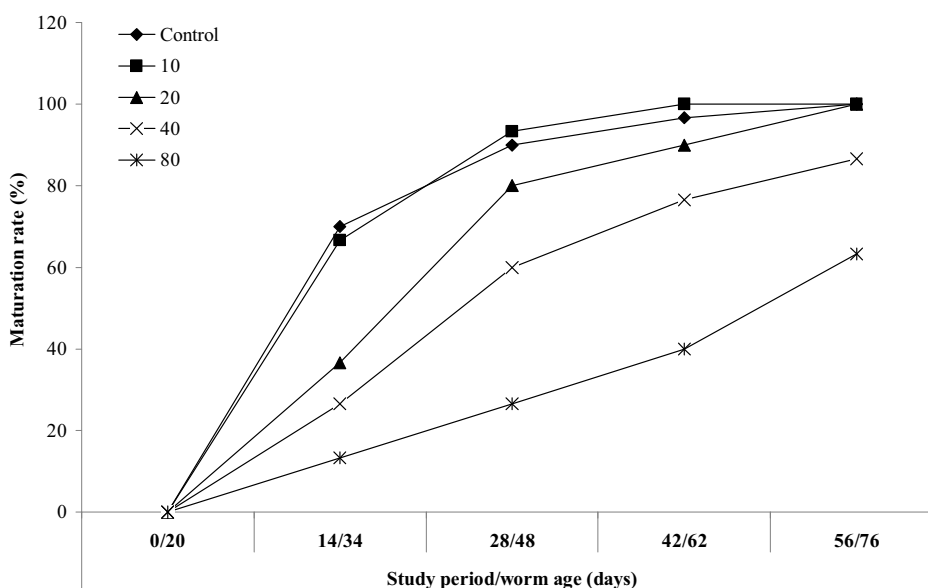

Fig. 4 Percentage of maturation rate of the earthworm *E. fetida* (*n*=30) exposed to different concentrations of Cr(VI) (mg/kg).

Table 2 Mean number of cocoons produced per worm, percentage of hatching success of cocoons and mean number of hatchlings produced (per worm) over a period of 56 days by the earthworm *E. fetida* (P generation) exposed to Cr(III) and Cr(VI) in organic substrate.

Reproductive parameter	Cr (III) concentration in mg/kg					Cr (VI) concentration in mg/kg				
	Control	50	100	200	400	Control	10	20	40	80
Cocoons / worm [a]	7 ± 0.30	$5 \pm 0.87^{**}$	$5 \pm 0.70^{**}$	$3 \pm 0.56^{**}$	$1 \pm 0.47^{**}$	7 ± 0.30	$5 \pm 1.00^{**}$	$4 \pm 0.75^{**}$	$2 \pm 0.58^{**}$	$1 \pm 0.26^{**}$
Hatching success (%) [b]	64	61^{**}	42^{**}	32^{**}	26^{**}	64	63^{**}	36^{**}	35^{**}	30^{**}
Hatchlings produced per worm [c]	5 ± 0.52	5 ± 0.52	$2 \pm 0.11^{**}$	$1 \pm 0.30^{**}$	$0.4 \pm 0.40^{**}$	5 ± 0.52	5 ± 0.60	$2 \pm 0.15^{**}$	$1 \pm 0.05^{**}$	$1 \pm 0.17^{**}$

[**] $P<0.01$
[a] mean ± SD (*n*=30)
[b] Hatching success (%) = (number of cocoons hatched/total number of cocoons) x 100
[c] Hatchlings produced per worm = [total number of hatchlings/Total number of worms (*n*=30)]

(**Table 2**). Hatching success was significantly reduced at the lowest treatment concentrations of 50 mg of Cr(III)/kg and 10 mg of Cr(VI)/kg, but total failure of hatching of cocoons was not observed. In the studies of Reinecke and Venter (1985), Venter and Reinecke (1988), Hartenstein *et al.* (1980), Vail (1974) and Watanabe and Tsukamoto (1976), between 33 and 88% of the cocoons of *E. fetida* hatched in control worms and worms grown in metal added substrate. Control worms and worms exposed to 50 mg Cr(III)/kg and 10 mg Cr(VI)/kg produced 5 hatchlings per worm, and hatchlings per worm were significantly (P<0.01) lower above these concentrations (**Table 2**). Until now, no effect of either of the species of Cr on the hatching success of cocoons had been reported in any earthworm species.

