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Effects of high copper concentrations on soil invertebrates (earthworms and oribatid mites):

Experimental results and a model

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Abstract. Data in the literature on the toxicity and uptake of copper by soil invertebrates are contradictory. Copper toxicity and bioaccumulation studies were therefore performed using earthworms and oribatid mites. Field-simulating experiments in soil-filled plastic containers showed that earthworms try to escape moderately toxic situations and that they are much more sensitive than oribatid mites to temporary high Cu^{2+} concentrations in soils. The total copper concentration in the bodies of the earthworm species *Octolasium cyaneum* was measured in experiments with different soil types and different amounts of added $CuSO_4$. The copper concentrations in the earthworms increased in response to the higher concentrations of the copper fraction extractable with 2.5% acetic acid in the soil. Furthermore, internal copper concentrations showed a slight tendency to oscillate. The worms died when the concentrations within their bodies exceeded about 100–120 ppm, calculated on a dry weight basis. To interpret the experimental results, a compartment model is proposed which describes the dynamics of different fractions of copper in worms living in varying soil environments. Applying this model, the different reports on toxicity and uptake of copper in the literature no longer contradict each other.

Copper and other metals are added to agricultural soils in the form of pesticides, fertilizers, sewage sludge, or field irrigations. Copper, usually found in concentrations between 4 and 150 ppm in soils (Hopps 1974, Scheffer et al. 1979, Thornton 1981, Chang et al. 1984), may in this way be increased up to several hundred ppm or even more. The heavy metal concentrations finally encountered in the soil may pose a potential hazard to animals and plants through direct toxic action or via food-chain effects (Cotton and Curry 1980a, b, Hartenstein et al. 1980b, Hunter and Johnson 1982). Another source of high copper concentrations are natural or non-natural deposits of copper. At these localities, vascular plants may accumulate large quantities of copper and constitute a copper-rich diet source for consumer populations (Larcher 1980). On sites of copper deficiency, on the other hand, an upward regulation of copper concentration may become essential for animals.

Terrestrial habitats present different paths of copper uptake into animal populations. Oral uptake is one of two paths of entry. Direct influx through the skin is another, and is especially important in animals that expose permeable skin structures to environmental agents. In earlier studies on aquatic animals, we showed that diffusionary uptake and elimination rates of pesticide transport between the environment and the biota outweighed foodchain path rates at least for many pesticides, and that steady state equilibrium values are predominantly explained through the action of diffusionary exchange processes, taking place at skin and gill areas (Streit 1979, Gunkel and Streit 1980). We hypothesized that average turnover rates of diffusing molecules within animals are intimately related to the specific habitat adaptations of a species (Streit 1982a). These and other published data (e.g., Stumm and Morgan 1981, Sanders et al. 1983) suggest that explanatory models of the environmental behavior of contaminants have to take into account the chemical configuration, in which the chemical is acting, and the degree of diffusionary exposition of the animal's body.

A cuticular or skin structure that prevents diffusionary inflow of material into the body to a high degree may prevent or slow down passive inflow of soluble metal fractions. Inflow through the gut-wall may then become a major path of entry of a metal into the body. On the other hand, animals with highly permeable cuticles (such as earthworms) may need an effective regulation mechanism to maintain internal homeostasis.

Literature data on the toxicity of heavy metals for soil organisms are often contradictory. Copper concentrations being reported as toxic for earthworms and other organisms showed no toxic effects to similar or even to the same species in other studies. For example, according to van Rhee (1975), 110 ppm Cu in soil are toxic to earthworms, whereas in the study of Hunter and Johnson (1982), worms are still abundant at about 250 ppm Cu and sparse at 2,000–3,000 ppm. Mortality in *Eisenia* was found to occur only at about 22,000 ppm by Hartenstein et al. (1981). Different opinions are also encountered in the literature concerning the existence of internal regulation of copper in earthworms. Van Rhee (1975) found a significant correlation between total copper concentration in soils and worms; Ireland (1979), however, proposed a regulation mechanism to maintain a constant internal copper concentration, independent of environmental concentrations.

According to a preliminary communication of our own (Streit and Jaeggy 1983), earthworms, unlike many arthropods and vertebrates, seem to be subject to an immediate response in their internal copper concentration, if copper concentration in the surroundings is raised. Uptake of cop-

9379

per therefore is potentially possible both externally through the skin and through the intestines by contaminated food. They may thus resemble their aquatic relatives, such as the tubificid worms and many other aquatic invertebrates (Coombs 1974, Brown 1977).

In this paper, we first measure the different behaviors of individuals and communities as found in arthropods (oribatid mites) and earthworms (*Octolasium cyaneum* Sav.) in field-simulating experiments. We show that the earthworm species studied is far more sensitive to the addition of copper in the soil than are the oribatid mites. Then we proceed to a more detailed experiment on the uptake of copper by worms, differentiating the types of copper in the soil ("copper speciation" in the chemical terminology). Finally we propose a simple mathematical model, which summarizes the results of the time-dependent internal copper concentrations and the observed regulation patterns, and in which the importance of different fractions of environmental copper is integrated.

