COPPER CONTENTS IN MEDITERRANEAN ECOSYSTEMS IN SOUTH AMERICA

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Abstract. Copper mining activities are very important in central Chile. Like other metals, copper is a nutrient for most plants at low concentration levels; however, at higher levels it may become toxic. At present, copper abundance in mediterranean ecosystems is unknown both for vegetation and soils. In this study, two localities of the Coastal Range in central Chile were studied. Copper content was found to be mainly related to site type, and secondly to the components of the ecosystem. Copper contents are similar to other mediterranean ecosystems of the world. Soil was found to represent the main source of data variation.

1. Introduction

The main agent of pollutant discharges to the environment is anthropogenic activity. Industrial and mining activities are one of the most important sources of contamination with metal elements. Trace metals such as Cr, Zn, As and Cu are required by organisms but they may be deleterious at high concentrations (Alloway, 1990; Benjamin and Honeyman, 1993; Kabata-Pendias and Pendias, 1984). One of the most relevant features that distinguish metals from other pollutants is that they are not biodegradable, therefore they may accumulate in the ecosystems with deleterious consequences which impose a challenge to the development of society.

Central Chile is characterized by large copper mining activities. Consequently, a high level of copper is expected to be found in these ecosystems in relation to more remote ecosystems. In central Chile, the main vegetation types are the sclerophyllous–evergreen forests, extensive at low altitude. In south-facing slopes at midle and higher elevation southern deciduous beech forests become predominant. Copper occurs in crustal rocks at concentrations ranging from about 10 to a few hundred ppm with an average of 70 ppm (Alloway, 1990; Hodgson, 1963). The principal copper minerals are primarily sulfides, hydroxides, and carbonate of which chalcopyrite is the most common (Benjamin and Honeyman, 1993).

A general survey of nutrient content in mediterranean-type ecosystems carried out by Gray and Schlesinger (1981) and by Specht (1988) included copper content in leaves of some selected tree species. However, there is no data related to copper content of South American mediterranean ecosystems. This basic information is crucial to perform future dynamics studies dealing with the copper cycle in mediterranean ecosystems. In this study, we provide information about copper content in two distant forest areas of the mediterranean ecosystem of central Chile. In addition, we compare these results with other forest ecosystems of the world. Additionally, we provide some explanation for the most relevant documented patterns.

In terrestrial ecosystems, nutrient cycling is dominated by soil–plant interactions. These cycles include uptake by plants and returns to soil through litter fall, foliar and stem leaching, decomposition and ash deposition from fire.

Bruns *et al.* (1991) used the system approach and proposed a model for environmental monitoring in wilderness areas. This model represents a heuristic tool that allows the identification of compartments of primary concern to delineate potential pollutant pathways through the system, and to identify potential critical receptors. In this study three compartments of the model are used: (i) vegetation, (ii) litter, and (iii) mineral soil. Each compartment was subdivided in low-level components according to the following criteria: (a) vegetation is dominated by woody plants with modular construction integrated in the architecture of plants (Harper, 1977; Hallé *et al.*, 1978), and since different structures have their own dead rates, wood (trunks or branches), twigs and leaves were defined as different components; (b) litter compartment was separated in leafy litter, sticks and partially decomposed material (Waring and Schlesinger, 1985) using structure-type and fragment size as criteria; (c) the superficial non-organic horizon was considered as mineral soil. Figure 1 shows the ecosystem model with the compartments and components considered in this study.

2. Methods

2.1. Study sites

The study was conducted in two sites of the coastal range in central Chile, at a distance ca. 250 km from each other (Figure 2). The northern site, El Roble mountain, is located at La Campana National Park (32° 55′ lat. S and 71° long. W). The southern site, Pantanillo Forest Station (a small farm of the University of Chile), is located near Empedrados (35°30′ lat. S and 72°20′ long. W). In both sites, we studied a deciduous and an evergreen forest. We selected two stands in a regeneration phase after cutting with trees about 10 m high in both forest-types. At El Roble, the deciduous forest is characterized by *Nothofagus obliqua* (Fagaceae), located in south-facing slopes ca. 1600 m above sea level. The evergreen forest is dominated by *Cryptocarya alba* (Lauraceae), in eastern-facing slopes at 800 m above sea level. At Pantanillo, the deciduous forest is characterized by the southern beech (*Nothofagus glauca* (Fagaceae) and the evergreen forest is dominated by *C. alba*. Both forest types exist in north-facing slopes at ca. 450 m and 400 m above sea level.

