ORGANIC MATTER REDUCES COPPER TOXICITY FOR THE EARTHWORM *Eisenia fetida* IN SOILS FROM MINING AREAS IN CENTRAL CHILE

Gonzalo Ávila[1], Hernán Gaete[2], Sébastien Sauvé[3], and Alexander Neaman[1, 4*]

ABSTRACT

The Aconcagua River basin (Central Chile) is one of the most important agricultural areas in the country. However, several copper (Cu) mining operations are located in the basin. The objective of the study was to determine Cu toxicity for the earthworm *Eisenia fetida* (Savigny 1826) in the agricultural soils of the basin. We determined the production of cocoons and juveniles of earthworms in the studied soils. The soils differed in the concentrations of organic matter (OM, range 2-6%), pH (range 7.3-8.3), texture (from loamy sand to clay loam), and total Cu concentrations (range 230-960 mg kg^{-1}). Concentrations of Cu and OM in the soils were the variables that determined the earthworms' biological response. In contrast, pH and texture did not affect this response. Cocoon and juvenile production decreased considerably in soils with elevated Cu concentrations (> 500 mg kg^{-1}), regardless of OM concentrations. Cocoon production decreased in the soils with Cu concentrations below 500 mg kg^{-1} when OM concentrations were below 3.5%. In contrast, cocoon production did not vary when OM concentrations were above 3.5%. The same effect of OM was observed on juvenile production. In this case, the threshold for OM concentration was 2.5%. It was concluded that it is important to consider OM concentrations in order to predict the biological response of earthworms in these soils.

Key words: *Eisenia fetida*, Aconcagua River, ecological risk assessment, Cu mining, trace elements.

INTRODUCTION

Copper (Cu) mining is the most important economic activity in Chile. However, the environmental problems historically associated with copper mining are widely known, particularly in relation to the contamination of agricultural soils by trace elements such as Cu (González *et al*., 2008; De Gregori *et al*., 2003). Although Cu is an essential element for all organisms, it becomes toxic at high concentrations (Sauvé *et al*., 1998).

The Aconcagua River basin in Central Chile is one of the most important agricultural areas in the country. On the other hand, several copper mining industries are located in the agricultural areas of the basin. There is little information available about the toxicity of copper for organisms and crops in agricultural soils of Chile.

Knowing the total concentration of a trace element in a soil is not sufficient to predict the potential ecological risk that it represents (Sauvé *et al*., 1998). Ecological risk is more related to the bioavailability of the element that, in turn, is related to the chemical form in which it is found in the soil. The National Research Council (NRC, 2003) defines bioavailability as the fraction of the total element that is available to the receptor organism.

Chile currently does not have any legislation on the maximum acceptable concentrations of toxic elements in soils. In the opinion of the authors based on what is outlined above, any future legislation should distinguish between soils where trace elements are present but do not represent a risk from those that, at similar concentration of trace elements, do represent significant ecological risks.

An approximation that can be used to solve this problem is carrying out toxicity bioassays with soil macroorganisms. Standardized bioassays that determine the acute and chronic toxicity with earthworm *E. fetida* are particularly suitable (OECD, 2000). *E. fetida* is considered representative of soil macrofauna and of earthworms in particular (OECD, 2000). The objective of the present study was to determine the toxicity of trace elements for earthworms in agricultural soils from mining areas in Central Chile.

[1]Pontificia Universidad Católica de Valparaíso, Facultad de Agronomía, Casilla 4-D, Quillota, Chile.
*Corresponding author (alexander.neaman@ucv.cl).
[2]Universidad de Valparaíso, Facultad de Ciencias, Av. Gran Bretaña 1111, Valparaíso, Chile.
[3]Université de Montréal, Département de Chimie, CP 6128 Centre-ville, Montréal, QC, H3C 3J7, Canada.
[4]Centro Regional de Estudios en Alimentos Saludables (CREAS), Blanco 1623, Of. 1402, Valparaíso, Chile.

MATERIALS AND METHODS

Site selection and soil sampling

The selection of sampling sites was based on the results of a previous study on the distribution of copper in agricultural soils in the Aconcagua River basin (R. Aguilar, unpublished results). This study revealed that the Catemu Creek sub-basin has the largest surface area of soils with high concentrations of Cu.