Methods

Container experiment (Annelida vs. Arthropoda toxicity)

Toxicological studies were carried out to compare the effects of equal concentrations of added copper to earthworms and oribatid mites during a six weeks' study. We used four plastic containers, each filled with soil from the original habitat of these animals. This soil was a loess-derived brown earth soil with local gley horizons at a beech-oak-pine forest site in the northern part of Switzerland (Bruderholz site, described in Streit 1982b). Each container had a volume of $35 \times 26.5 \times 29$ cm = 27 l. The soil-filled containers were left in an aerated greenhouse at outdoor temperatures and remained undisturbed, but regularly rinsed, for 18 months. Some herbaceous plants and tree seedlings began to grow in this period. At the end of this period, 6–7 individual earthworms were put into each vessel, where they crawled immediately into the soil. The original arthropod fauna was left unaltered and consisted almost entirely of microarthropods.

At the beginning of a six week-experiment, the four containers were irrigated once with varying amounts of copper sulfate solution in one half of the container, whereas the other half received an equal amount of distilled water only. After this one-time treatment, the pots received no further irrigation. Mean soil copper concentrations in the copper-irrigated half of the containers rose, on an average, by 40–200 ppm soluble copper. This average value was calculated from the difference of applied copper and recovered copper in the drainage water at the bottom of the containers (measured with neutron activation analysis, cf. below). Soil samples were taken at weekly intervals and put into Berlese funnels to collect oribatid mites. At the end of the experiment, 6 weeks after irrigation, all the earthworms (whether living or dead) were taken out from the container soils and the numbers of living and dead worms were counted.

Copper uptake experiment with earthworms

For studies of copper uptake and regulation, the worms were put into glass dishes of 19×9 cm, into which two different soil types had been introduced: (1) the brown earth soil from the original habitat (C: 3.3%, N: 0.15%, Ca: 0.13%, background total copper: 51.6–54 ppm, pH 4.24 (1 M KCl) and 4.78 (H_2O) [cf. also Streit 1982a]), (2) a commercial peat soil (C: 42.6%, N: 1.41%, Ca: 0.441%, background total copper 13.4–15.4, pH 4.5 (color.)). The worms were kept in a dark room at 17–18° C. Moisture was held constant throughout the whole acclimation and experimental period by replacing daily the water evaporated, which was estimated from the total weight of the dishes and soil. The worms did not try to escape under these circumstances. No additional food was offered apart from that represented by the organic part of the soil material, to avoid the development of non-homogenous copper concentrations in the experimental vessels.

Copper was added as a $CuSO_4$ solution to provide concentrations between 100 ppm and 2,700 ppm relative to the soil dry weight, and was quickly and thorougly mixed with the soil particles. Variation in the content of copper in various parts of the experimental soil was tested by measuring different replicates. The standard deviation of single measurements was less than 4% from the mean value, which indicated a good mixture of the added copper.

Chemical analysis

Total copper contents in the soils and in the worms were determined by use of a neutron activation analysis method, carried out in the Institute of Physics at the University of Basel.

Copper contents in different soil fractions were determined in part by the anodic stripping method and also by neutron activation analysis. The soluble fractions we used were 2.5% acetic acid (=0.42 M) and 1 M hydrochloric acid. According to McLaren and Crawford (1973a, b), the 2.5% acetic acid fraction should contain soil solution, exchangeable copper, and copper mainly bound to inorganic sites (so-called specific sorption). Specific sorption is important in controlling the concentration of copper in the soil solution, which itself normally shows rather low values. Different from the recommendations of McLaren and Crawford (1973a, b), yet consistent with Neuhauser and Hartenstein (1980), we used 1 M hydrochloric acid as a second solvent. Extraction with 1 M hydrochloric acid probably recovers copper bound at organic sites as well as some free oxides. Extractions were all performed for 1 h and in amounts of 1 g soil dry mass per 30 ml solvent. Residues from these extraction procedures may mainly represent copper within lattice structures of primary and secondary soil minerals.

An individual sample of soil and worms, collected in the Franciscan complex area of Jasper Ridge, Biological Preserve of Stanford University, California, was analyzed using an atomic absorption spectrometer, Perkin Elmer Model 303.

Soil carbon content was determined by a wet oxidation method, using $K_2Cr_2O_7$, recovering mainly organic carbon. Total nitrogen was determined by using a semiautomatic BUECHI Kjeldahl system. pH was determined in H_2O and in 1 M KCl with an electric pH meter, and also in part by use of a colorimetric technique.

The influence of different amounts of $CuSO_4$ on soil pH values was relatively small. Even after adding 1,200 ppm Cu, the decrease of pH was not more than about 0.5 to 0.7 (Jaeggy and Streit 1982).