The NOEC values of growth and number of hatchlings per worm were found to be 50 mg/kg for Cr(III) and 10 mg/kg for Cr(VI). The NOEC values of cocoon production were < 50 mg/kg for Cr(III) and <10 mg/kg for Cr(VI). Van Gestel *et al.* (1992) reported a 21-day NOEC value of 320 mg/kg dry soil for the effect of Cr(III) on cocoon production by *E. andrei*, and Lock and Janssen (2002a) reported a 21-day NOEC value of 560 mg Cr(III)/kg dry artificial soil for cocoon production in *E. fetida*. The present study recorded comparatively low NOEC values. The observed differences between the NOEC values in different studies may be due to differences in the substrates and/or the period of NOEC measurement. The NOEC values for the effects of Cr(III) and Cr(VI) on growth and reproduction show that cocoon production is more sensitive than growth and number of hatchlings. Van Gestel *et al.* (1992) concluded that

Table 3 EC$_{50}$ values (Median effective concentration) (mg/kg) for the effects of Cr(III) and Cr(VI) on the growth and reproduction of *E. fetida*.

Cr species	Growth of worms (Study period/worm age in days)				Reproduction	
	14/34	28/48	42/62	56/76	Cumulative cocoon production	Total number of hatchlings
Cr(III)	308.07 (-)	458.12 (-)	378.52 (-)	332.11 (-)	130.70 (83.08 - 188.78)	106.80 (63.42 - 164.93)
Cr(VI)	67.59 (-)	89.56 (-)	77.29 (-)	68.08 (-)	21.73 (13.32 - 31.34)	22.95 (12.93 - 37.40)

95% confidence limit values are given in parentheses.

Table 4 Biota to soil accumulation factors (BSAF).

	Cr(III) conc. (mg/kg)			Cr(VI) conc. (mg/kg)		
Exposure concentrations	Total chromium content (mg/kg fresh weight)	BSAF [a]	Exposure concentrations	Total chromium content (mg/kg fresh weight)	BSAF [a]
Control	4.6	1.991	Control	4.6	1.991
50	10.7[**]	0.213	10	9.2[**]	0.920
100	14.1[**]	0.140	20	13.2[**]	0.659
200	13.4[**]	0.067	40	13.1[**]	0.327
400	21.0[**]	0.052	80	21.5[**]	0.268
Mean	12.76		Mean	12.32	

[**] $P<0.01$
[a] BSAF = Cr content in worm/Cr content in media

for some chemicals (cadmium, paraquat, fentin, benomyl, phenmedipham), cocoon production is the most sensitive parameter, while for others (pentachlorophenol, parathion, carbendazin), cocoon hatchability is most likely to be affected. Additionally, as an overall parameter to describe the effects of chemicals on earthworm reproduction, the total number of juveniles produced per worm per week appeared to be useful. In particular, this parameter is the most sensitive in the case of Cr(III), being a factor of 10 more sensitive than cocoon production. However, in the present study such a wide difference was not observed between the NOEC values for the effects of either of the species of Cr on cocoon production or number of juveniles produced for the entire study period of 56 days.

The EC$_{50}$ value for the effect of Cr(III) on growth was more than five times greater than the EC$_{50}$ values computed for Cr(VI) (**Table 3**). The EC$_{50}$ values for the effects of Cr(III) on cocoon production and number of hatchlings were more than four times higher than the values recorded in Cr(VI) (**Table 3**). The difference between the Cr(III) and Cr(VI) values for EC$_{50}$ and NOEC in terms of the growth of the earthworm was more than five-fold. The differences in the EC$_{50}$ and NOEC values recorded in the present study between Cr(III) and Cr(VI) in terms of their effects on cocoon production were also around five-fold, providing evidence that Cr(VI) is more toxic against reproduction than is Cr(III). For invertebrates, a stress-related decrease in adult body weight has been positively correlated with a reduction in the number of eggs per female, indicating reduced reproductive success (Buikema *et al.* 1980). Van Gestel *et al.* (1992) have reported an EC$_{50}$ value for the effect of Cr(III) on *E. andrei* cocoon production of 155 mg/kg dry soil. Molnar *et al.* (1989) found a 50% reduction of the 56-day *E. fetida* reproduction at around 250 mg Cr(III)/kg wet weight in a substrate of peat mixed with horse manure. According to Lock and Janssen (2002a), the 21-day EC$_{50}$ value for the effect of Cr(III) on cocoon production by *E. fetida* was 892 mg/kg dry weight of artificial soil, and cocoon production ceased at 1000 mg/kg dry weight. Differences exist between the periods of EC$_{50}$ assessment in this study and others; hence, it is difficult to compare the toxicity data to arrive at meaningful conclusions.

