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Laboratory Studies on the Effect, Uptake and Distribution of Chromium in *Eisenia foetida* (Annelida, Oligochaeta)

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With 5 Figures and 1 Table

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Key words: *Eisenia*, chromium, toxicity, accumulation

Abstract

The effect of chromium as chromic and chromate salts on the growth and reproduction of *E. foetida*, and the uptake and distribution of this metal were examined using both radiotracer methods and electron microscope X-ray microprobe analysis. Results indicated that both growth and reproduction were significantly retarded by sublethal concentrations of chromium. The magnitude of the effect was concentration dependent. Freshly introduced chromium (VI) was more toxic than chromium (III), but the toxicity of chromium (VI) decreased after a delay period.

E. foetida was capable of taking up and retaining considerable amounts of chromium. More chromium was accumulated in the gut-chloragogenous system than in the tissues of the body wall. The accumulated chromium could be eliminated by ejection of eleocytes through the dorsal pores. The chromium content of *E. foetida* showed positive relation with the chromium content of the substrate, however, the relation between the ratios of storage and environmental concentrations was inverse. Maximal accumulation of chromium was found in juveniles which hatched and grew in polluted substrate.

Introduction

Chromium is an essential trace element involved in glucose and lipid metabolism (HWANG et al. 1987, LANGARD 1982). However, in high dosages chromium (VI) is considered to be a highly cytotoxic substance, and its mutagenic and carcinogenic effects are well documented (COSTA et al. 1984, FORNACE et al. 1981, LANGARD 1982). As a primary effect, chromate may reduce the electrical potential across the cell membrane, and this will result in a reduced capability to transport essential metabolites, and may contribute, with other intracellular effects, to the overall cytotoxicity of chromate (BRUN et al. 1987).

Chromate is taken up readily by cells, while chromic complexes are only transported to a limited extent (COSTA et al. 1984, LANGARD 1982, SANDERSON 1976). Chromate is a strong oxidizing agent, which has been shown to be reduced by cytoplasmic extracts. When cells have been treated with chromate, only the chromic form was found to be present intracellularly (JENNETTE 1979, LANGARD 1982, SANDERSON 1976). Chromium (III) has a strong tendency to form coordination complexes with a very slow rate of

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ligand exchange. In cell-free systems, chromic but not chromate salts were found to produce DNA-protein crosslinks, suggesting that the trivalent state may be the final active form. Therefore, the ultimate carcinogen might be chromium (III) (COSTA et al. 1984, FORNACE et al. 1981).

Whereas the chromic ion cannot pass through the cell membrane, cultured cells exposed to chromate have the ability to concentrate chromium intracellularly, against a concentration gradient, by reduction of chromate to chromic. This maintains a gradient for chromate whilst the reduced trivalent chromium cannot pass out of the cells again (SANDERSON 1976).

Chromium is widely used in the metal finishing and tanning industries. As these industries are often widely dispersed, supervision and control of chromium waste disposal is often difficult. Large amounts of anthropogenic heavy metals, including chromium, may be introduced into soils through waste-waters and through land-disposal of sewage sludges. Earthworms are heavily exposed to soil pollutants (ANDERSEN and LAURSEN 1982, BEYER 1981, CARTER et al. 1983, FLECKENSTEIN and GRAFF 1982, HELMKE et al. 1979, NEUHAUSER et al. 1984), and form an important link in natural food chains. In addition to their role in soil processes, they can accumulate and transfer beneficial and potentionally hazardous materials alike from soil to predators (BEYER 1981, IRELAND 1977, 1983). Among heavy metals, little is known about the uptake and retention of chromium by earthworms. The accumulation of heavy metals may depend not only on the extent of soil contamination alone, but also on a number of soil- and species-related variables (IRELAND 1983, MA 1982). *E. foetida* is widely used in the management of sludge, but only sparse informations are available concerning chromium uptake and their sensitivity to chromium.

The purposes of the present study were to provide information on 1. toxicity, 2. uptake and accumulation, and 3. the distribution and elimination of chromium in *E. foetida* when the metal was used in chromate and chromic forms.

Materials and Techniques

Eisenia foetida (Savigny) was obtained from stock cultures of our laboratory. The culture medium contained uncontaminated peaty soil and horse manure as food. Experiments were carried out at room temperature.

