

# Zinc and copper availability in herbage and soil of a *Pinus radiata* silvopastoral system in Northwest Spain after sewage-sludge and lime application

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## Abstract

Silvopastoral systems are ancient farming systems in the world, consisting of the combination of a woody component (trees or shrubs) and crops and/or animals within the same land-management unit. In various European Union (EU) countries, the possibility of using sewage sludge as a fertilizer is under consideration as a viable method of disposal, considering the increase in sewage-sludge production in recent years and the restrictions imposed by European policy on the usual methods of disposal. The concern is the concentration of heavy metals, which can reach humans through the food chain. In Spain, R.D. 1310/1990, as well as European Directive 86/278, limit the total in-soil heavy-metal concentration, but not the solubility changes, which directly affect plant absorption and leaching of heavy metals throughout the soil profile. The objective of this experiment was to compare, in a silvopastoral system over a period of 3 years, the effect of applying three doses of sewage sludge combined with and without liming, on total and available soil Zn and Cu and their concentration in plants. Liming did not affect Zn and Cu availability; however, sewage sludge increased Zn and Cu availability, though total in-soil Zn was increased only in November 2000. In-plant Zn concentration was increased by sewage sludge in the last 2 years of the study. In all cases, the quality of forage obtained and measured with regard to the concentrations of Zn and Cu was adequate for animal consumption. With respect to sewage-sludge application as a fertilizer, the management of heavy-metal availability must be included in the policy, because environmental risk could then be adequately evaluated.

**Key words:** acid soil / agroforestry system / heavy metal / organic fertilization

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## 1 Introduction

Silvopastoral systems, in which trees and pasture production are combined, are more complex ecosystems than exclusively agronomic systems (Rigueiro-Rodríguez et al., 2007). Adequate silvopasture management should increase positive interactions or synergies and reduce negative interactions among their components (soil, trees, grass, and cattle) to increase global system productivity. Once the trees are established, the most important management practices will be related to pasture cultivation within the silvopastoral system, fertilizers being the most commonly used. Fertilizer application is the simplest management practice for increasing production of pasture in a predictable way, and either organic or inorganic fertilizers can be used.

In some EU countries, the use of sewage sludge as organic fertilizer (European Directive 86/278/CEE, DOCE n° L 181; European Union, 1986) is encouraged because of its organic-matter content (SOM) and concentration of nutrients, especially nitrogen. In silvopastoral systems in Northwest Spain, the benefits of the use of sewage sludge on plant and

tree growth, on the botanical composition of pasture, on the in-soil macronutrient levels (N, P, K, Ca, and Mg), and on the plants themselves have previously been evaluated (Mosquera-Losada et al., 2006; López-Díaz et al., 2007; Rigueiro-Rodríguez et al., 2007). However, the use of this residue as a fertilizer must take into consideration the heavy-metal concentration that sewage sludge contains, especially Zn, Cu, and Cr concentrations that are higher than are normally found in soils. Sewage sludge usually contains other heavy metals in lower proportions, such as Cd, Pb, and Hg. Care should be taken to ensure that grazing animals are not exposed to undesirable concentrations of heavy metals, in order to protect the human food chain.

The heavy-metal availability in soil depends on different factors, *i.e.*, its origin, concentration, soil pH, and SOM concentration. The heavy-metal problem is usually more important in acidic soils because heavy-metal solubility is normally increased when soil pH is reduced (Smith, 1996). Moreover, the effect of soil pH may be greater on the availability of



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applied heavy metals with different residues, including sewage sludge (Barbarick et al., 1998; Illera et al., 1999), than in current forms in the soil (Barber, 1995; Reddy et al., 1995), especially on very acidic soils. The increment of heavy-metal availability usually increases their concentration in plants (Krebs et al., 1998). The modification of heavy-metal availability caused by sewage-sludge application in very acidic soils, as a result of the high sewage pH, has not been studied in depth. Changes in soil properties that affect the form and bio-availability of heavy metals, mainly those that are derived from sewage sludge, should be considered in decisions related to the adequate use of this residue as a fertilizer (Allo-way, 1995; Hillman et al., 2003; López-Mosquera et al., 2005).

The aim of this study is to investigate the effects of various doses of sewage sludge and inorganic fertilizer, with or without liming, on total heavy-metal concentrations in soil. This study also attempts to address the availability of the most important heavy metals of sewage sludge (*i.e.*, Zn and Cu) and their plant concentrations in a silvopastoral system located on very acidic soil.