The increase in earthworm body Cr concentration correlated with an increasing range of substrate Cr concentrations. The mean Cr concentration in the control worms (4.6 mg/kg) was significantly (P<0.01) higher when compared to both Cr exposures (**Table 4**). The BSAF values were < 1 in all Cr concentrations except in the control. Further, the BSAF values showed a decrease with an increase in the amendment concentration of both species of Cr. Chromium's toxicity is dependent on its chemical form, and chromate, Cr(III), tends to be less toxic than dichromate, Cr(VI) (Sivakumar and Subbhuraam 2005; Frederic *et al.* 2008). The uptake of Cr(III) is largely a passive process,

whereas the uptake of Cr(VI) is mediated by the sulphate carrier but with lower affinity (Skeffington *et al.* 1976). This is based on the fact that metabolic inhibitors (e.g., sodium oxide and dinitrophenol) inhibit Cr(VI) uptake by barley seedlings but not that of Cr(III) (Skeffington *et al.* 1976). In addition, it has been reported that the interconversion of different Cr forms is quite frequent in soil and water (Cervantes *et al.* 2001). The questions remaining to be answered are: If the prevailing views that Cr(III) compounds are scarcely capable of penetrating biological membranes and are very poorly absorbed by the intestine (Donaldson and Barreras 1966; Langard 1982) are accepted, how did chromium chloride exert lethal action on the earthworm? Are earthworm skin and/or intestine permeable to Cr(III)? If they are not permeable, is the observed accumulation of Cr in the body of the earthworm exposed to chromium chloride the consequence of its oxidation to Cr(VI)? Though it is not known either how or in what form Cr(III) entered the earthworm and exerted its toxicity, the observed Cr accumulation in the body of the earthworm exposed to chromium chloride in the present study indicates that Cr(III) ions as a pollutant can enter through the earthworm's skin; however, it required a much higher concentration (5-fold) to exert toxic action comparable to that of Cr(VI). Sivakumar and Subbhuraam (2005) reported the lowest LC$_{50}$ values for both species of Cr in organic medium (1635 mg/kg for Cr(III) and 219 mg/kg for Cr(VI)) compared to the values recorded in different physicochemical characteristic soils (1656 to 1902 mg/kg for Cr(III) and 222 to 257 mg/kg for Cr(VI)), further complicating the situation in that Cr(VI) would be reduced by the organic matter. Although it is known that interaction between Cr(III) and organic ligands leads to the formation of mobile, organically-bound Cr(III) (Srivastava *et al.* 1999), the difference maintained in the LC$_{50}$ values between Cr(III) and Cr(VI) in the organic medium as in soils (Sivakumar and Subbhuraam 2005) is intriguing, and further study is required to find out the actual mechanism by which both species enter the earthworm body.