Growth and reproduction studies

The toxicity and the effect of chromic and chromate compounds on the growth and reproduction of *E. foetida* were studied by adding different concentrations (250—2500 mg · kg^{-1} wet wt) of chromium supplements to the culture medium. $K_2Cr_2O_7$ was used for chromium (VI), and $KCr(SO_4)_2$ for chromium (III) experiments. To evaluate the effects of chromium on the growth and reproduction of the worms over eight weeks plastic bags containing 1000 g wet weight substratum were used. Five worms were placed in each bag and three replicates of each experiment were made. The average initial age of the introduced worms was six-seven weeks, the average live-weight was 208 ± 26 mg. The number and the live-weight of the worms, the number of the cocoons and hatchlings were counted bi-weekly. Full cocoons and hatchlings were counted together as the number of progenies.

Two alternative studies were used in chromate studies. Earthworms were introduced into the toxicated culture medium immediately or after a two-week delay.

^{51}Chromium uptake studies

Uptake of chromite by the worms during exposure was determined by incubation with $^{51}CrCl_3$ containing $KCr(SO_4)_2$ solution. In chromate uptake studies $Na_2{}^{51}CrO_4$ containing K_2CrO_4 was used. Chromium solutions were mixed with the culture medium and clitellated adults of *E. foetida* were

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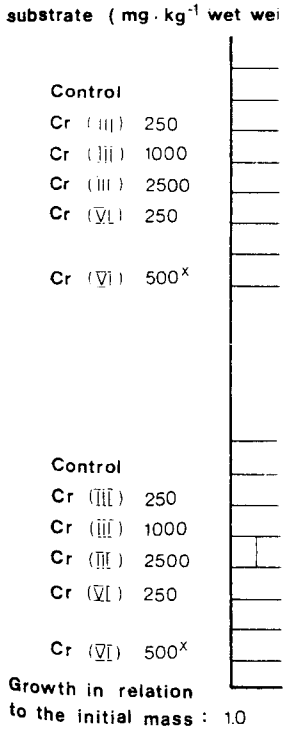

Fig. 1. The effect

introduced immediately. The ^{51}chromium content of the whole body of the adults, cocoons and juveniles were determined three times during the six week experiment. The ^{51}chromium concentrations of the body wall and of the gut-chloragogenous system (alimentary canal) of the dissected adults were determined.

To determine the amounts of incorporated ^{51}Cr, worms fed on the ^{51}Cr labelled substratum were dissected, and the gut content carefully removed. Dissected worms were washed three times in tap-water. Cocoons were analysed without dissection. Samples were dried overnight at 120 °C, then ashed at 600 °C for six hours. The ash was measured in a shielded counter with automatic energy selective scaler. The equipment was standardized with a known activity of ^{51}Cr.

X-ray microprobe analysis (EMMA)

For X-ray microprobe analysis of the chloragosomes air-dried smear preparations were used. Both preparation technique and X-ray microprobe analysis were performed as described in 1980 (FISCHER and TROMBITÁS). Earthworms eject coelomic fluid through the dorsal pores, in response to mechanical irritation. The eleocytes of the ejected coelomic fluid were prepared with the same technique. EMMA spectra both of the chloragosomes and eleocyte granules prepared from untreated and from long-term (three months) chromate treated earthworms were compared.

Results

The effects of chromium on growth and reproduction

Among the concentrations used, only the freshly prepared 500 mg · kg^{-1} wet wt chromate containing substratum, caused significant mortality (Fig. 1). However, sublethal