## 2 Materials and methods

The experiment was carried out in Lugo (NW Spain) located in the Atlantic bio-geographic region of Europe, at 748 m above sea level, over a period of 3 years. Annual accumulated precipitation and mean temperature are usually around 1200 mm and 12°C, respectively. Periods of drought probably affect pasture production, and these occurred in June and August of 1998 and 2000 and in July 1999. Periods with low mean temperatures (<6°C) occurred in January of all 3 years and in December 1998.

The experiment was conducted on a 5 year-old *Pinus radiata* D. Don plantation, with a density of 1667 trees ha<sup>-1</sup>. The experimental design consisted of completely randomized blocks with three replicates. In November 1997, the vegetation was cleared, the soil was plowed, and experimental plots were established. The plots (96 m<sup>2</sup> each) consisted of a perfect square with 5 × 5 trees, which were sown with a mixture of 25 kg ha<sup>-1</sup> of *Lolium perenne* var. Brigantia, 10 kg ha<sup>-1</sup> of *Dactylis glomerata* var. Artabro, and 4 kg ha<sup>-1</sup> *Trifolium repens* cv. Huia after plowing. They were initially fertilized with 52.4 kg P ha<sup>-1</sup> and 166 kg K ha<sup>-1</sup>. Eight treatments included: no fertilizer (NF), three sewage-sludge doses based on nitrogen application (S1: 160 kg total N ha<sup>-1</sup>; S2: 320 kg total N ha<sup>-1</sup>; S3: 480 kg total N ha<sup>-1</sup>) taking into account that around 25% of the total nitrogen will be available during the first year (Environmental Protection Agency, 1994), with or without liming applied in 1998 before sowing (2.5 t CaCO<sub>3</sub> ha<sup>-1</sup>). A mineral-fertilizer treatment (MIN) was also included in the trial. It consisted of the application of 500 kg 8:11:13 (N : P : K) mineral fertilizer ha<sup>-1</sup>, following conventional practice for fertilizing pastures. All fertilizer treatments were applied at the start of the growing season in 1998, 1999, and 2000.

Pastures were harvested eight times during the study: in July and December 1998, and in May, July, and November of 1999 and 2000. Before harvesting, four pasture samples

were taken in each plot (0.09 m<sup>2</sup>) using a hand clipper at a height of 2.5 cm. At the same time, a composite soil sample per plot was randomly taken at a depth of 0–5.5 cm (for determining organic matter and available Zn and Cu concentrations) and at a depth of 0–25 cm (for determining Zn and Cu total heavy-metal concentration). In both cases (0–5.5 cm; 0–25 cm), four samples were taken to create the composite sample.

Soil samples were air-dried, passed through a 2 mm sieve, and ground with an agate mortar. Total heavy-metal concentration was analyzed with the VARIAN 220FS spectrophotometer using atomic absorption (VARIAN, 1989), after a nitric acid digestion made in a CEM MDS-2000 microwave (CEM, 1994). Available Zn and Cu were measured after extraction with Mehlich 3 (Mehlich, 1985) with VARIAN 220FS spectrophotometer, using atomic absorption (VARIAN, 1989). Pasture samples were dried in an oven at 80°C to determine dry matter and were ground for chemical analyses. In-plant heavy-metal concentrations (Zn and Cu) were measured with the VARIAN 220FS spectrophotometer using atomic absorption (VARIAN, 1989), after a nitric acid digestion made in a CEM MDS-2000 microwave (CEM, 1994). The results obtained were analyzed with one-way ANOVA and means separated by a Duncan's multiple-range test (SAS, 2001).

## 3 Results

Sewage-sludge composition in 1998, 1999, and 2000 is presented in Tabs. 1 and 2. The sewage sludge originated from the urban residues of the nonmanufacturing town of Lugo, which explains the low level of heavy-metal levels found in this residue (Zn, Cu, Cr, Pb, Cd, Hg, Ni). Heavy-metal sewage-sludge concentrations (Tab. 2) were below Spanish legislated limits for use in agriculture (R.D. 1310/1990: BOE 01/11/1990; Ministerio Agricultura, Pesca y Alimentación, 1990).

The experiment was located in an Umbrisol soil (50 cm depth) with sandy-clay-loam texture (FAO, 2006). The soil bedrock is slate. The soil was acidic (initial soil pH in water [1:2.5: 4.97], with a high initial concentration of SOM [123.2 g kg<sup>-1</sup>], nitrogen [5.2 g kg<sup>-1</sup>], and saturated aluminum percentage [55%], and low Olsen-P soil concentration [0.003 g kg<sup>-1</sup>]). All soil heavy metals (Tab. 3) were below the limits for using sewage sludge as a fertilizer in Spain (R.D. 1310/1990; BOE 01/11/1990; Ministerio Agricultura, Pesca y Alimentación, 1990).