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Table 1. Fractionation of copper in the original brown earth soil (column 1: original habitat of *Octolasium cyaneum* and the mites) and after addition of 400 ppm Cu in form of $CuSO_4$ to this soil (column 2) and to the peat soil (column 3)

Fraction	[1] original loess-derived brown earth-soil (partially gley)	[2] addition of 400 ppm Cu to the brown earth soil [3.2% carbon]	[3] addition of 400 ppm Cu to the peat soil [42.6% carbon]
1. 2.5% acetic acid	18% = 9.5 ppm	50% = 226 ppm	8% = 33 ppm
2. 1 M hydrochloric acid	4% = 2.2 ppm	18% = 81 ppm	64% = 265 ppm
3. sum of fraction 1. + 2.	22% = 11.7 ppm	68% = 307 ppm	72% = 298 ppm
4. residue	78% = 39.9 ppm	32% = 144 ppm	28% = 116 ppm
5. overall total	100% = 51.6 ppm	100% = 451 ppm	100% = 414 ppm

Experimental results

In the natural habitat of the earthworms and the oribatid mites, the total amount of 51.6 ppm Cu was partitioned within different fractions according to the values presented in column [1] of Table 1. The addition of copper ions (columns [2] and [3]) did not lead to a proportional rise in the concentration of the acetic acid fraction alone: In column [2], only slightly more than half the added amount of 400 ppm Cu was actually found as additional copper in this fraction (increasing from 9.5 to 226 = 216.5 ppmCu). The rest was split up into the hydrochloric acid fraction (increasing from 2.2 to 81 = 78.8 ppm Cu), and the residue fraction (increasing from 39.9 to 144 = 104.1 ppm Cu). In column [3], adding 400 ppm Cu to the organic soil again resulted in a different partitioning of recovered copper, in that now the hydrochloric acid fraction was dominant.

Oribatid vs. earthworm sensitivity

The mite communities in the four containers evolved differently from each other, which corresponds to a high variability in species associations also found in the natural soil habitat. Yet, within each container half side (whether contaminated or not), species composition was rather constant (though showing a high standard deviation due to small-scale patches of abundance for single species). Between 40 and 150 ppm Cu, no significant effects of the copper could be detected in species assemblage. We therefore report only the results from the 200 ppm experiment in the following.

Comparing total number of individuals and species between the control side and the 200 ppm side of the container, the observed reduction in numbers was not significant ($P > 0.1$). A significant decrease was found only in the number of juveniles ($P < 0.02$), which was mainly due to reduction in a single species (*Platynothrus peltifer*). This species was also the only one which showed a significantly lower number of adults in the contaminated area (Table 2). On a whole, we conclude that the influence of even 200 ppm additional soluble copper on the oribatid mite community during a 6 weeks' study does not give conclusive evidence of negative effects at a community level (6 species not affected vs. 1 affected).

The earthworm species studied (*Octolasium cyaneum*) was greatly affected by copper addition. In the contaminated half of all four containers, even in the one with the lowest copper concentration added (40 ppm), all the living earthworms had either emigrated to the non-contaminated side by the end of the experiment (i.e., after 6 weeks) or were killed. We found dead specimens in the contaminated areas of the two highest concentrations (150 and 200 ppm copper added).

Toxicity values as tested in additional experiments showed that different soil types, varying in their organic carbon content, show great differences in toxicity to earthworms when equal amounts of $CuSO_4$ are added. LC_{50} values measured after 4 days in soil of poor organic material (3.2% C) were about 181 ppm. In peat soil (42.6% C), however, it was as high as 2,760 ppm. Detailed values of these analyses are given elsewhere and shall not be repeated here (Jaeggy and Streit 1982, Streit and Jaeggy 1983).

Copper uptake studies in earthworms

$CuSO_4$ was added in three different concentrations (100 ppm Cu, 400 ppm Cu, 1,200 ppm Cu) to the glass dishes. This had the effect of establishing variable concentrations in the copper extracting acid fractions from the soil (cf. Table 1).

When earthworms were introduced into experimental vessels filled with uncontaminated soil or with only a low concentration of copper, they began to crawl into the soil immediately and disappeared within 1–2 min. They showed an escape behavior if touched by a pincette. On the other hand, in high copper concentrations which would lead finally to death, the worms showed a very different behavior: they wound in all directions and formed knots in their bodies. At the same time they began to push out feces. They

Table 2. Arithmetic means of individual numbers in the 7 predominating oribatid mite species from four 200 cm^3 samples from the non-contaminated (left) vs. the contaminated half of the container (right). Because of the patchy distribution, the standard deviations are rather high. A significant difference, based on t-test statistics (* $P < 0.05$) is found only with *Platynothrus peltifer*. On an overall basis (6 species not affected, 1 affected), a significant effect of the copper on the adult oribatid mite community cannot be detected

	Non-contaminated	Contaminated with 200 ppm CU	
Oppiella quadricarinata	94.67 (s = 40.50)	59.25 (s = 14.89)	NS
Oppiella nova	46.33 (s = 16.17)	36.50 (s = 16.30)	NS
Brachychthonius berlesei	36.33 (s = 34.21)	35.25 (s = 24.45)	NS
Suctobelbella sp.	14.67 (s = 5.51)	12.50 (s = 14.39)	NS
Oppiella obsoleta	10.00 (s = 6.25)	4.50 (s = 2.65)	NS
Platynothrus peltifer	4.00 (s = 1.73)	0.25 (s = 0.50)	*
Tectocepheus sp.	0.33 (s = 0.58)	2.25 (s = 3.86)	NS