With the objective of obtaining samples with a wide range of total Cu concentration, 13 localities of the Catemu Creek sub-basin were sampled (Table 1). In each locality, 10 kg of soil were obtained, from a soil depth of 0 to 20 cm, following the removal of the existing vegetation. The soils were then taken to the laboratory of the Faculty of Agriculture, Pontifical Catholic University of Valparaiso, where they were dried at 60 ºC for 2 days. Then, the soils were disaggregated in a porcelain mortar and sieved to 2 mm.

Physical-chemical analysis of the soils

The total concentrations of Cu, lead (Pb) and zinc (Zn) were determined by atomic absorption spectrophotometry (GBC, model 902, Dandenong, Victoria, Australia) following acid digestion of the soils with a mixture of fluorhydric and perchloric acids (Maxwell, 1968). The determination of total arsenic (As) in the soils was carried out using neutron activation analysis. To ensure the quality of the results, reference samples were analyzed. For all the cases, the percentage of difference between the obtained values versus the certified values did not exceed 10%.

The soil texture was determined using the simplified hydrometer method according to Sheldrick and Wang (1993). The concentration of organic matter (OM) was determined according to Sadzawka *et al.* (2006). Electrical conductivity (EC) and pH were determined in saturated paste extracts (Sadzawka *et al.*, 2006). Soluble Cu concentration and activity of free Cu^{2+} ion (pCu^{2+}) were determined in the same extract by atomic absorption spectrophotometry and by ion selective electrode (Rachou *et al.*, 2007a), respectively.

Bioassays of toxicity

To determine chronic toxicity, bioassays with the earthworm *Eisenia fetida* were carried out using the protocols of the OECD (2000). Specifically, 500 g of soil was adjusted to a humidity of 40% w/w and placed in experimental glass containers of 750 mL. Ten adult earthworms (with visible clitellum) were incubated in each container. Earthworms were previously washed with distilled water, blotted dry and weighed. Five grams of cow manure were moistened with 5 mL of distilled water and added to each container. Eight replicates were made with each soil. The design was randomized. After 4 weeks of exposure, the weight, adult survival and number of cocoons were determined. Then, the cocoons were incubated in the same soil for additional 4 weeks and the number of juveniles was determined. Room temperature was maintained within the range of 22 to 24 °C, with illumination of 200 lux and a photoperiod of 12 h of light and 12 h of darkness. Moisture was maintained by the application of 40 mL of distilled water once a week. Soil 41 was considered as a control because of the lowest concentrations of Cu among the studied soils (Table 1).

Table 1. Physico-chemical characteristics of the studied soils.

Soil	Texture	OM	Cu	Pb	Zn	As	CE	pH	pCu^{2+}	Cu
		%	———————— mg kg^{-1} ————————				dS m^{-1}			mg L^{-1}
18	Sandy loam	5.2	959	542	923	44	2.3	7.3	7.3	2.70
27	Sandy loam	3.5	847	81	209	19	2.4	7.9	7.9	0.74
28	Loam	2.2	382	81	244	17	1.3	8.0	8.0	0.28
29	Loam	4.0	431	30	156	21	0.8	8.3	8.3	0.67
30	Clay loam	5.2	426	46	135	30	2.2	7.6	7.6	0.29
31	Loam	5.3	354	36	117	24	2.9	8.1	8.1	0.56
37	Loamy sand	2.0	354	36	97	30	2.6	7.9	7.9	0.38
38	Loam	3.5	434	40	117	28	2.5	7.9	7.9	0.22
41	Loam	6.0	226	36	148	29	4.8	7.8	7.8	0.60
49	Loam	5.6	707	47	134	37	4.1	8.0	8.0	0.83
50	Clay loam	4.0	650	63	146	45	1.5	8.2	8.2	0.46
52	Loam	2.8	597	47	133	36	2.1	8.2	8.2	0.48

OM: organic matter. CE: electrical conductivity. pCu^{2+}: - log (activity of the free Cu^{2+} ion).

Statistical analysis

Using the Dunnett test, comparisons between the responses of the earthworms in the studied soils and the control soil have been made. Simple and multiple regressions were made between the responses of the earthworms and the physico-chemical characteristics of the soils. Also, simple regressions were made between the concentrations of Pb, Zn or As and those of Cu. We used the Minitab 3.1 and Excel 2003 for statistical analysis.