**Table 1:** pH (in water), carbon-to-nitrogen (C : N) ratio, and dry matter (%DM), organic matter (g SOM kg<sup>-1</sup>), N, P, and K (g [kg DM]<sup>-1</sup>) sewage-sludge concentrations in the years 1998, 1999, and 2000.

Year	pH	C : N	DM	SOM	N	P	K
1998	6.9	8.8	25.0	490.0	32.1	9.3	2.5
1999	6.9	7.1	25.0	392.5	32.1	5.2	2.5
2000	6.9	6.1	23.5	441.2	42.3	16.5	2.6

**Table 2:** Zn, Cu, Cr, Cd, Hg, Ni, and Pb (mg [kg DM]<sup>-1</sup>) sewage-sludge concentration in the years 1998, 1999, and 2000 and legal limits in EU European Directive 86/278 (European Union, 1986) and Spain R.D. 1310/1990 (Ministerio Agricultura, Pesca y Alimentación, 1990). Limits depend on soil pH (minimum: soil pH < 7, maximum: soil pH > 7).

Years	Zn	Cu	Cr	Cd	Hg	Ni	Pb
1998	821.0	244.0	39.0	5.0	1.5	21.0	203.0
1999	746.0	154.0	141.0	1.0	1.5	30.0	94.7
2000	1320.0	241.0	74.0	1.5	1.5	49.0	184.0
Law limits	2500–4000	1000–1750	1000–1500	20–40	16–25	300–400	750–1200

**Table 3:** Zn, Cu, Cr, Cd, Hg, Ni, and Pb (mg [kg DM]<sup>-1</sup>) soil concentration, at the beginning of the experiment (September 1997), and legal limits in EU European Directive 86/278 (European Union, 1986) and Spain R.D. 1310/1990 (Ministerio Agricultura, Pesca y Alimentación, 1990). Limits depend on soil pH (minimum: soil pH < 7, maximum: soil pH > 7).

	Zn	Cu	Cr	Cd	Hg	Ni	Pb
mg kg <sup>-1</sup>	17.2	8.2	13.2	<1	0.13	<1	<1
Legal limits	150–300	50–140	100–150	1.0–3.0	1.0–1.5	30–75	50–300

In Fig. 1, the mean total soil concentrations of Zn and Cu in November 2000 are shown. Results of the first 2 years (1998 and 1999) are not presented, because the effects of different treatments were minimal and increases were not observed over this period. In general, in the final sampling (November 2000) Zn in-soil concentration was increased by sewage-sludge application (from 17.1–17.7 mg kg<sup>-1</sup> in NF to 14.7–29.9 mg kg<sup>-1</sup> in sewage-sludge treatment), with or without lime, except S3 from limed plots. However, in-soil Cu values with sewage sludge were always lower or similar to the NF treatment, both in the limed or unlimed plots. Cu results obtained with mineral fertilization were similar to control.

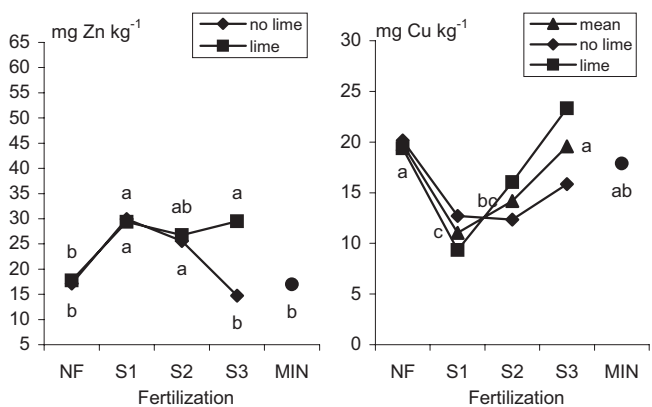
The range of available Zn was between 1 and 27 mg Zn kg<sup>-1</sup> (Tab. 4). In general, the sewage-sludge application improved Zn availability, mainly after 1999, although the response to

the high dose (S3) was observed in December 1998, due to the higher quantity of Zn that was added with the sewage-sludge application with respect to the other heavy metals. In July (mg Zn kg<sup>-1</sup> = 11.07 × N<sup>2</sup> + 3.46 × N + 1.16; r<sup>2</sup> = 0.99) and December 1998 (mg Zn kg<sup>-1</sup> = 29.46 × N<sup>2</sup> + 10.71 × N + 2.05; r<sup>2</sup> = 0.99) and May 1999 (mg Zn kg<sup>-1</sup> = 9.14 × N + 1.14; r<sup>2</sup> = 0.87), Zn concentration was proportional to the nitrogen applied with sewage sludge. On the other hand, mineral fertilizer produced similar values to the NF treatment and lower values than the sewage-sludge treatments.