Table 3. Correlation analyses of total copper in worms (from the two different soils and different $CuSO_4$ concentrations) vs. different fractions of extractable copper after various times of exposure. Refer to text for explanation. Significance: * $P<0.05$, ** $P<0.01$, *** $P<0.001$). The highest correlation coefficients at any observation time are underscored and exclusively found in the acetic acid fractions

	2.5% acetic acid fraction	1 M hydrochl. acid fraction	Acetic and hydrochl. acid fraction	Total Cu
1 h	0.487	0.333	0.109	0.115
3 h	0.897*	0.843*	0.415	0.383
8 h	0.987***	0.952**	0.668	0.642
1 Day	0.989***	0.067	0.644	0.680
4 Day	0.988**	0.582	0.950*	0.965**
Last day (26–96)	0.9996***	0.749	0.988***	0.994***

also tried to escape from the experimental vessels. After about 15 min, the worms began to dig into the earth and disappeared from the surface. After some time the worms remained motionless in the soil. (This occurred after 1 day at 400 ppm Cu added in the 3.2% C soil, but after only 1 h at 1,200 ppm Cu added.) They had turned to a reddish color around the clittellum by that time. When they were touched in this condition, they just sluggishly moved their fore-ends and exhibited some kind of searching behavior, without any escape reactions. Dead worms were red to violet around the whole fore part of their bodies, especially around the clitella. They had emptied most of their gut contents; their back ends were flat.

Principally, any physiological or behavioral responses of animals through the effect of copper occurs either by irritating or penetrating the outer skin or after an assimilation process during digestion in the gut. The immediate response behavior of the worms after contact with highly contaminated soils indicates that primary irritation effects acted – at least at higher concentrations – on the outer surface of the earthworms.

Evidence for the uptake of copper directly through the skin comes from the observation that worms in highly contaminated soils, which definitely did not feed, accumulated copper from the soil. Furthermore, since these worms could be assumed to have non-functioning (or far inadequate) regulation mechanisms, their diffusion constants could be estimated. They turned out to be similar to the initial "diffusion constants", as measured from the initial increase in total copper concentration in the non-fatal cases until regulation was initiated. We therefore conclude that in highly polluted areas and at the beginning of exposure even in less polluted areas, the net-uptake of copper through the skin is quantitatively dominant, compared to uptake through the gut wall. At longer exposures, however, equilibria will probably also be affected through the uptake in the digestive organs.

We calculated correlation coefficients between each copper fraction and total copper concentrations in the worms, as measured after different times of exposure. The results presented in Table 3 show that the acetic acid fraction is the most important in determining total copper concentrations in the worms. The successive increase in the correlation coefficients of total copper in worms vs. the soluble fraction from rather low values ($r=0.487$) to very high values ($r=0.9996$) shows that initially the equilibrium copper concentrations to the acclimation soil were still maintained.

All the earthworms had originally been taken from the same site of brown earth soil. Under non-polluted conditions, equilibrium concentrations in the bodies of the earthworms always turned out to be higher when they had been maintained in the original soil during the acclimation period than when they were kept in the peat soil. The average concentration in the first case was 48.2 ppm Cu (SD = 4.3), in the second case it was 39.8 ppm Cu (SD = 4.6 ppm). Adding copper to both soil types in three different concentrations (100, 400, and 1,200 ppm) raised the acetic acid fraction from 9.5 ppm (original value in the brown earth soil; lower in the peat soil, but not measured) up to several hundred ppm. Adding equal amounts of $CuSO_4$ led to different patterns of copper concentrations in the worms (vertical bars in Fig. 1). The concentrations converged to a final value with a slight tendency to oscillate. The most obvious differences were found when adding 400 and 1,200 ppm copper sulfate to the soils: These concentrations were still harmless for the worms in the peat soil (as far as survival is considered), but fatal in the original soil with a low percentage of organic material.

Elimination and detoxification studies in earthworms

We used the situation described in Fig. 1c, (which finally caused death of the worms) to determine whether worms could recover and regain their original internal concentration after putting them back into non-contaminated soil. After a 24 h exposure in brown earth soil contaminated with 400 ppm additional $CuSO_4$ and accumulating up to 94.5 ppm Cu (on a dry-weight basis), five individual worms were replaced into the original soil in which they had lived until 24 h before. Two out of the five individuals died within 7 h after transferring and showed no regulation pattern. Their Cu concentration was 135 ppm (SD = 6.2) thereby corresponding to the values of non-transferred animals (cf. Fig. 1c). Another 2 individuals survived for another full day and slightly eliminated part of the accumulated copper (down from 94.5 [SD 3.1] to 89.0 [SD 4.5] ppm Cu). The surviving individual seemed to recover totally and lived till the end of the observation period, i.e. at least another full month.

Model of copper regulation in earthworms

To explain the course of the copper concentrations in the worms after different times of exposure, as presented by the bars in Fig. 1, we introduce a mathematical model which describes the dynamics of the copper concentration in earthworms living in various soil environments. The scheme of the model is illustrated in Fig. 2.