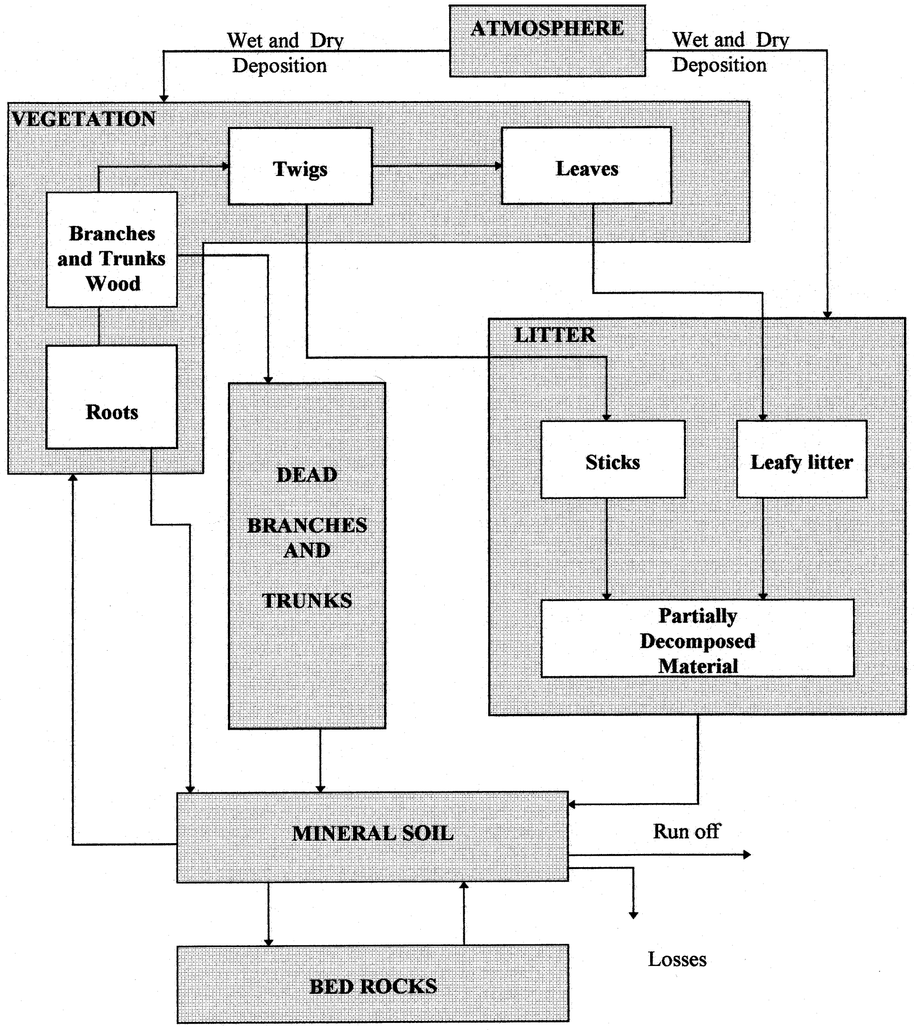

Figure 1. Components defined in a general model of a mediterranean-type ecosystem. Names in the boxes are the components; the arrows show the fluxes between components.