Plant Zn concentrations ranged from 9.6 to 52.3 mg kg<sup>-1</sup> (Fig. 2), but until July 1999, the value range was between 10.8 and 20.9 mg Zn kg<sup>-1</sup> (data not presented). There was no significant response of in-plant Zn concentration to sewage-sludge application until November 1999. In that harvest, the sewage sludge increased the proportion of in-plant Zn (from 15.7 with the NF treatment to 20.9–51.7 mg kg<sup>-1</sup> with sewage sludge). The same response was obtained in July (mg Zn kg<sup>-1</sup> = 12.47 × N + 15.43; r<sup>2</sup> = 0.86) and November 2000 (from 19.1 and 13.2 to 23.5–25.5 and 29.7–34.1 mg kg<sup>-1</sup> in July and November 2000, respectively). Mineral fertilizer produced values of Zn similar to the NF treatment and, sometimes, Zn soil concentration values were lower than in soil treated with sewage sludge.

Copper extracted with Mehlich 3 was between 0.1 and 2.7 mg kg<sup>-1</sup> (Fig. 3). In general, there was no significant response to liming and MIN treatments applied individually. These values were similar to un-limed and NF treatment and lower than those obtained with sewage-sludge treatments.

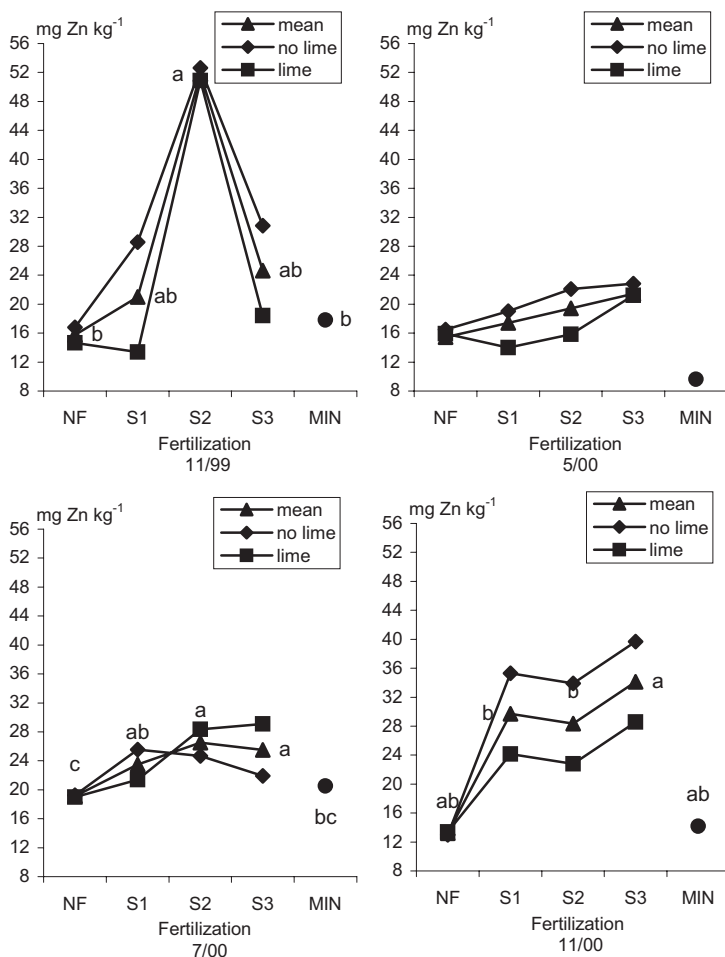
Sewage-sludge application improved Cu extractability with the higher dose (S3) from the end of 1998 and in the other organic treatments (S1 and S2) from 1999. Copper extracted with Mehlich 3 was directly proportional to the applied doses in the final two years. It increased from 0.2 and 0.4 mg kg<sup>-1</sup> with the NF treatment to 0.6–1.5 and 0.9–2.4 mg kg<sup>-1</sup> with sewage sludge, in 1999 and 2000, respectively. Plant Cu concentration was between 1 and 9.3 mg kg<sup>-1</sup> (Fig. 4). In general,