In this model, the copper in the soil compartment consists of three different forms, an "inorganic" form (X_s, corresponding to the content extractable with 2.5% acetic acid), an "organic complex" form (X_{so}, extractable with 1 M hydrochloric acid), and a mineral form (X_{min}, residue). Only X_{si} is assumed to have the toxic effect and enter the worm. The copper in the worm compartment is assumed to exist in three states in the body of the earthworm: [1] A small fraction of a highly soluble "transport form" (des

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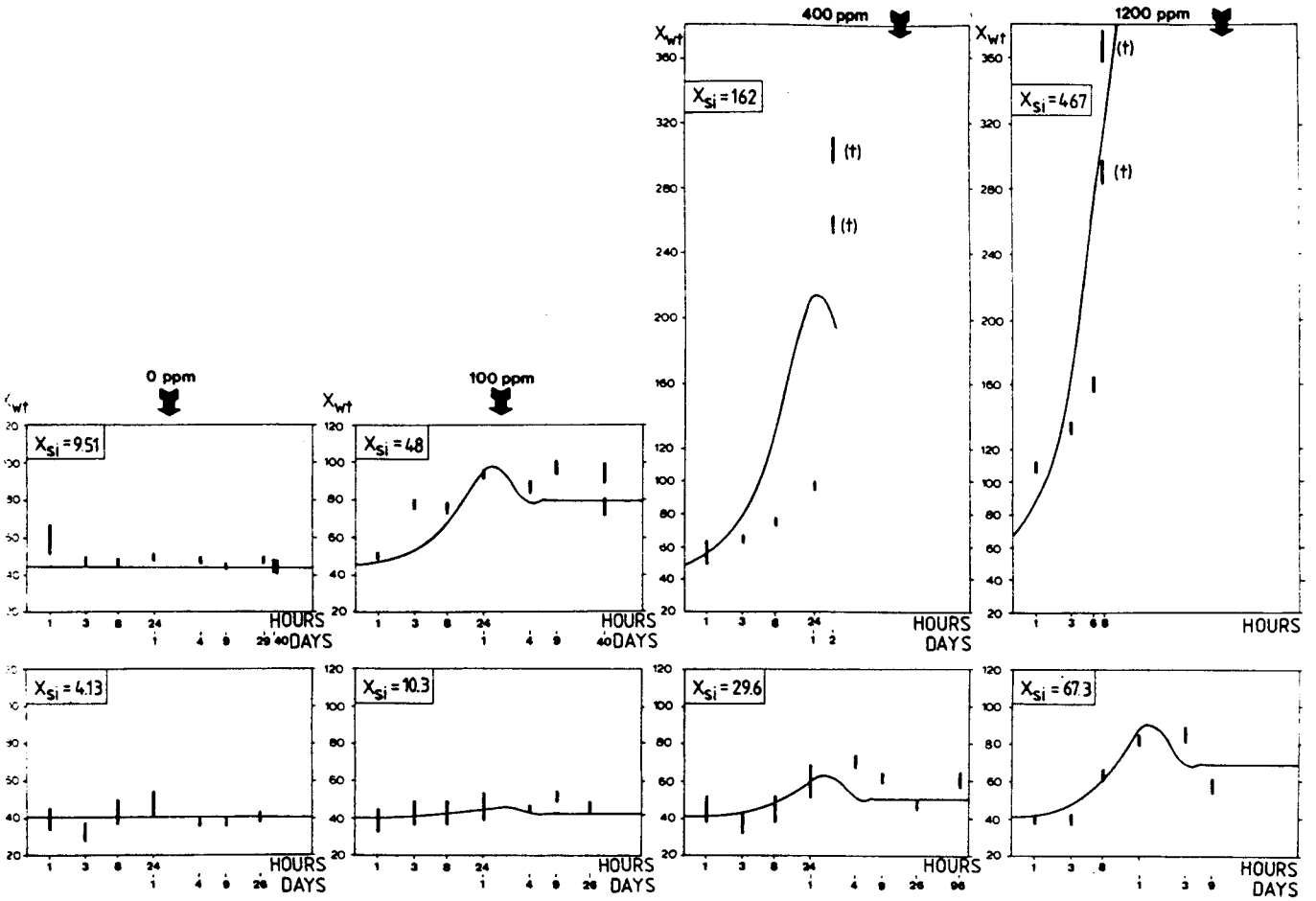

Fig. 1a–h. Concentrations in *Octolasium cyaneum*. **a–d** [above]: earthworms living in loess (3.2% C) from the original habitat. **e–h** [below]: earthworms living in peat soil (42.6% C). Added amount of Cu^{2+} is: 0 ppm in the 1st column, 100 ppm in the 2nd, 400 ppm in the 3rd, 1,200 ppm in the 4th. Corresponding extractable concentrations are given in each graph. Vertical bars indicate range of copper values within one standard deviation (based on three individuals, measured separately each time), found in worms (X_{wt}). Lines correspond to model-based values, using the following parameters: [above]: $m=0.2$, $e=2$, $x_{crit}=4$, $c=0.1$, $b/a=9$, $X_{wi}(0)=4.49$, $Y(0)=2.18$. [below]: $m=0.1$, $e=2$, $x_{crit}=4$, $c=0.1$, $b/a=9$, $X_{wi}(0)=4.10$, $Y(0)=0.49$