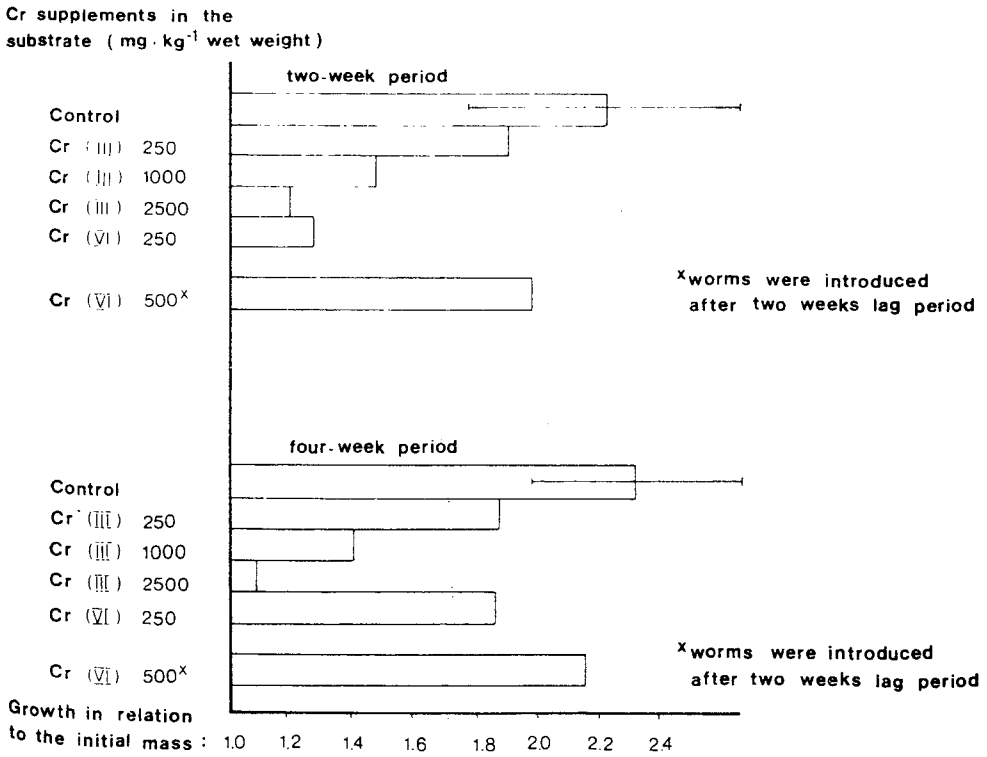

Fig. 1. The effect of chromium on the mass gain of juvenile *Eisenia foetida*

effects, manifested by retarding of growth, were observed in both chromium (VI) and chromium (III) containing media. The growth retarding effect of chromium (III) depended on the concentration of the metal, and proved to be a long-term influence. The growth retarding effect of freshly prepared chromium (VI) containing media showed greater magnitude than those of chromium (III), but decreased significantly during the experiments. In experiments in which worms were introduced into the 500 mg chromium (VI) added substratum after a two-week delay, the lethal effect was diminished and the retarding effect on growth was reduced significantly (Fig. 1).

Reproduction of *E. foetida* was substantially decreased by all concentrations and forms of chromium used in these experiments. The inhibitory effect of chromium (III) on the reproduction was concentration dependent (Fig. 2).

Uptake and distribution of chromium

Metal uptake studies using ^{51}chromium indicated that chromium (VI) could be accumulated in greater amounts than chromium (III) (Fig. 3). Chromium concentrations in the adult worms depended on the environmental concentration of this metal (Fig. 3