RESULTS AND DISCUSSION

Soil characterization

The studied soils were different in OM concentrations (range of 2 - 6%), EC (range of 0.8 - 4.8 dS m^{-1}), pH (range of 7.3 - 8.3) and texture (from loamy sand to clay loam). The soils presented a wide range of total Cu concentration (from 230 to 960 mg kg^{-1}) (Table 1). These high Cu concentrations are mainly due to mining activities, while application of copper-based products in agriculture represents a minor source (R. Aguilar, unpublished results). Simple regressions revealed that the relations between the concentrations of Pb, Zn and As (Table 1) versus those of Cu were not significant (P > 0.05).

Validity of the control soil

The OECD (2000) established the following criteria for validity of the control soil: (1) each repetition (10 adult earthworms) should produce at least 30 juveniles at the end of the bioassay, (2) the variation coefficient in the reproduction parameters must be less than 30%, and (3)

adult mortality must be less than 10%. Additional to this, Spurgeon *et al.* (2003) propose that weight loss should be less than 15%. These four criteria were satisfied in the control soil (Table 2).

Survival and weight loss

In all the soils used, the earthworm survival was higher than 98% and weight loss did not exceed 20% (Table 2). The data of survival presented a very narrow range (98-100%) and, thus, it was not possible to carry out regressions with the physico-chemical characteristics of the soils. On the other hand, the physico-chemical characteristics of the soils did not explain weight variation. As shown below, the survival and weight loss are variables that less sensitive to Cu toxicity, in comparison to the reproduction variables.

Identification of the variables that affected reproduction

Bioassays with earthworms have been widely used to determine the toxicity of trace elements in soils (Spurgeon *et al.*, 2003). However, the technique has been criticized for not adequately representing real environmental conditions and, consequently, not being relevant from an environmental point of view (Davies *et al.*, 2003). The criticism is based on the fact that the OECD (2000) proposes the use of artificial soils (composed of peat, clay, and sand) enriched with solutions of metals at increasing concentrations. In fact, it has been observed that the toxicity of trace elements for earthworms is considerably higher in artificially-contaminated soil media than in field-collected soils. This is explained by a greater bioavailability of trace

Table 2. Results of chronic toxicity bioassays (OECD, 2000). Soil 41 was considered as a control (C).

Soil	Survival	Weight loss	Number of cocoons	Number of juveniles	Cu in earthworms
	———————— % ————————				mg kg^{-1}
41 (C)	100 ± 0	(-)14 ± 3.8	28 ± 3.2	54 ± 4.4	23 ± 0.8
18	100 ± 0	(-) 4 ± 8.9	11 ± 6.5*	19 ±17*	61 ± 31*
27	99 ± 3.5	(-) 8 ± 5.4	6 ± 4.0*	14 ± 9.0*	64 ± 4.4*
28	100 ± 0	(-)10 ± 9.7	9 ± 5.6*	8 ± 9.8*	37 ± 0.4
29	100 ± 0	(-)15 ± 3.5	26 ± 5.2	50 ± 12	37 ± 3.8
30	98 ± 4.6	(-) 8 ± 5.8	18 ± 3.0*	36 ± 4.4*	39 ± 1.9
31	100 ± 0	(+) 1 ± 3.9*	20 ± 4.1*	41 ± 5.7	51 ± 4.5*
37	100 ± 0	(-) 8 ± 4.5	15 ± 6.3*	23 ± 7.9*	41 ± 2.9
38	99 ± 3.5	(+) 2 ± 6.8*	15 ± 5.5*	41 ± 17	59 ± 3.5*
49	100 ± 0	(-)19 ± 8.0	9 ± 5.1*	24 ± 12*	46 ± 0.6
50	98 ± 4.6	(-) 6 ± 6.8	4 ± 3.1*	14 ± 7.8*	46 ± 0.6
52	100 ± 0	(+) 2 ± 12*	5 ± 4.3*	13 ± 6.8*	37 ± 27

* Significantly different from the control according to the Dunnett test (P < 0.05).
±: Standard deviation. (-) = Weight loss. (+) = Weight increase.

elements in artificially-contaminated soils in comparison to those collected in the field (Spurgeon and Hopkin, 1995). As a result, recent studies highlight the importance of using field-collected soils to evaluate ecological risk of trace elements present in the soil (Nahmani *et al.*, 2007a; 2007b).