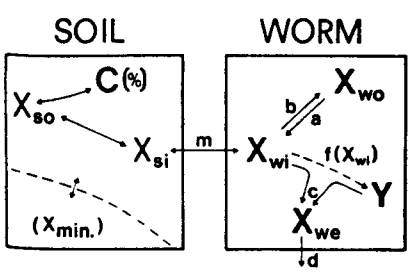

Fig. 2. Compartment model of copper exchange rates. The expression $f(X_{wi})$ is chosen as

$$f(X_{wi})=\begin{cases}0, & \text{if } X_{wi}<x_{crit}\\ e(X_{wi}-x_{crit}), & \text{if } X_{wi}>x_{crit};\end{cases}$$

where x_{crit} designates the critical concentration of X_{wi}, above which regulation takes place. The total concentration of copper in the worms is $X_{wt}=X_{wi}+X_{wo}+X_{we}$, which, under steady state conditions, is designated as $\hat{X}_{wt}$

ignated as X_{wi}), that is assumed to be exchanged with the X_{si} fraction in the soil by a passive transport process through the skin, the coefficient of which is denoted by m; [2] A higher amount of a more complexed form (X_{wo}); [3] an excretion form (X_{we}, this again in a low amount). Let Y be the concentration of a chemical substance for copper excretion (which may occur either through the gut wall, the excretory system, or the sliming behavior). The dynamics we study in the following are then:

$$dX_{wi}/dt=m(X_{si}-X_{wi})+(aX_{wo}-bX_{wi})-cX_{wi}Y \quad (1a)$$

$$dX_{wo}/dt=-aX_{wo}+bX_{wi} \quad (1b)$$

$$dX_{we}/dt=cX_{wi}Y-dX_{we} \quad (1c)$$

$$dY/dt=f(X_{wi})-cX_{wi}Y \quad (1d)$$

The total amount of copper in the body, denoted by X_{wt}, is the sum of three forms

$$X_{wt}=X_{wi}+X_{wo}+X_{we}. \quad (2)$$

It was only this form which could be measured in the experiments.

The flow of inorganic copper due to passive transport is probably reduced as much as possible to avoid the toxic effect. Also the body's reaction of active excretion after an environmental increase of copper needs some time. In contrast, the excretion of the compound X_{we}, and the bal-

ance between X_{wi} and X_{wo} in the body, may be established faster. We here assume that the reaction coefficients a, b, and d in the Eqs. (1a, d) are much greater than m, c and $f(X_{wi})$. The dynamics of X_{wo} and of X_{we} converge rapidly for fixed values of the other variables. The quasi-stationary states corresponding to the fixed values of other variables are calculated by setting the time changes in Eqs. (1b) and (c) equal to zero.

$$X_{wo} = (b/a)X_{wi} \tag{3a}$$

and

$$X_{we} = (c/d)X_{wi} \text{ assumed negligible} \tag{3b}$$

Equation (2) becomes

$$X_{wt} = (1 + b/a)X_{wi}. \tag{4}$$

Hence, we have a reduced dynamical system composed of two variables only:

$$dX_{wi}/dt = m(X_{si} - X_{wi}) - cX_{wi}Y, \tag{5a}$$

$$dY/dt = f(X_{wi}) - cX_{wi}Y \tag{5b}$$

The steady state of this system is determined by setting the rate of change of variables (X_{wi} and Y) equals to zero. From Eqs. (5a, and b), we have

$$0 = m(X_{si} - \hat{X}_{wi}) - c\hat{X}_{wi}\hat{Y}, \tag{6a}$$

$$0 = f(\hat{X}_{wi}) - c\hat{X}_{wi}\hat{Y} \tag{6b}$$

where variables with a hat are the values at the steady state. Cancelling $\hat{Y}$ from these, we have

$$\hat{X}_{wi} = X_{si} - \frac{1}{m} \cdot f(\hat{X}_{wi}) \tag{7}$$

Experimental results at steady state concentrations (based on values used for Table 3) indicated that $f(X_{wi})$ is a two-part linear function:

$$f(X_{wi}) = \begin{cases} 0 & \text{if } X_{wi} < x_{crit} \quad (8a) \\ e\,(X_{wi} - x_{crit}) & \text{if } X_{wi} > x_{crit} \quad (8b) \end{cases}$$

where "x_{crit}" is a critical concentration, below which the active excretion process is deactivated [Eq. (8a)]. Above x_{crit} [Eq. (8b)], the rate parameter "e" determines the rate of the elimination process.

The parameter values were chosen according to the following calculations or assumptions:

(1) $b/a = 9$, so that the factor for conversion from soluble to total copper (cf. (Eq. 4)) is $(1 + b/a) = 10$. (Value adapted from percentage of firmly-bound copper in terrestrial snails, as found by Moser [1978, cited by Wieser 1979].)

(2) $m = 0.1$ (lower row of Fig. 1) -0.2 (upper row of Fig. 1). This coefficient of passive transport of copper between the soil and the worm's body was estimated from the increase in internal copper concentrations of the worms at the beginning of the worms' exposure to elevated concentrations, applying a simple diffusion equation.