**Figure 1:** Mean total concentrations of Zn (mg Zn kg<sup>-1</sup>) and Cu (mg Cu kg<sup>-1</sup>) in soil under the different fertilizer and lime treatments in November 2000 (Zn) and in May, July, and November of the year 2000 (Cu). Liming dose was 2.5 t CaCO<sub>3</sub> ha<sup>-1</sup>; NF: no fertilizer; S1: low sewage-sludge doses (160 kg total N ha<sup>-1</sup>); S2: medium sewage-sludge doses (320 kg total N ha<sup>-1</sup>); S3: high sewage-sludge doses (480 kg total N ha<sup>-1</sup>); MIN: mineral fertilizer. Different letters indicate significant differences between fertilizer treatments (◆: no lime; ■: lime) or the mean of both (▲) (p < 0.05).

**Table 4:** Mean available (Mehlich 3) zinc concentrations (mg Zn kg<sup>-1</sup>) in soil under the different fertilizer and lime treatments in 1998, 1999, and 2000. Liming dose was 2.5 t CaCO<sub>3</sub> ha<sup>-1</sup>; NF: no fertilizer; S1: low sewage-sludge doses (160 kg total N ha<sup>-1</sup>); S2: medium sewage-sludge doses (320 kg total N ha<sup>-1</sup>); S3: high sewage-sludge doses (480 kg total N ha<sup>-1</sup>); NFL: no fertilizer + lime; S1L: low sewage-sludge doses (160 kg total N ha<sup>-1</sup>) + lime; S2L: medium sewage-sludge doses (320 kg total N ha<sup>-1</sup>) + lime; S3L: high sewage-sludge doses (480 kg total N ha<sup>-1</sup>) + lime; MIN: mineral fertilizer. Different letters indicate significant differences between fertilizer treatments (small letter: no lime; capital letter: lime) ( $p < 0.05$ ).

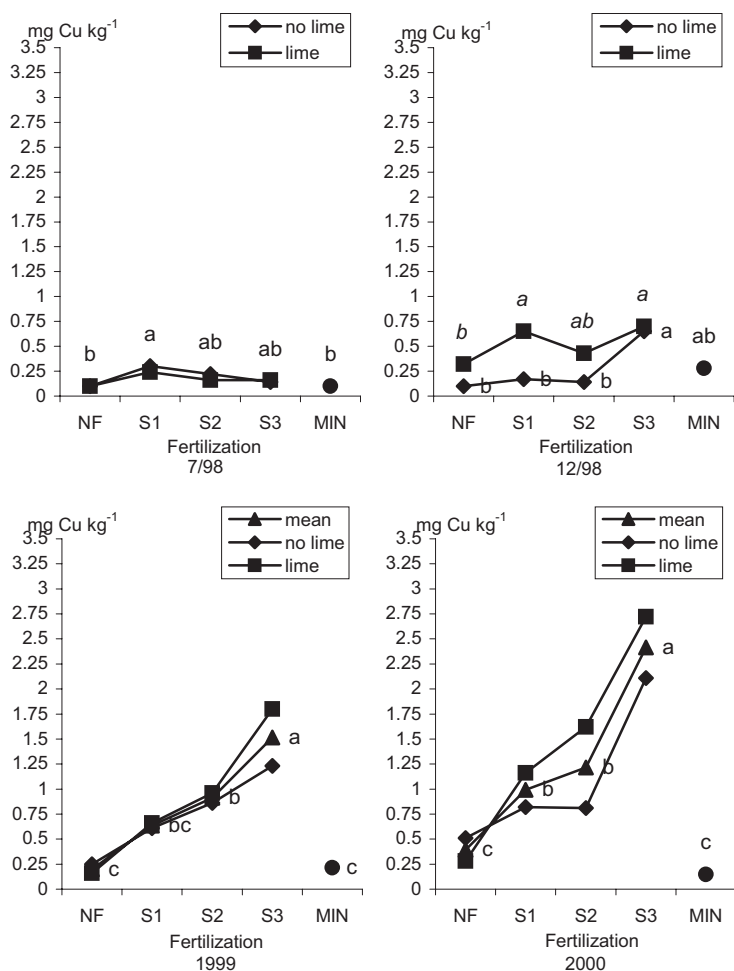
Date	NF	S1	S2	S3	MIN	NFL	S1L	S2L	S3L
07/98	0.9	1.4	1.3	1.4	1.2	1.2	0.9	1.9	2.1
12/98	1.1c	1.6b	1.6b	3.1a	1.3b	2.1B	1.1C	1.6BC	3.7A
05/99	1.3c	3.8ab	4.4a	5.1a	1.5bc	1.1	2.6	4.1	5.5
07/99	1.0b	3.3ab	5.2ab	5.7a	0.8b	0.8C	2.0BC	3.8B	11.1A
11/99	1.3c	2.1c	4.6ab	8.4a	1.5b	1.6B	3.0B	6.8A	7.2A
05/00	1.2c	8.9b	8.1b	15.7a	1.0c	1.2B	7.3AB	10.3AB	13.7A
07/00	2.0c	6.3c	10.6b	14.9a	1.5c	1.6B	9.0B	8.9B	20.8A
11/00	3.0d	6.5c	8.9b	27.1a	1.6d	1.2B	5.9B	12.9AB	24.7A