2.2. Sampling methods

Copper content in the vegetation components were determined from samples collected from ten randomly selected trees. A branch inserted directly on the trunk was selected. Twigs were taken from the distal part of this branch. A sample from the basal part of the branch was obtained for wood analysis. Immediately under the selected trees, we collected the litter components, that is leafy litter (formed by leaves of last fall season), sticks (formed by twigs of several fall seasons), and par-

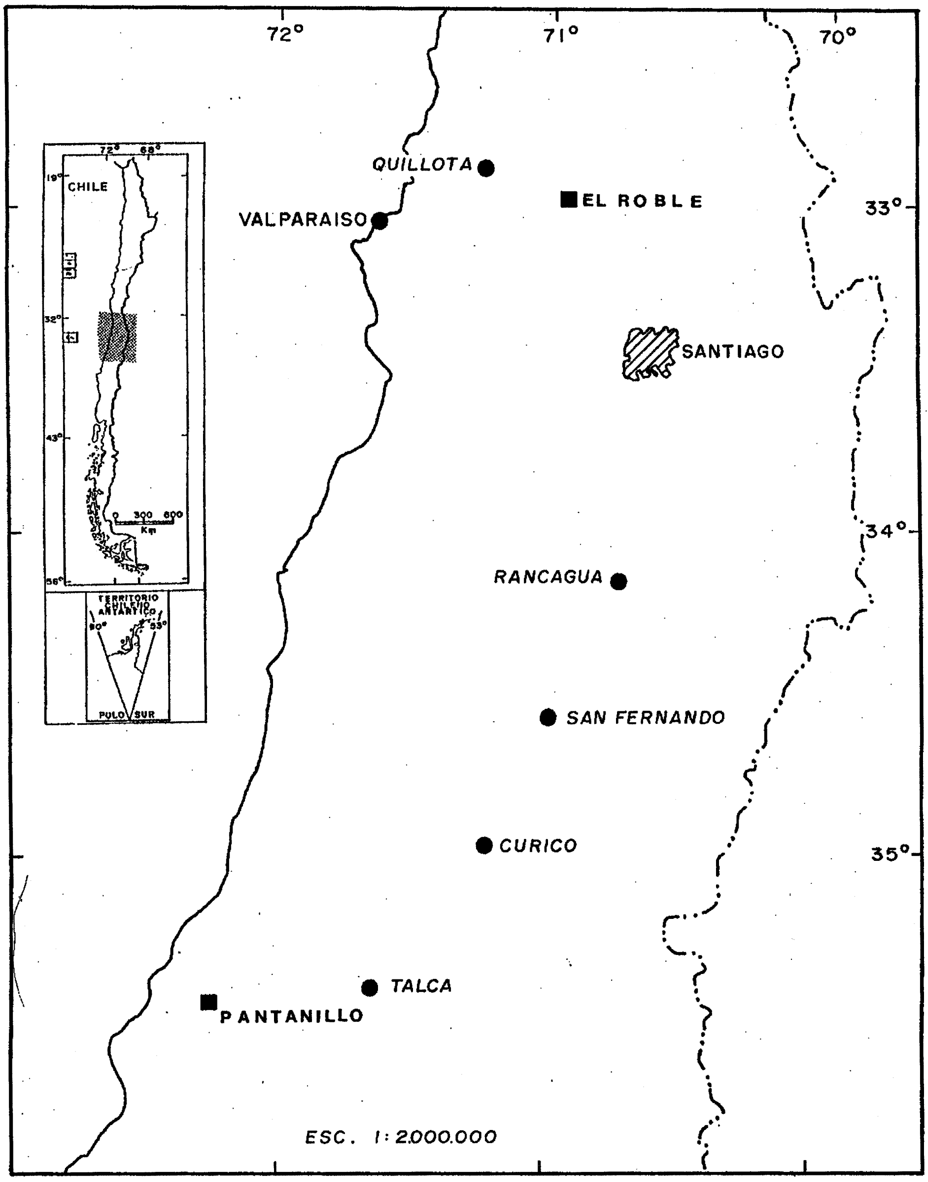

Figure 2. Location of study sites in central Chile.

tially decomposed material (formed by leaves and twigs finely fragmented) were also collected. The upper 0–5 cm of the non-organic soil horizon was considered as mineral soil component for all sampling points. Latex gloves were used to take each sample. Sampling was performed in winter before the start of the new growth season to avoid physiological activity that may generate changes in the copper

Table I

Variables and sample size for studying the copper content in six components of mediterranean forest ecosystem type in central Chile