(3) $e = 2$. Using experimental values for Eq. (8b), applied in Eq. (7), we determined a mean value of $e = 1.187$, which, however, showed a high variability. Any value between 1 and 2 seemed acceptable.

(4) $x_{crit} = 4$. The exact calculation, applying Eqs. (7) and (8), resulted in $x_{crit} = 4.088$.

(5) c was the only value, for which no experimental data or published information was available. Numerical calculations showed that the result is not very sensitive to the value of c. We here used $c = 0.1$.

Discussion

There are only very few papers discussing copper uptake and accumulation in earthworms in relation to different fractions of copper in the soil. None so far has presented data together with an explanatory model. In the following we will discuss: first, copper fractionation in the soil system, second, the validity of predicting toxicity of Cu and internal steady state concentrations from a single soil fraction, and third, possible physiological or evolutionary responses of earthworm populations to high environmental concentrations of Cu.

[1] Mullins et al. (1982), fractionating soil copper after a long term $CuSO_4$ application, found very low values of Cu in either soil solution or as exchangeable copper (<0.1 ppm), but greatly increased values in the "specifically absorbed copper", the "organically bound copper", and the "hydrous-occluded copper". With the exception of the last fraction, all are potentially available for plant uptake. The increase in the "organic fraction", as found by these authors, is consistent with values from our studies, where the amount in the 1 M HCl fraction was much elevated after $CuSO_4$ application though to a different degree in different soils (as shown in Table 1).

[2] Beyer et al. (1982), according to our knowledge, were the first to demonstrate that copper concentration in worms is best correlated with extractable copper and not subject to bioconcentration by earthworms relative to soil (i.e., similar to Pb and Ni, but in contrast to Cd and Zn). They found values of 9–57 ppm Cu (relative to dry weight) in *Lumbricus terrestris* and *Aporrectodea* spp. sampled from both untreated and sewage sludge treated areas. Fractionation using DTPA is somewhat different from our fractionation using 2.5% acetic acid. Being aware of this fact, we nevertheless plotted their values (using squares) together with our own findings (open and filled circles and triangles) in Fig. 3.

All steady state values are found to be significantly related on a double logarithmical scale to the X_{si} values in the respective soil. Open circles correspond to dead individuals, no longer capable of regulating. They are found approximately on a line $X_{wt} = X_{si}$. The dashed area at 110–120 ppm total copper in worms corresponds to maximum values found in living earthworms in literature data (Hartenstein et al. 1980a) or our own experiments. (Hartenstein et al. (1980b) report a short-term maximum value of ca. 150 ppm in *Eisenia foetida*, similar to our short-term values, as represented in, e.g. Fig. 4.) The lowest value for total copper in earthworm species seems to be about 8.8 ppm (Helmcke et al. 1979, cf. also Hartenstein et al. 1981, Beyer et al. 1982). The black triangle represents values found for an undetermined earthworm species encountered on the Franciscan complex area of Jasper Ridge, Biological Preserve of Stanford University, California, collected in Winter 1982/83.

The regulation pattern of copper in earthworms at high concentrations is similar to that in mammals fed copper-rich diets. Both groups exhibit a regulation of copper concentration, yet with a general rise in tissue copper concentrations at increased copper uptake rates. Hunter and Johnson (1982) detected values of 4–31.1 ppm Cu in different

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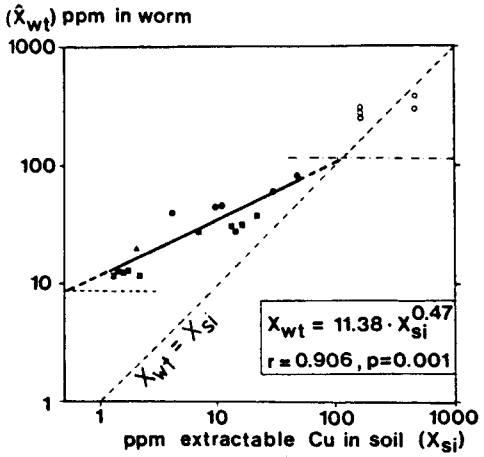

Fig. 3. Relationship between total copper in worms and extractable copper in soil under steady state conditions ($\hat{X}_{wt}$), as found at varying concentrations of environmental copper extracted with either 2.5% acetic acid (circles, dots, and triangles) or DTPA (squares). • Own data of living worms from loess and peat soil experiments (*Octolasium cyaneum*). o Own data of dead worms. ▲ Earthworms found on Jasper Ridge, Biological Preserve of Stanford University [undetermined species]. ■ Data from Beyer et al. (1982), (*Lumbricus terrestris, Aporrectodea* spp.)