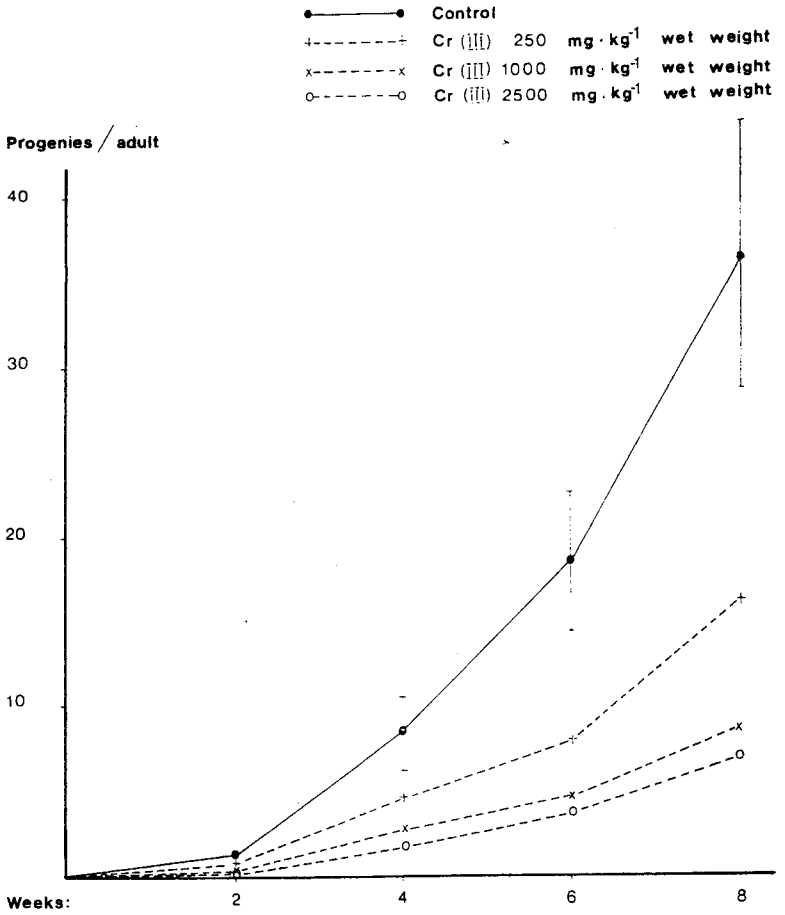

Fig. 2. The effect of chromium on the reproduction of *Eisenia foetida*

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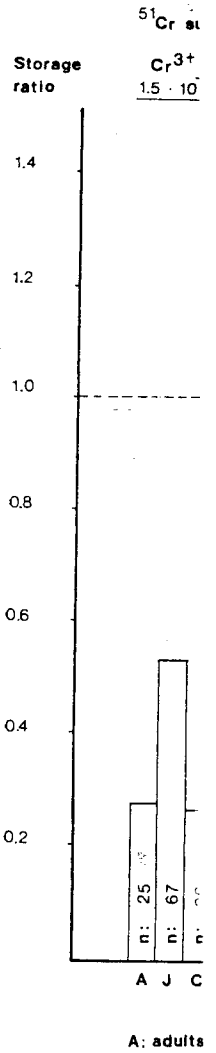

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and 4). The chromium content of the adults was increased by elevated environmental chromium concentrations, at least in the sublethal ranges, while concentration factors (i.e. storage ratios) decreased (Fig. 4). Cocoons also accumulated chromium. Juveniles, hatched and grown in chromium containing media contained more metal than worms which were introduced as adults. Concentration factors of the juvenile were greater than 1.0 in two of the chromium (VI) treatments if calculated on a dry-weight basis (Fig. 3).