Source of variation								
Site	RO	RO	RO	RO	PA	PA	PA	PA
Forest type								
	DE	DE	EV	EV	DE	DE	EV	EV
	No	No	Ca	Ca	Ng	Ng	Ca	Ca
Components								
Wood	5	5	5	5	5	5	5	5
Twigs	5	5	5	5	5	5	5	5
Leafy litter	5	5	5	5	5	5	5	5
Sticks	5	5	5	5	5	5	5	5
Pdm	5	5	5	5	5	5	5	5
Soil	5	5	5	5	5	5	5	5

RO, El Roble mountain; PA, Pantanillo Forest Station; DE, deciduous; EV evergreen; No *Nothofagus obliqua* Ng, *Nothofagus glauca*; Ca, *Cryptocarya alba*. Components: wood, twigs, leafy litterm sticks, partially decomposed material (pdm), and soil.

content of plant tissues. These procedures were performed in both forest types and in the two study sites. Leaves were not included in the analysis as, in the deciduous forest, trees remained defoliated during the sampling period.

The samples were labeled, sealed and kept in clean polyethylene bags in the field to avoid contamination, and transported to the laboratory. The sampling designs is summarized in Table I, including 2 sites, 2 forest types (deciduous and evergreen), and 6 components. The number of replicates per component was 5, with a total of 240 ecological samples.

2.3. Analytical procedure

All samples were oven dried at 105 °C, 3–4 h after arrival to the laboratory to avoid decomposition. Afterwards, samples were labeled and kept in polyethylene bags until copper content determinations. Latex gloves were used to handle samples. Each sample was pulverized and homogenized. The homogenized material was dried until constant weight was obtained. Three replicates (200–250 mg) from each sample were digested with a mixture of nitric and sulfuric acids (12 + 3 ml respectively) using a flowing back/vacuum system Gerhardt (Kjeldatherm). Soil samples were subjected to a similar procedure but samples were sieved through a 1.0 mm sieve before digestion. Subsamples were then taken by a quartering method. Each sample was transferred quantitatively to a 25 ml volumetric flask by washing the digestion container with distilled deionized water after digestion, and mixed thoroughly to ensure homogeneity. Soil samples were previously filtered to eliminate silica. Samples were stored at 4 °C in 100 ml polyethylene bottles for further analysis.

2.4. Anodic stripping voltammetry (ASV)

A recent review shows the main advantages and disadvantages of advanced electroanalytical techniques versus modern atomic absorption spectrometry and other analytical techniques (Besier *et al.*, 1994). For copper determinations it is concluded that ASV is an efficient technique when environmental analysis is performed (Florence, 1986). Anodic stripping voltammograms were recorded using a voltammograph instrument CV-27 with a three electrodes assembly. The working electrode was glassy carbon mercury film-coated electrode, the counter electrode was a platinum coil and the reference electrode Ag/AgCl (KCl saturated). Before each determination the glassy carbon electrode was polished with alumina on a felt wetted with deionized water. An aliquot of 1 ml of the sample solution was added into a volumetric cell containing 10 ml 0.1 M sodium chloride plus 2×10^{-4} M Hg^{++} solution. Solutions were deoxygenated by purging oxygen-free nitrogen during 2 min. Copper determination was carried out by anodic stripping voltammetry under the following conditions: electrolysis potential = -1.1 V vs Ag/AgCl, scan rate = 35 mV/s, preconcentration time = 180 s (stirred), equilibrium periods = 60 s (unstirred). The amount of copper of the sample was quantified by the standard addition method using the peak which appears at – 0.15 mV vs Ag/AgCl.

2.5. Atomic absorption spectrometry (ASS)

Atomic absorption measurements were performed using a Perkin Elmer 360 Spectrophotometer. The determination of copper was carried out under standard experimental conditions.

2.6. Statistical analysis

To evaluate the existence of the effects of independent variables on copper content a three-way ANOVA was performed. Copper content was the dependent variable and site (S), forest type (F), and components (C) were the independent variables. Three analytical measurements were performed on each of the 240 samples and the average of these three values was considered an ecological replicate. An averall ANOVA was first performed and then two independent ANOVA, one per study site.