On the other hand, the use of field-collected soils presents several difficulties. First, in areas near copper mining activities, soils have high concentrations of several trace elements (Cu, Pb, Zn, Cd and As, among others; De Gregori *et al.*, 2003; Ginocchio *et al.*, 2004). In this case, it could be difficult to distinguish between the effects of different trace elements on the response of the earthworms. Second, agricultural soils can contain other types of chemical compounds, such as pesticides and/or fungicides, which can affect the response of the earthworms (Slimak, 1997). Finally, the intrinsic physico-chemical characteristics of the soil, such as pH, texture and OM content, among others, also affect the degree of toxicity of the trace elements present in the soil (Kennette *et al.*, 2002, Nahmani *et al.*, 2007a).

In the present study, the variables that affected earthworm reproduction were identified using simple and multiple regressions between earthworm responses and the physico-chemical characteristics of the soils. The regressions ruled out any evident effects of Pb, Zn, and As on the response of earthworms. The simple and multiple regressions between earthworm reproduction and the physico-chemical characteristics of the soils indicated that pH, texture, EC, and soluble Cu concentration did not affect the response of the earthworms. On the other hand, a significant regression was observed between pCu^{2+} and earthworm reproduction (Table 3). The effect of free Cu^{2+} ion will be discussed in detail below.

The best prediction of the earthworm response was obtained by considering total Cu concentrations together with OM (Table 3). The regression coefficients increased upon considering both variables together, in comparison to total Cu alone. This effect of OM on Cu toxicity will be discussed in detail below.

Thus, the effects observed on earthworm reproduction are mainly due to Cu and OM, explaining about 70% of the variance (Table 3). Nevertheless, the studied soils could contain other undetermined compounds (for example, pesticides and/or fungicides) that have affected earthworm reproduction.

Effect of OM on the toxicity of copper

The earthworm *E. fetida* lives in environments rich in OM (OECD, 2000). Despite this, the regressions between OM concentrations and earthworm reproduction were not significant. This suggests that OM does not have a direct effect on the reproduction parameters. This concurs with Spurgeon and Hopkin (1999) who indicated that *E. fetida* was not able to obtain sufficient nutrients from mineral soils (with OM < 20%), requiring the addition of food to the soils used in the bioassays.

The multiple regressions show that OM promotes cocoon and juvenile production, while total Cu decreases earthworm reproduction. Consequently, it is necessary to consider OM content to predict the biological responses of earthworms in soils contaminated with trace elements.

The soils with more than 50% of inhibition in the production of cocoons or juveniles are considered as toxic for earthworms (Hund-Rinke and Wiechering, 2001; Hund-Rinke *et al.*, 2005). The soils with total Cu concentrations higher than 500 mg kg^{-1} were toxic, independent of the OM concentrations (Figure 1). In contrast, in the soils with total Cu concentrations below 500 mg kg^{-1}, OM concentrations determined Cu toxicity. In the case of cocoon production, the soils with total Cu concentrations below 500 mg kg^{-1} were toxic when OM concentrations were lower than 3.5%. The opposite was observed in the case of OM concentrations above 3.5% (Figure 1). In the case of juvenile production in soils with total Cu concentrations below 500 mg kg^{-1}, the critical threshold for OM concentration was about 2.5% (Figure 2).

The combined effect of total Cu and OM on the response of earthworms is due to the control that these two variables exert on the activity of the Cu^{2+} ion that

Table 3. Regressions between the number of cocoons/juveniles and soil copper concentrations.