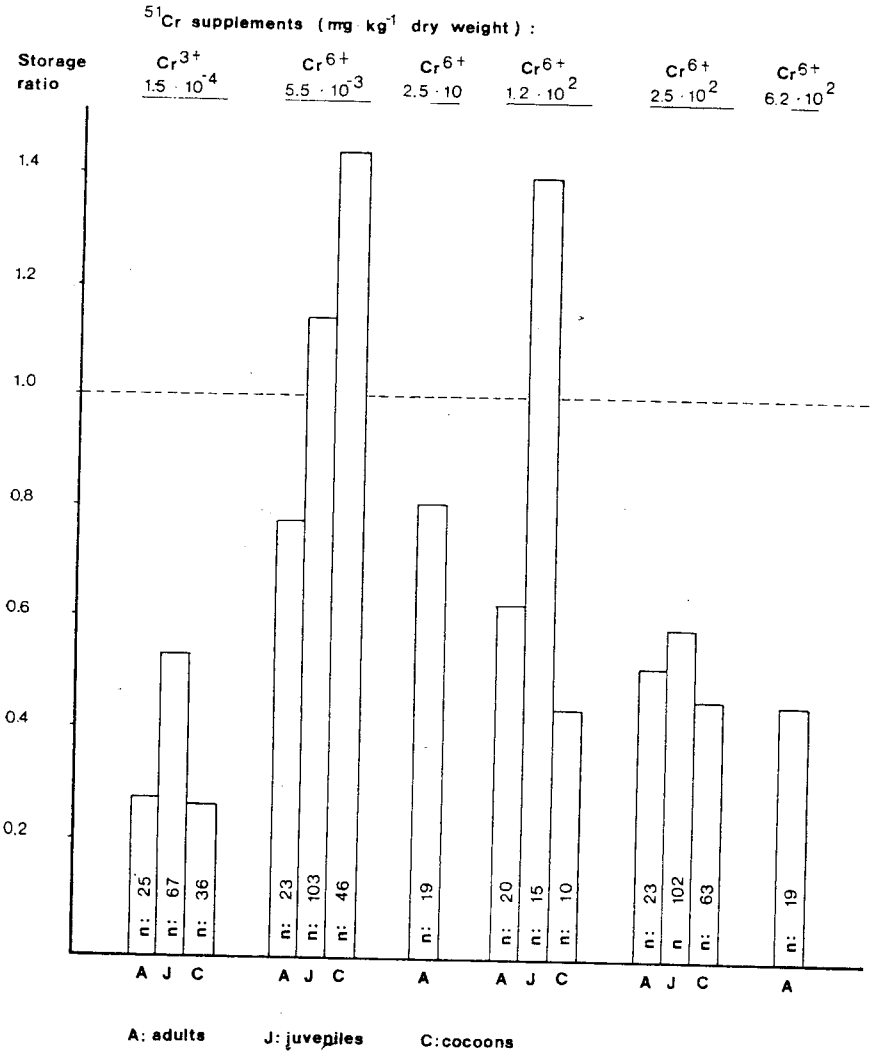

Fig. 3. Influence of the state and the concentration of chromium in the soil on the chromium content of *Eisenia foetida*

The ^{51}Cr concentration in the dry matter of the gut-chloragogenous system was 1.93 times greater than those of the body wall. About 55.6% of the accumulated ^{51}Cr was found in the gut-chloragogenous system, and 44.4% in the body wall tissues.

Distinct Cr signals appeared on the EMMA spectra in some of the chloragosomes and eleocyte granules of the long-term treated worms, however, Cr signals were absent in those of the controls (Fig. 5).

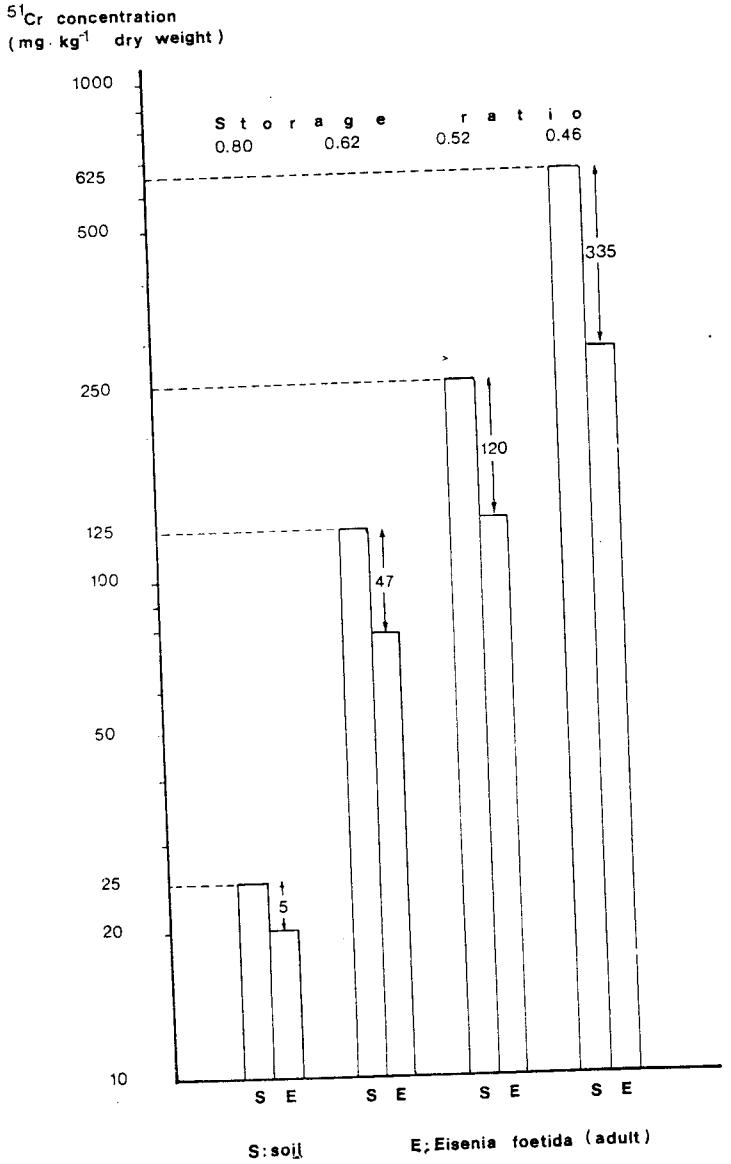

Fig. 4. Relation between the ^{51}chromium contents of *Eisenia foetida* and their environments