Table II

Analysis of variance of copper content based on three variables and six components at two sites in mediterranean forest ecosystem type in central Chile

Source of variation	df	SS	MS	F	P
Sites (S)	1	49838.83	49838.83	213.12	<<0.01
Forest type (F)	1	91.85	91.85	0.39	0.5315
Components (C)	5	63898.90	12779.78	54.65	<<0.01
Interactions					
S × F	1	118.00	118.00	0.50	0.4782
S × C	5	52749.95	10549.99	45.31	0.0000
S × F × C	5	3622.85	724.57	3.10	0.0100
Error	216	50511.6	233.85		
Total	239	225563.53	943.78		

df, degree of freedom; MS, mean squares; SS, sum squares.

Table III

Global analysis of copper content (ppm) in mediterranean forest ecosystem type. Tukey HSD test, variable: copper content. Probabilities for post-hoc test between components wood, twigs, leafy litter, partially decomposed material (pdm), soil

	Wood	Twigs	Leafy litter	Stick	Pdm	Soil
Wood	1.0000	–	–	–	–	–
Twigs	0.0341	1.0000	–	–	–	–
Leafy litter	0.1129	0.9978	1.0000	–	–	–
Stick	<<0.01	0.5718	0.2968	1.0000	–	–
Pdm	<<0.01	<<0.01	<<0.01	<<0.01	<<0.01	–
Soil	<<0.01	<<0.01	<<0.01	<<0.01	0.1094	1.0000

3. Results Discussion

3.1. Global Analysis

There was a significant effect of site on copper content (ANOVA test, Table II). This result was expected on the base of the differences of the chemical composition of the bed rocks between the two sites. El Roble mountain is located over a geological substrate of granite and mica-schist bed rock, with chalcopyrite-dyke, a mineral rich in copper. Pantanillo Forest Station is also located on a granite and mica-schist bed rock and quartz-dike, not associated with copper minerals. Forest types did not differ in copper content (ANOVA test, Table II). Nevertheless, the components differed significantly (ANOVA, Table II). According to the a posteriori tests (Tukey HSD test, Table III), twigs, leafy litter and sticks were not different and consequently were included as a whole group. Wood was not

Table IV

Mean values of copper contents (ppm) in the ecosystem components for site analysis, in mediterranean forest ecosystem type in central Chile. Components: wood, twigs, leafy litter, sticks, partially decomposed material (pdm), soil

Components	Global	Northern site, El Roble	Southern site, Pantanillo
Wood	1.1561	1.8240	0.4882
Leafy litter	9.8382	15.5705	4.1060
Twig	11.3487	15.4565	7.2410
Sticks	16.8565	27.5970	6.3160
Pdm	38.2235	59.8170	17.2700
Soil	46.9490	91.3100	2.5880

different from leafy litter, partially decomposed material was not different from soil and this last component was not different from partially decomposed material (Table III). In consequence, wood partially decomposed material and soil may be considered another homogeneous group. Another notable result is that the partially decomposed material component was significantly different in relation to the other components (Table III). These results suggest that this component should express a particular accumulation dynamics and it could be used for monitoring the behavior of copper in the ecosystem functioning.

Interestingly, partially decomposed material and soil have the highest copper contents in relation to the other components of the ecosystem (Table IV), suggesting a process of copper accumulation coming from the rocks and the decomposition of organic matter. On the other hand, wood is in the last ranking of copper content in relation to the other component of vegetation (Table IV). This result may be explained because leafy litter and twigs are originated from biological tissue with high metabolic activity whereas the wood tissue remains inert during plant activity.

It has been suggested that copper (and other metal elements) tend to be bound by organic substances, through percolation at a lower rate to the soil profile (Zunino and Martin, 1977a, b). It also may be immobilized by microbial biomass (Laskowski *et al.*, 1995). This property of organic matter which retains copper into its structure may explain the higher copper values found in the partially decomposed material components for all the analyzed samples. The same tendency has been reported in dead matter in the European oak forests (Laskowski *et al.*, 1995).