Production of cocoons	R^2	P	Production of juveniles	R^2	P
PC = 26 + 0.02 CuT	0.44	0.02	PJ = 50 - 0.04 CuT	0.34	0.05
PC = 15 - 1.6 CuS	0.21	0.12	PJ = 30 - 3 CuS	0.23	0.10
PC = 16 - 0.03 CuT + 2.8 OM (0.005) (0.03)	0.68	0.01	PJ = 24 - 0.05 CuT + 6.8 OM (0.01) (0.01)	0.68	0.01
PC = -69 + 8.5 pCu^{2+}	0.43	0.02	PJ = -118 + 15 pCu^{2+}	0.35	0.04

PC: Production of cocoons. PJ: production of juveniles. OM: organic matter (%). CuT: total copper (mg kg^{-1}). CuS: soluble copper (mg L^{-1}). pCu^{2+}: - log (activity of the free Cu^{2+} ion). R^2: regression coefficient. P: probability. Number in parenthesis indicates the P value of the variable in multiple regressions.
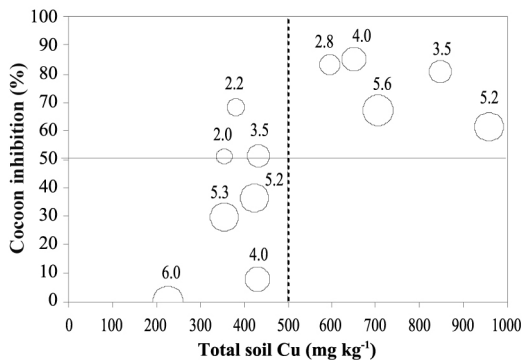
Figure 1. Effect of total copper and organic matter on cocoon production. The size of the circles and the values represent the percentage of organic matter in each soil.
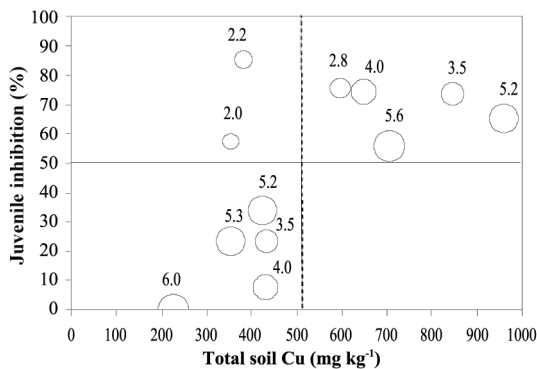

Figure 2. Effect of total copper and organic matter on juvenile production. The size of the circles and the values represent the percentage of organic matter in each soil.

is considered as the bioavailable form of Cu in the soil (Sauvé *et al*., 1998; Thakali *et al*., 2006). McBride *et al*. (1997) and Sauvé *et al*. (1997) proposed an empirical equation that describes the effect of the physico-chemical properties of the soil on the activity of the Cu^{2+} ion: pCu^{2+} = a + b pH + c log CuT + d log OM. In agreement with this postulate, in the present study, the activity of the Cu^{2+} ion in the saturated paste extracts was controlled by the concentrations of OM and total Cu (Table 4). In turn, pH did not affect pCu^{2+}, probably because of the narrow pH range in the studied soils (pH of 7.3 to 8.3).

Also, the effect of OM in reducing Cu toxicity for earthworms is probably due to a change in the mobilization of copper from the solid phase to the soil solution. According to Rachou *et al*. (2007b), the kinetics of the mobilization of the elements from the solid phase to the soil solution decreases with increasing concentration of OM. Thus, the decrease in the flow of Cu from the solid phase to the soil solution can, in turn, reduce its toxicity for the earthworms.

Effect of different forms of copper on reproduction

The bioavailability of a trace element can be estimated through a chemical analysis that extracts a fraction of the element. Diverse extractants were proposed to simulate the bioavailability of trace elements for plants and soil organisms. It is often considered that the bioavailable fraction of a trace element corresponds to its soluble form (Posthuma *et al*., 1997; Kabata-Pendias, 2004). Nevertheless, the regressions between earthworm reproduction and Cu concentrations in the saturated paste extract, corresponding to the soluble form of Cu (Table 3), indicated that this form of the element did not affect the earthworms' reproduction.

On the other hand, the Cu^{2+} ion is the bioavailable form of Cu both in soil and water (Thakali *et al*., 2006). The activity of the Cu^{2+} ion is often considered to be the best variable to predict Cu toxicity for plants, organisms and microbial processes in the soil (Sauvé *et al*., 1998). In accordance with these postulates, in the present study, the regressions between pCu^{2+} in saturated paste extract and earthworms' reproduction were significant (Table 3).