Earthworms exposed to more than 200 mg Cr(III)/kg and 40 mg Cr(VI)/kg did not produce enough hatchlings for further study of F$_1$ generation worms. The reduction in the number of cocoons produced, their hatching success and number of hatchlings produced as the substrate amended concentration of Cr(III) and Cr(VI) was increased, is suggestive of a progressively reduced allocation of energy for cocoon production and differential energy allocation among those cocoons produced; resulting in the production of fewer healthy juveniles per worm. The F$_1$ offsprings hatched from Cr(III) and Cr(VI) exposed worms exhibited no significant differences in their growth (**Fig. 5**), maturation (**Fig. 6**), cocoon production, hatching success of the cocoons and hatchlings produced per worm (**Table 5**) compared to the F$_1$ offsprings produced from control worms in P generation.
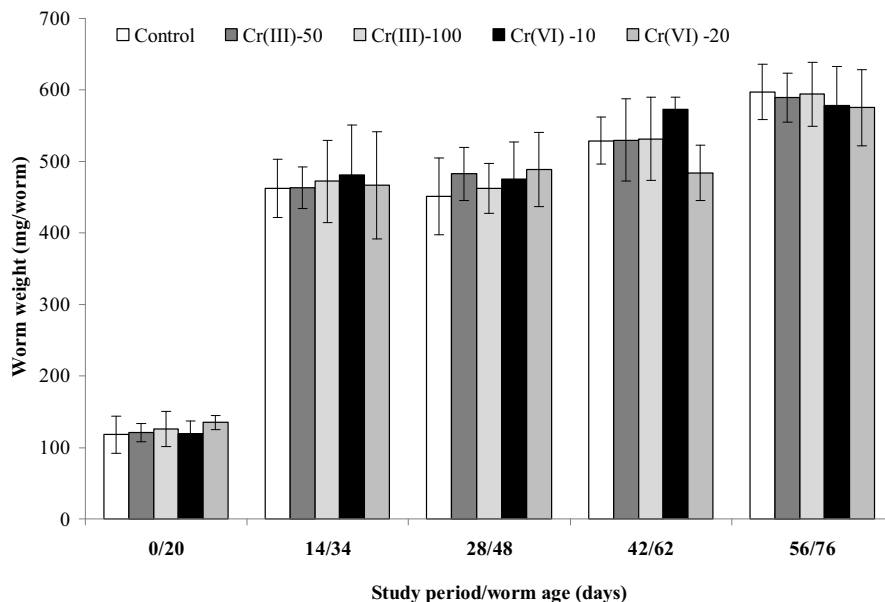
Fig. 5 The growth rates of the F_1 generation earthworms in Cr unamended substrate. Concentrations in this figure indicate parental generation earthworm exposure.
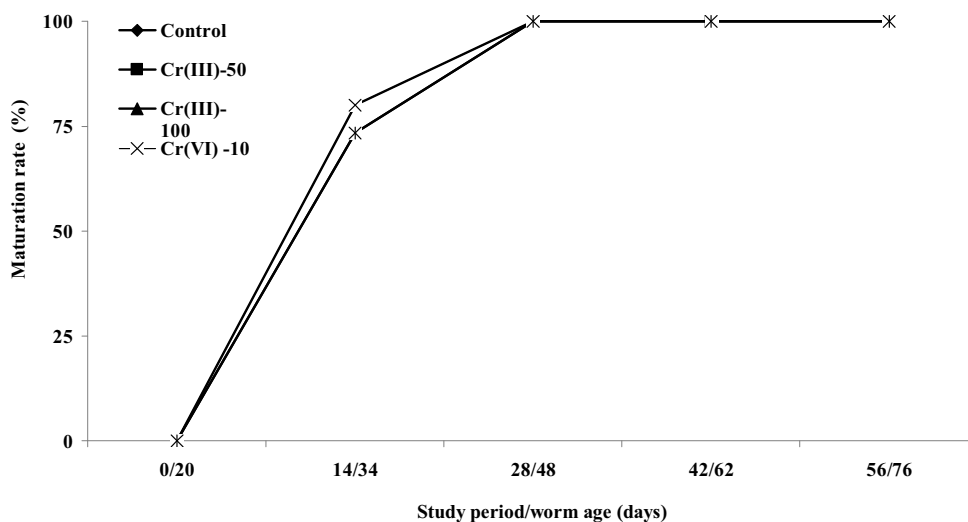

Fig. 6 The maturation rates of the F_1 generation earthworms in Cr unamended substrate. Concentrations in this figure indicate parental generation earthworm exposure.

Table 5 Reproduction of the F_1 generation earthworms in Cr unamended substrate.

Reproduction	Control	Cr(III) concentration (mg/kg)[*]		Cr(VI) concentration (mg/kg)[*]	
		50	100	10	20
No. of cocoons per worm [a]	7 ± 0.46	7 ± 0.70	6 ± 0.80	6 ± 0.57	7 ± 0.70
Hatching success (%) [b]	61	60	64	60	64
Hatchlings per worm [c]	5 ± 0.40	5 ± 0.50	5 ± 0.41	5 ± 0.52	5 ± 0.42

[*] Parental generation earthworms exposure concentrations
[a] mean ± SD (n=30)
[b] Hatching success (%) = (number of cocoons hatched/total number of cocoons) x 100
[c] Hatchlings produced per worm = [total number of hatchlings/Total number of worms (n=30)]

CONCLUSIONS

In conclusion, the result of the present investigation shows that both species of Cr (III and VI) affected the growth, maturation, reproduction and survival of the earthworm *E. fetida*. The NOEC values for the effects of Cr(III) and Cr(VI) on growth and reproduction show that growth is less sensitive than reproduction. The difference between the EC_{50} values of Cr(III) and Cr(VI) for growth was five-fold and cocoon production was four-fold. The difference between the Cr(III) and Cr(VI) values for EC_{50} and NOEC for growth and cocoon production were $\geq$ five-fold, providing evidence that both Cr species are more toxic for reproduction than growth. Further study is required to determine the actual mechanism by which both Cr species' entered the earthworm body. The growth, maturation rate and reproduction of the F_1 generation worms produced by Cr-exposed worms did not alter significantly from those of the control earthworms.

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