Strong differences have been detected in the mean copper content of components between El Roble and Pantanillo (Table IV). For instance, the copper content in the soil is nearly 35 times higher in the northern sites. However, this difference is not reflected in the same proportion if we compare the different components between sites. For example, concentration ratio in wood is only 3-7, in twigs about 2, in leafy litter about 4, in sticks about 4 and in partially decomposed material only

Table V

Analysis of variance of copper content based in two variables and six components of El Roble and Pantanillo sites separately, in mediterranean forest ecosystem type in central Chile

El Roble

Source of variation	df	SS	MS	F	P
Forest type (F)	1	209.04	209.04	0.47	0.4965
Components (C)	5	113400.00	22680.00	50.51	<<0.01
Interactions					
F × C	5	8166.04	1633.32	3.64	0.0044
Error	1	209.04	209.04	0.47	0.4965
Total	119	18108.23	152.17	–	–

Pantanillo

Source of variation	df	SS	MS	F	P
Forest type (F)	1	0.81	0.81	0.04	0.8345
Components (C)	5	3471.75	694.35	37.23	<<0.01
Interaction					
F × C	5	106.85	21.37	1.15	0.34
Error	108	2219.35	18.65	–	–
Total	119	5785.16	48.61	–	–

df, degrees of freedom; MS, mean squares; SS, sum squares.

3.4 (Table IV). Copper uptake is a active process, since it is reduced by metabolic inhibitors (Dokiya *et al.*, 1964). These results suggest that when the copper level in the soil is low as in Pantanillo, vegetation components (wood, twigs) accumulate this element in order to satisfy the physiological requirements of plants (Pantanillo site). In poor copper soil it is necessary to assume that complexed as well as ionic copper is absorbed by plant root (Barber, 1995). However, when the environment is richer in copper, we suggest that plants may select it, but there is not a clear mechanism. In consequence, the uptake of copper into the plants does not reflect its abundance in the environment. The effects of variation between habitats in environmental factors other than soil composition on uptake and translocation of heavy metals in plants can be as important as the effects of differences in availability of metals in the soils (Otte and Wijte, 1993).

Table VI

Analysis of variance of copper content in two sites in mediterranean forest ecosystem type. Tukey HSd test, variable: copper content. Probabilities for post-hoc test between components wood, twigs, leafy litter, sticks, partially decomposed material (pdm), soil

(a) El Roble

	Wood	Twigs	Leafy litter	Sticks	Pdm	Soil
Wood	1.0000	–	–	–	–	–
Twigs	0.3301	1.0000	–	–	–	–
Leafy litter	0.3209	1.0000	1.0000	–	–	–
Stick	0.0028	0.4627	0.4735	1.0000	–	–
Pdm	0.0001	0.0001	0.0001	0.0002	1.0000	–
Soil	0.0001	0.0001	0.0001	0.0001	0.0001	1.0000

(b) Pantanillo

	Wood	Twigs	Leafy litter	Sticks	Pdm	Soil
Wood	1.0000	–	–	–	–	–
Twigs	0.0001	1.0000	–	–	–	–
Leafy litter	0.0946	0.2050	1.0000	–	–	–
Sticks	0.0007	0.9841	0.5885	1.0000	–	–
Pdm	0.0001	0.0001	0.0001	0.0001	1.0000	–
Soil	0.6410	0.0117	0.8757	0.0077	0.0001	1.0000

3.2. Site analysis

In both El Roble and Pantanillo sites the copper content differed significantly (ANOVA test, Table V). However, the forest type did not differ in copper content in the two sites (ANOVA test, Table V). A posteriori analysis (Tukey HDS test; Table VIa) indicated that twig and leafy litter components were almost identical in copper content. Moreover, wood and twig components did not differ between each other. Sticks, twigs and leafy litter did not differ also. Based on their similarity, these four components may form a group. Finally, partially decomposed material and soil were different relative to the other components. According to Tukey HDS test (Table VIb) twigs, stick and leafy litter were similar in copper content. Soil and wood were also similar. Partially decomposed material differed from all the other components.