Bioaccumulation of copper

Earthworms can actively excrete Cu assimilated in their tissues (Spurgeon and Hopkin, 1999). This implies extra energy costs, generating a reduction in energy available for growth and development. This, in turn, affects sexual maturity and production of cocoons and juveniles (Spurgeon and Hopkin, 1996). Likewise, the efficiency of excretion probably decreases with increasing concentrations of Cu, resulting in increased bioaccumulation (i.e., the concentration of assimilated Cu in earthworm tissue) in the soils with higher Cu concentrations (Svendsen and Weeks, 1997; Scott-Fordsmand *et al*., 2000).

In the present study, the concentrations of total Cu in the soil explained 53% of the variance (P = 0.001) in the bioaccumulation of Cu. In contrast, other soil properties like pH, OM and other forms of Cu (soluble or free) did

Table 4. Effect of total copper, organic matter and pH on pCu^{2+}.

	R^2	P
pCu^{2+} = 15 - 2 log CuT	0.34	0.04
pCu^{2+} = 14 - 2 log CuT + 1.9 log OM	0.59	0.01
(0.018) (0.042)		
pCu^{2+} = 7 - 2 log CuT + 2 log OM + 0.8 pH	0.72	0.01
(0.020) (0.014) (0.093)		

CuT: total copper (mg kg⁻¹). OM: organic matter (%). pCu^{2+}: - log (activity of the free Cu^{2+} ion).
R^2: regression coefficient. P: probability. Numbers in parenthesis indicate the P value of the variable in multiple regressions.

not affect its bioaccumulation. The concentrations of Cu in the tissues were in the range of 23 to 64 mg kg^{-1}. The effect of these concentrations on the earthworm reproduction is discussed in detail below.

The normal range of Cu concentration in earthworm tissues can be determined through the use of biomarkers of the stress induced by this element. For example, Svendsen and Weeks (1997) used the stability of the lysosomal membrane as a biomarker of sub-cellular stress in the earthworms *E. andrei* exposed to increasing concentrations of Cu. The degree of damage induced by Cu on the lysosomal membrane depended on its bioaccumulation. No damage to tissue was detected at concentrations of 8 to 25 mg kg^{-1}. Concentrations of 25 to 55 mg kg^{-1} produced medium damage, while concentrations higher than 55 mg kg^{-1} provoked severe damage. Likewise, Scott-Fordsmand *et al.* (2000) reported the critical threshold of 50 mg kg^{-1} of bioaccumulation of Cu by *E. fetida*, using the same biomarker of damage to the lysosomal membrane. Similarly, the species *Lumbricus rubellus* did not show a decrease in the production of cocoons with a Cu bioaccumulation below the critical threshold of 40 mg kg^{-1} (Ma, 2005).

In the present study, the bioaccumulation of 23 mg Cu kg^{-1} in the earthworms present in the control soil can be considered as normal, in accordance with Svendsen and Weeks (1997). A higher bioaccumulation of Cu (in the range of 37 to 64 mg kg^{-1}) caused a reduction in cocoon production. Svendsen and Weeks (1997) proposed that earthworms can present individual differences in the efficiency of excreting assimilated Cu. Consequently, bioaccumulation of Cu is not a good biomarker of stress induced by this element, as is reflected in the low regression coefficient ($R^2 = 0.37$, $P < 0.05$) between the parameters of reproduction and bioaccumulation.

CONCLUSIONS

The majority of the studied soils in the Aconcagua River basin in Central Chile presented toxic effects on earthworms, inhibiting the production of cocoons and juveniles.

The regression analysis ruled out any evident effects of Pb, Zn, and As on the response of earthworms.

The observed effects on earthworm reproduction are mainly due to Cu and OM, explaining 70% of the variance.

Reproduction of earthworms is not determined solely by Cu, but also by OM. It is necessary to know OM concentrations to correctly predict the response of macrofauna in soils contaminated by Cu.