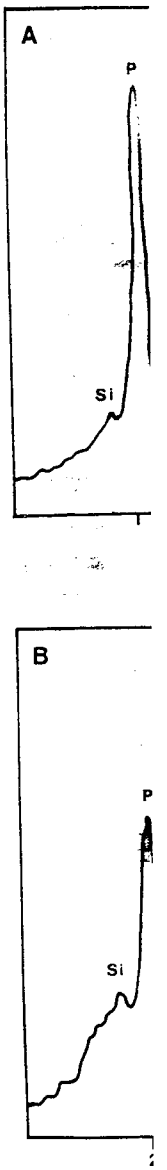

Fig. 5. Energy
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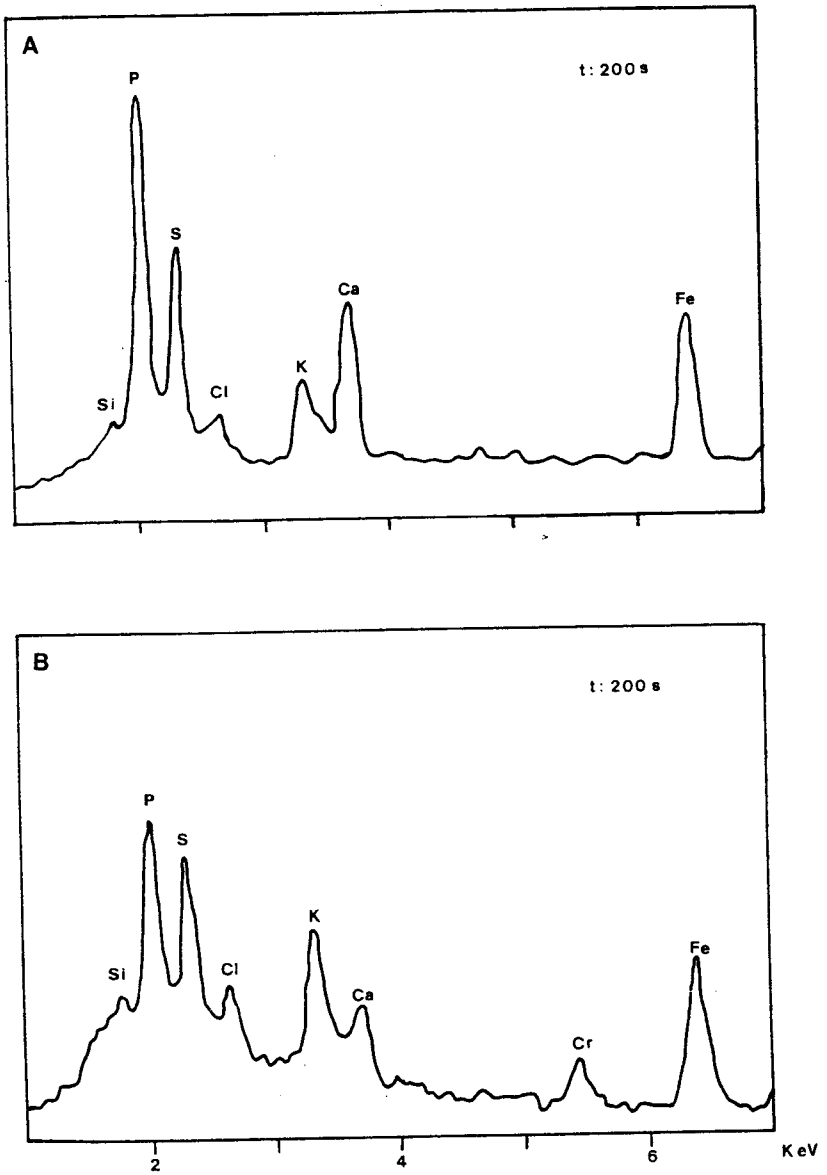