3.3. Copper content in relation to other ecosystems

The mean copper content in the component of the mediterranean forest ecosystem type are shown in Table VII. Copper in wood is low for both sites and species with means ranging between 0,5 ppm and 2 ppm, but twigs show values clearly

Table VII

Copper content ppm in different components of mediterranean forest ecosystem-type for two sites in central Chile. Components: wood, twigs, leafy litter, sticks, partially decomposed material (pdm) soil

	El Roble			Pantanillo		
	$\bar{X}$	se	sd	$\bar{X}$	se	sd
Deciduous forest type						
Wood	1.94	0.18	0.58	1.70	0.31	1.01
Twigs	17.00	1.38	4.37	7.06	0.50	1.60
Leafy litterWood	18.12	2.18	6.91	3.92	0.32	1.02
Sticks	12.90	1.98	6.26	4.70	0.32	1.02
Pdm	70.37	4.13	13.08	18.41	3.69	11.68
Soil	82.56	15.49	48.98	3.76	1.26	4.00
Evergreen forest type						
Wood	1.70	0.31	1.01	0.34	0.09	0.28
Twigs	13.90	1.22	3.87	7.41	0.48	1.51
Leafy litter	13.01	1.39	4.41	4.29	0.18	0.59
Stick	41.98	3.65	11.56	7.92	1.12	3.55
Pdm	48.07	5.77	18.27	16.12	2.24	7.09
Soil	100.05	14.87	47.04	1.40	0.34	1.10

$\bar{X}$, mean; Se, standard error; Sd,, standard deviation.

higher with a means of 7–17 ppm (Table VII). Copper content in twigs and wood in southern beech forest of *Nothofagus pumilio* in southern Chile ranges between 3 ppm and 5 ppm and 2–5 ppm respectively (Caldentey, 1992). The copper in leaves of *Cryptocarya alba* ranged between 5 ppm and 7 pppm and 3–5 ppm in northern and southern sites respectively (Serey, unpublished data). These values are very similar to those reported by Specht (1988) for foliar analyses in several tree species in mediterranean ecosystems of Australia, France and California. Leaves of *Fagus sylvatica* in Europe show copper content means of 14 ppm (Lepoutre and Teissier Du Cros, 1979). The partially decomposed material components had the second highest, with means between ppm 70 and 16 ppm. Wiersma *et al*. (1990, 1992) reported copper values reaching 5 ppm in *Nothofagus pumilio* forest for mosses and lichens at Torres del Paine National Park (southern Chile). Leafy litter were similar between sclerophyllous forest of Australia and Pantanillo with values ranging between 2 ppm and 7 ppm. Clearly, this compnent was higher at El Roble (Table VII). Stick component was higher in both El Roble and Pantanillo in relation to those values observed in sclerophyllous forests of Australia which ranged between 5 ppm and 6 ppm (Lee and Correl, 1978). In a deciduous forest of

Nothofagus pumilio, litter presented a copper content of 39 ppm at Torres del Paine National Park (Wiersma *et al*., 1988), at least two times higher than those reported in deciduous *Nothofagus* forests in this study (Table VII).

One relevant feature of the results is the strong difference of copper content in the soil between El Roble and Pantanillo. In El Roble, this value was ca. 92 ppm and at Pantanillo it was 2.6 ppm (Table IV). On the other hand, the mean values estimated for crustal rock in this area is ca. 70 ppm (Benjamin and Honeyman, 1992). That is, copper content in El Roble enriched relative to the availability of this element in the rocks. Wiersma *et al*. (1988) reported soil copper content in *Nothofagus pumilio* forests, ranging between 3 ppm and 4 ppm. These values are very similar to those found in both deciduous and evergreen forests at Pantanillo (Table VII). Copper level of uncontaminated soil in Africa shows values ranging from 7–80 ppm and a geological substrate with similar copper values as those reported for Chile (Onianwa *et al*., 1986). In this study soil copper content ranged between similar values, that is 4–100 ppm. This evidence suggests that human activities have played a minor role in the determination of the soil copper content of this component in the two study sites.

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