RESUMEN

Materia orgánica reduce la toxicidad del cobre para la lombriz *Eisenia fetida* en suelos de áreas mineras en Chile Central. La cuenca del Río Aconcagua (Chile Central) es una de las más importantes áreas agrícolas en el país. Por otro lado, varias industrias de la minería de cobre (Cu) se encuentran ubicadas en esta cuenca. El objetivo del estudio fue determinar la toxicidad de Cu para la lombriz *Eisenia fetida* (Savigny 1826) en los suelos agrícolas de la cuenca. Se determinó la producción de capullos y juveniles de la lombriz en suelos estudiados. Los suelos se diferenciaron por las concentraciones de materia orgánica (MO, rango 2-6%), pH (rango 7,3-8,3), textura (entre arenoso franca y franco arcillosa) y concentraciones totales de Cu (rango 230-960 mg kg^{-1}). Las concentraciones de Cu y MO en los suelos fueron las variables que determinaron la respuesta biológica de las lombrices. En contraste, pH y textura no afectaron a esta respuesta. La producción de capullos y juveniles disminuyó considerablemente en suelos con altas concentraciones de Cu (> 500 mg kg^{-1}), independientemente de las concentraciones de MO. La producción de capullos disminuyó en suelos con concentraciones de Cu inferiores a 500 mg kg^{-1} cuando las concentraciones de MO fueron inferiores a 3,5%. Por el contrario, la producción de capullos no varió cuando la concentración de MO fue superior a 3,5%. El mismo efecto de MO fue reconocido sobre la producción de juveniles. En este caso, el umbral crítico de la concentración de MO fue de 2,5%. Se concluye la importancia de considerar las concentraciones de MO para predecir las respuestas biológicas de lombrices en estos suelos.

Palabras clave: *Eisenia fetida*, Río Aconcagua, evaluación del riesgo ecológico, minería de Cu, elementos traza.

LITERATURE CITED

Davies, N.A., M.E. Hodson, and S. Black. 2003. Is the OECD acute worm toxicity test environmentally relevant? The effect of mineral form on calculated lead toxicity. Environ. Pollut. 121:49-54.

De Gregori, I., E. Fuentes, M. Rojas, H. Pinochet, and M. Potin-Gautier. 2003. Monitoring of copper, arsenic and antimony levels in agricultural soils impacted and non-impacted by mining activities, from three regions in Chile. J. Environ. Monitor. 5:287-295.

Ginocchio, R., G. Carvallo, I. Toro, E. Bustamante, Y. Silva, and N. Sepulveda. 2004. Micro-spatial variation of soil metal pollution and plant recruitment near a copper smelter in Central Chile. Environ. Pollut. 127:343-352.

González, I., V. Muena, M. Cisternas, and A. Neaman. 2008. Acumulación de cobre en una comunidad vegetal afectada por contaminación minera en el valle de Puchuncaví, Chile Central. Rev. Chil. Hist. Nat. 81:279-291.

Hund-Rinke, K., M. Lindemann, and M. Simon. 2005. Experiences with novel approaches in earthworm testing alternatives. J. Soils Sediments 5:233-239.

Hund-Rinke, K., and H. Wiechering. 2001. Earthworm avoidance test for soil assessments. An alternative for acute and reproductive tests. J. Soils Sediments 1:15-20.

Kabata-Pendias, A. 2004. Soil-plant transfer of trace elements: An environmental issue. Geoderma 122:143-149.

Kennette, D., W. Hendershot, A. Tomlin, and S. Sauvé. 2002. Uptake of trace metals by the earthworm *Lumbricus terrestris* L. in urban contaminated soil. Appl. Soil Ecol. 19:191-198.

Ma, W. 2005. Critical body residues (CBRs) for ecotoxicological soil quality assessment: copper in earthwors. Soil Biol. Biochem. 37:561-568.

Maxwell, J. 1968. Rock and mineral analysis. 584 p. Pergamon Press, Oxford, UK.

McBride, M., S. Sauvé, and W. Hendershot. 1997. Solubility control of Cu, Zn, Cd and Pb in contami-nated soils. Eur. J. Soil Sci. 48:337-346.

Nahmani, J., M.E. Hodson, and S. Black. 2007a. Effects of metals on life cycle parameters of the earthworm *Eisenia fetida* exposed to field-contaminated, metal-polluted soils. Environ. Pollut. 149:44-58.

Nahmani, J., M.E. Hodson, and S. Black. 2007b. A review of studies performed to assess metal uptake by earthworms. Environ. Pollut. 145:402-424.