Fig. 5. Energy-dispersive X-ray emission spectra of the eleocyte granules of *Eisenia foetida*.
A) control; B) long-term chromium treated

Discussion

Our experiments indicated, that the growth and reproduction of *E. foetida* were seriously retarded by sublethal concentrations of both chromate and chromic salts. Chromate was more toxic than chromic form, however, the toxicity of the chromium (VI) treated substratum decreased during a delay time. Decreasing toxicity of chromium (VI) may be explained by its reduction to chromium (III) and interaction with the substratum. Published data suggest (COSTA et al. 1984, LANGARD 1982) that the most stable state of chromium is chromium (III), and that this trivalent form is less toxic than the hexavalent form.

Table 1. Levels of chromium in the earthworms and their environments ($mg \cdot kg^{-1}$ dry weight except where stated otherwise) Storage ratio $= \frac{\text{concentration in earthworms}}{\text{concentration in environment}}$

Conditions	Environment	Earthworms	Storage ratio	References
Control	61		0.068	HELMKE et al. (1979)
Sludge 34.35 kg Cr/ha		$\bar{x}$ 11.23		
68.70 kg Cr/ha		$\bar{x}$ 2.80		
137.40 kg Cr/ha		$\bar{x}$ 7.06		
Control		38.0		HARTENSTEIN et al. (1980a)
Sludge	108	63.0	0.58	
Sludge	200–650	1–13		HARTENSTEIN et al. (1980b)
Soil with waste	10–130		$\bar{x}$ 0.074	MA (1982)
Control	65	33.0	0.50	LINÉRES et al. (1985)
Garbage	159	37.2	0.23	
Natural soil	$\bar{x}$ 12.7	$\bar{x}$ 3.94	$\bar{x}$ 0.32	BEYER et al. (1987)
Mining area	$\bar{x}$ 20.38	$\bar{x}$ 7.17	$\bar{x}$ 0.35	
Industrial area	$\bar{x}$ 25.02	$\bar{x}$ 12.99	$\bar{x}$ 0.52	
Galvanized towers	$\bar{x}$ 10.05	$\bar{x}$ 29.25	$\bar{x}$ 2.91	
Laboratory experiment with ^{51}chromium (VI)	25	20	0.80	This work
	125	78	0.62	
	250	130	0.52	
	625	290	0.46	

There are a few data concerning the chromium content of earthworms collected from unpolluted and polluted soils or sludge (Tab. 1). The maximum chromium concentration of the polluted sludge found in the cited works was 650 $mg \cdot kg^{-1}$ (HARTENSTEIN et al. 1980b), and 63 $mg \cdot kg^{-1}$ in *E. foetida* (HARTENSTEIN et al. 1980a) on a dry weight basis. In this work the maximum concentrations were 625 $mg \cdot kg^{-1}$ ^{51}Cr in the substratum, and 290 $mg \cdot kg^{-1}$ ^{51}Cr in the worms, without reference to the natural chromium content.

Chromium (III) was accumulated to a lesser extent and a lower storage radio than chromium (VI). This is in agreement with the known fact, that cell membranes are impermeable to chromium (III), but are permeable to chromium (VI) as Cr_4O^{2-} which may be transported by the sulfate transport system (COSTA et al. 1984, LANGARD 1982).

The chromium content of *E. foetida* at sublethal concentration values was positively related to the environmental chromium content, when other environment-related variables were identical. However, the relation between the storage ratios and environmental concentrations was inverse. Worms in all developmental stages accumulated chromium. Maximum storage ratios were found in juveniles, which hatched and grew in polluted substrata. Storage ratios of other metals were also higher in juveniles than in adults (FLECKENSTEIN and GRAFF 1982).

The chromium accumulation potential of the gut-chloragogenous system appeared to be twice than that of the body wall tissues. This may be explained by the fact that chloragocytes and their granules (chloragosomes) are capable of taking up and retaining certain heavy metals (ANDERSEN and LAURSEN 1982, IRELAND 1983). Our X-ray microanalytical results proved that chromium was accumulated in a number of chloragosomes and eleocyte granules. The chromium content of these granules could be discharged by ejection of eleocytes (liberated chloragocytes) through the dorsal pores.

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