**Figure 2:** Mean total zinc concentrations in forage (mg Zn kg<sup>-1</sup>) under the different fertilizer and lime treatments in 1999 and 2000. Liming dose was 2.5 t CaCO<sub>3</sub> ha<sup>-1</sup>; NF: no fertilizer; S1: low sewage-sludge doses (160 kg total N ha<sup>-1</sup>); S2: medium sewage-sludge doses (320 kg total N ha<sup>-1</sup>); S3: high sewage-sludge doses (480 kg total N ha<sup>-1</sup>); MIN: mineral fertilizer. Different letters indicate significant differences between fertilizer treatments (▲) ( $p < 0.05$ ).

there was no in-plant Cu-concentration response to liming. In 1998, only the high sewage-sludge dose (S3) increased Cu plant concentration (3.5 mg kg<sup>-1</sup>) compared to NF (2.7 mg kg<sup>-1</sup>). In May 1999, that response was observed with all the sewage-sludge treatments (3.9–9.3 mg kg<sup>-1</sup>). Furthermore, it was directly proportional to the applied doses of sew-

age sludge. However, in the final sampling (November 2000), S2 and S3 treatments had Cu concentrations (2.4–2.9 mg kg<sup>-1</sup>, respectively) significantly lower than NF (5.7 mg kg<sup>-1</sup>). The mineral fertilizer and NF treatments produced similar values of Cu, as was previously found in the soil.



**Figure 3:** Mean available (Mehlich 3)-copper concentrations (mg Cu kg<sup>-1</sup>) in soil under the different fertilizer and lime treatments in 1998, 1999, and 2000. Liming dose was 2.5 t CaCO<sub>3</sub> ha<sup>-1</sup>; NF: no fertilizer; S1: low sewage-sludge doses (160 kg total N ha<sup>-1</sup>); S2: medium sewage-sludge doses (320 kg total N ha<sup>-1</sup>); S3: high sewage-sludge doses (480 kg total N ha<sup>-1</sup>); MIN: mineral fertilizer. Different letters indicate significant differences between fertilizer treatments (◆: no lime; ■: lime) or the mean of both (▲) (*p* < 0.05).