NRC. 2003. Bioavailability of contaminants in soils and sediments: Processes, tools and applications. 420 p. Commission on Bioavailability of Contaminants in Soils and Sediments. National Academy Press, Washington, D.C., USA.

OECD. 2000. Draft guideline for the testing of chemicals. 17 p. Earthworm reproduction test (*Eisenia fetida/ andrei*). Organization for Economic Cooperation and Development (OECD), Paris, France.

Posthuma, L., R. Baerselman, R.P.M. Van Veen, and E.M. Dirven-Van Breemen. 1997. Single and joint toxic effects of copper and zinc on reproduction of *Enchytraeus crypticus* in relation to sorption of metals in soils. Ecotox. Environ. Safe. 38:108-121.

Rachou, J., C. Gagnon, and S. Sauvé. 2007a. Use of an ion-selective electrode for free copper measurements in low salinity and low ionic strength matrices. Environ. Chem. 4:90-97.

Rachou, J., W. Hendershot, and S. Sauvé. 2007b. Soil organic matter impacts upon fluxes of cadmium in soils measured using diffusive gradients in thin films. Commun. Soil. Sci. Plant Anal. 38:1619-1636.

Sadzawka, A., M.A. Carrasco, R. Grez, G. Mora, H. Flores, y A. Neaman. 2006. Métodos de análisis recomendados para los suelos de Chile. Serie actas INIA Nº 34. 164 p. Instituto de Investigaciones Agropecuarias, Santiago, Chile.

Sauvé, S., A. Dumestre, M. McBride, and W. Hendershot. 1998. Derivation of soil quality criteria using predicted chemical speciation of Pb^{2+} and Cu^{2+}. Environ. Toxicol. Chem. 17:1481-1489.

Sauvé, S., M.B. McBride, W.A. Norvell, W.H., and Hendershot. 1997. Copper solubility and speciation of in situ contaminated soils: Effects of copper level, pH and organic matter. Water Air Soil Pollut. 100:133-149.

Scott-Fordsmand, J., J. Weeks, and S. Hopkin. 2000. Importance of the contamination history for understanding toxicity of copper to earthworm *Eisenia fetida* (Oligochaeta: Anelida), using neutral-red retention assay. Environ. Toxicol. Chem. 19:1774-1780.

Sheldrick, B.H., and C. Wang. 1993. Soil sampling and methods of analysis. p. 499-511. *In* M. Carter (ed.) Particle size distribution. Canadian Society of Soil Science, Lewis Publishers, Boca Raton, Florida, USA.

Slimak, K.M. 1997. Avoidance response as a sublethal effect of pesticides on *Lumbricus terrestris* (Oligochaeta). Soil Biol. Biochem. 29:713-715.

Spurgeon, D., and S. Hopkin. 1995. Extrapolation of laboratory based OECD earthworm test to metal-contaminated field sites. Ecotoxicology 4:190-205.

Spurgeon, D., and S. Hopkin. 1996. Effects of metal-contaminated soils on the growth, sexual development, and cocoon production of the earthworm *Eisenia fetida*, with particular reference to zinc. Ecotox. Environ. Safe. 35:86-95.

Spurgeon, D.J., and S.P. Hopkin. 1999. Comparisons of metal accumulation and excretion kinetics in earthworms (*Eisenia fetida*) exposed to contaminated field and laboratory soils. Appl. Soil Ecol. 11:227-243.

Spurgeon, D., M. Weeks, and J. Van Gestel. 2003. A summary of eleven years progress in earthworm ecotoxicology. Pedobiologia 47:588-606.

Svendsen, C., and J.M. Weeks, 1997. Relevance and applicability of a simple earthworm biomarker of copper exposure. I. Links to ecotoxicological effects in a laboratory study with *Eisenia andrei*. Ecotox. Environ. Safe. 36:72-79.

Thakali, S., H. Allen, D. Di Toro, A. Ponizovsky, C. Rooney, F.J. Zhao, and S.P. McGrath. 2006. A terrestrial biotic ligand model. 1. Development and application to Cu and Ni toxicities to barley root elongation in soils. Environ. Sci. Technol. 40:7085-7093.