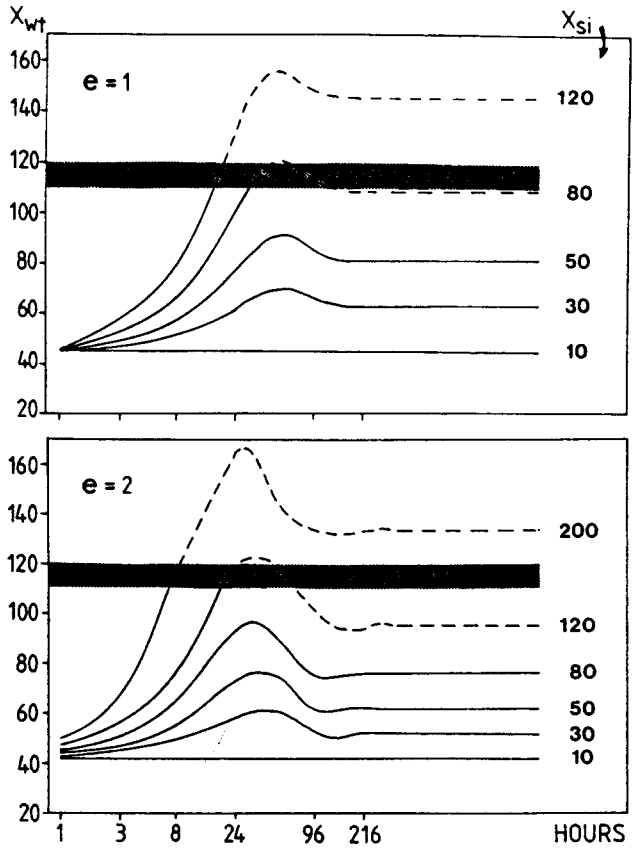

Fig. 4. Two families of time-dependent solutions of X_{wt} (total copper in worms) for different values of soluble copper (X_{si}), assuming $e=1$ in the upper graph and $e=2$ in the lower graph. All other parameters were chosen equal for both graphs or according to stable steady state conditions: $m=0.1$, $c=0.1$, $x_{crit}=4$, $(1+a/b)=10$, $X_{wi}(0)=4.5$ and $Y(0)=1.2$ [above], resp. $X_{wi}(0)=4.3$ and $Y(0)=1.3$ [below]. Establishment of higher rates of e would thus result in tolerance of higher X_{si} values. Dotted areas: threshold concentration, passing which would kill the worms (see text); dashed part of the curves therefore reflects purely mathematical calculations

tissues of rodents at control sites, 4.4–56.2 ppm at intermediate distances from a refinery (total Cu in soil: 246 ppm) and 4.2–77.8 ppm at the refinery site (with high concentration in many invertebrates; total Cu in soil: 2,480 ppm). Copper excretion in mammals occurs almost exclusively in feces (Owen 1964). Homeostatic regulation mechanisms control overall copper concentrations of mammals through a variety of interrelated mechanisms (Owen 1964, Evans 1973).

The reaction of arthropods to high copper concentrations, on the other hand, is often different. The concentration of tissue-copper in isopods starts at a given value, and the final amount is determinated by the copper content of food (Wieser et al. 1977). In a series of habitats with various copper contents, dry weight related Cu concentrations between 74 and about 1,740 ppm were found. These high values reflect mainly the capability of the hepatopancreas to store large amounts of Cu, mainly in specialized cells (Wieser 1979). Hopkin and Martin (1982), studying the distribution of copper within the woodlouse *Oniscus asellus* from uncontaminated and contaminated sites, found values of about 400–2,200 in the hepatopancreas in uncontaminated areas and up to 6,000 ppm in a contaminated area. The total amount of copper in the so called "rest parts", however, varied only between 50 and 65 ppm and may therefore reflect a regulation possibly similar to whole earthworm individuals.

Snails (*Helix aspersa*) were found to exhibit overall Cu concentrations between 30 and 87 ppm, the latter being the value at a heavy metal-rich site (Coughtrey and Martin 1977). They therefore seem to resemble earthworms rather than isopods.

Contrary to many arthropod-type animals in the soil, earthworms, snails and other invertebrates with permeable skin structures may suffer much more from a temporary increase in soluble copper in the environment and may also be highly exposed to soluble fractions. This difference is well demonstrated by the different response of *Octolasium cyaneum*, compared to the oribatid mite community, presented in this paper. "Earthworm-type" animals may take up copper through the skin as well as through their food and the final equilibrium may be established as an overall balance between intake and excretion. Since soluble fractions are much more likely to be subject to temporary variations than copper bound in food particles, fast-acting regulation capacities may be essential for these animals, even in undisturbed situations.

Studies by Emmerich et al. (1982) showed that the form of copper shifted to the free ionic form as the pH decreased. Decreases of pH through local or global instabilities of inorganic processes in the biosphere, including precipitation of acid rain, should be studied especially with respect to the release of toxic metals into the environment.

[3] The fact that not all of the values plotted in Fig. 3 fit equally well, may be due to [a] the different analytical methods involved, [b] the fact that any model will only partially reflect real situations and therefore will never be fully explanatory, [c] physiological or evolutionary responses of populations, which resulted in the shifting of parameter values of the model. Therefore, we calculated

two different configurations of parameters in our model, assuming that through physiological adaptation or selective pressure, the value of e has shifted from 1 to 2 or vice versa. The result, plotted in Fig. 4, shows that tolerable maximum concentrations as well as final steady state values are greatly affected by such simple rate changes. At present, we cannot conclude whether such parameter shifting may be of any significance in long-term or short-term adaptation patterns of populations.

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