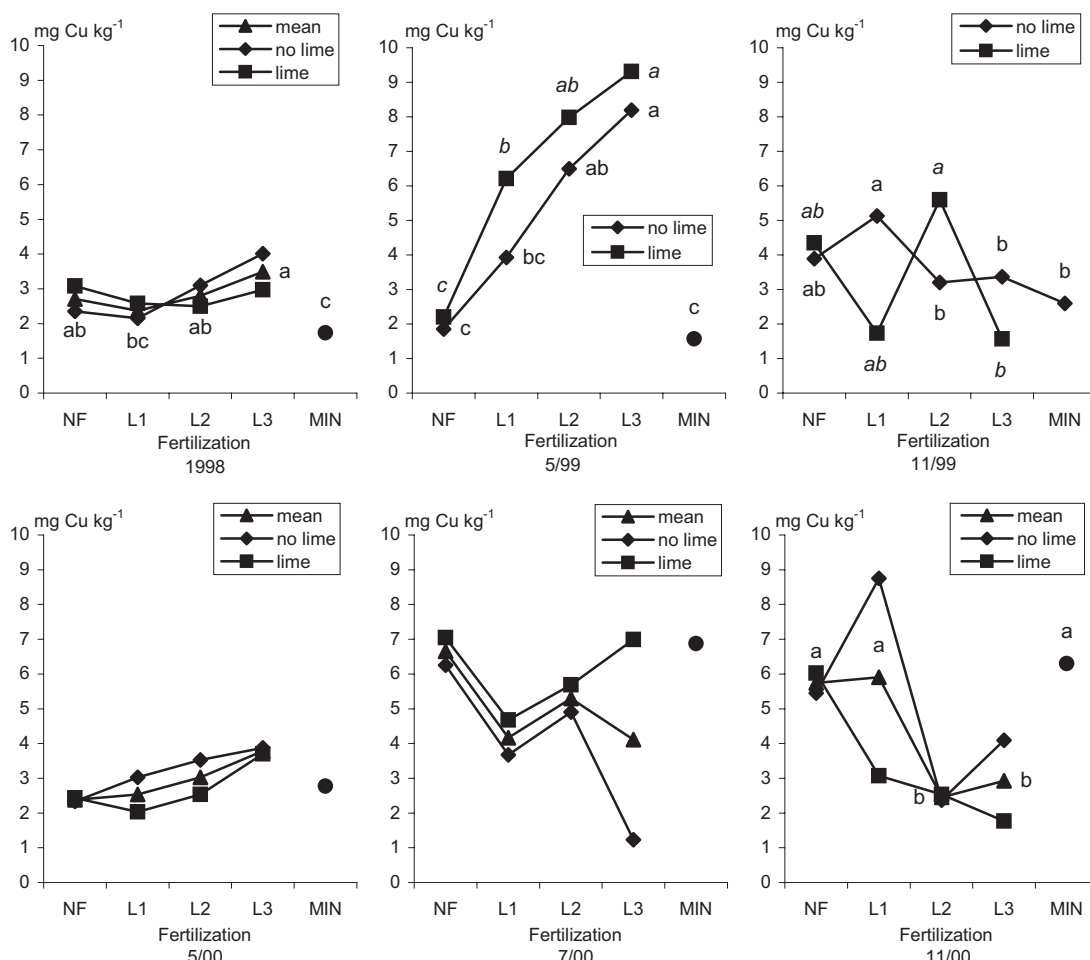
### 4 Discussion

The total in-soil values of Zn and Cu can be considered to be low when compared to the usual range for natural soils detected by several authors, such as Barber (1995; 10–300 mg kg<sup>-1</sup> for Zn, 1–50 mg kg<sup>-1</sup> for Cu) and Kabata-Pendias and Pendias (1985; 10–105 mg kg<sup>-1</sup> for Zn and 6–60 mg kg<sup>-1</sup> for Cu). Moreover, all values (Zn: 7.6–60.7 mg kg<sup>-1</sup>; Cu: 1.7–28.4 mg kg<sup>-1</sup>) were always very low compared to the Spanish regulation limits (Zn: 150 mg kg<sup>-1</sup>; Cu: 50 mg kg<sup>-1</sup>). This may be explained by the fact that this experiment was located in a woodland area without nearby pollution sources. The total in-soil Zn concentration was only significantly increased in the final year (2000), after three annual consecutive sewage-sludge applications, and in November (*p* = 0.01) after the high precipitation of spring and the high temperatures of summer, which contributed to the incorporation of the sewage sludge into the soil.

The effect of different treatments on the extractable Zn and Cu depended on the influence of these treatments on the factors that affect their availability, such as SOM and pH, as was observed by Chaudri et al. (2000), Hillman et al. (2003), McBride et al. (2004), and López-Mosquera et al. (2005). Extractable-Zn and -Cu values (Mehlich 3) were similar to those obtained by Monterroso et al. (1999) in this region, in

very acid mine soils (0.6–33.2 mg kg<sup>-1</sup> and 0.6–17.6 mg kg<sup>-1</sup>, respectively). The role of SOM in heavy-metal adsorption in acid soils has already been described (Kabata-Pendias and Pendias, 1985; Alloway, 1995). Higher levels of SOM reduce the negative effect of heavy metals, because SOM reduces their availability (Kabata-Pendias and Pendias, 1985). But, under our conditions and due to changes in pH caused by liming and sludge application, the availability of Zn and Cu applied with the sludge can be indirectly increased. This is due to the important effect of both treatments on mineralization, which mobilizes heavy metals and favors their incorporation into the soil. Later, heavy-metal availability is affected by the soil pH due to the higher availability of heavy metals in acid soils (Porta et al., 1999). It should be noted that Zn and Cu are the main heavy metals of sewage sludge, and therefore the impact of sludge application is higher for those elements than with others regulated by the R.D. 1310/1990 (BOE 01/11/1990; Ministerio Agricultura, Pesca y Alimentación, 1990), such as Hg, Cd, Ni and Pb (López-Díaz et al., 2007).

Sewage sludge very significantly increased Zn and Cu availability in the third year. In that year, the increase of Zn and Cu was roughly 100%–2300% and 186%–586%, respectively, compared to the NF treatment. In spite of this, the residue has an exceptionally high quality with respect to heavy-metal



**Figure 4:** Mean total copper concentrations in forage (mg Cu kg<sup>-1</sup>) under the different fertilizer treatments in 1998, 1999, and 2000. Liming dose was 2.5 t CaCO<sub>3</sub> ha<sup>-1</sup>; NF: no fertilizer; S1: low sewage-sludge doses (160 kg total N ha<sup>-1</sup>); S2: medium sewage-sludge doses (320 kg total N ha<sup>-1</sup>); S3: high sewage-sludge doses (480 kg total N ha<sup>-1</sup>); MIN: mineral fertilizer. Different letters indicate significant differences between fertilizer treatments (◆: no lime; ■: lime) or the mean of both (▲) (*p* < 0.05).

concentration according to EU and US standards (USEPA, 1993; Smith, 1996). This can be explained by the fact that the town of Lugo does not have a significant metal industry and the population is low (around 80,000 inhabitants).

In spite of the fact that the total in-soil heavy-metal concentration was not generally modified by fertilizer treatments, the response of Zn and Cu soil availability to sewage-sludge application appears to indicate that available Zn and Cu in acid soils with high SOM concentration is increased after consecutive applications. If the sewage sludge applied had a higher heavy-metal concentration, it is probable that this effect would have been more pronounced. It is important to evaluate the heavy-metal-solubility changes that occur when using sewage-sludge application over a long term. These directly affect plant absorption and the leaching of heavy metals through the soil profile. This has not yet been taken into consideration in either European or Spanish policy. The lack of response of Zn and Cu to mineral fertilizer could be explained by the fact that, despite the heavy-metal concentration of this fertilizer (Verloo and Willaert, 1990), the total amount applied was much lower than with the sewage-sludge treatments.

The in-plant range of Zn (9.6–52.3 mg kg<sup>-1</sup>) and Cu (1–9.3 mg kg<sup>-1</sup>) obtained was slightly below the usual in-plant range (25–250 mg kg<sup>-1</sup> for Zn, Jones, 1972; 10–80 mg kg<sup>-1</sup> for Cu, Loué, 1988) and below the threshold that indicates plant deficiency (25 mg kg<sup>-1</sup> for Zn, Jones, 1972; 5 mg kg<sup>-1</sup> for Cu, Kabata-Pendias and Pendias, 1985). Moreover, Zn values were only sufficient for plant development when sewage sludge was applied, which could be regarded as a benefit. Moreover, Zn and Cu concentrations were often lower than those obtained by Mosquera-Losada and González-Rodríguez (2004) in Galician pastures on more fertile agricultural land (23.2–48.1 mg kg<sup>-1</sup> for Zn; 5.7–11.2 mg kg<sup>-1</sup> for Cu), that had been fertilized with inorganic compounds. This finding is highly relevant because sewage-sludge input will improve levels of in-plant microelements, rather than causing detrimental effects. In-plant Zn concentration peaked in November 1999, but not in the other years studied. In the first year, this could be explained with the age of the pasture (recently sown). In the last year, however, the reduction in plant Zn could be explained with the increased presence of orchard grass (Rigueiro-Rodríguez et al., 2005), which does not accumulate Zn (Genevini et al., 1983; Mosquera-Losada et al., 2001). In Galicia, Mosquera-Losada and González-Rodríguez

(2004) also detected a peak in Zn concentration in November. In the present study, there were no seasonal variations of in-plant Cu concentration, as *García et al.* (1986) also observed.

In general, liming did not significantly modify the in-plant concentrations of Zn and Cu, as was also observed by *García et al.* (1986) and *Stevens and Laughlin* (1996) for Cu. Sewage-sludge treatments scarcely affected plant Zn (until November 1999) or Cu in the forage (*McBride et al.*, 2004) in spite of inputs made with the sewage-sludge application (especially Zn: 12.8–38.4 kg ha<sup>-1</sup>) and the effect on the increase of the availability of Zn and Cu in soil. The lack of response to sewage-sludge treatments was probably due to the low initial in-soil levels and the increase of the proportion of species that do not accumulate Zn and Cu with sewage-sludge treatments, such as orchard grass (*Genevini et al.*, 1983; *López-Díaz et al.*, 2007). Moreover, in 1998 and 1999, the increase in forage production caused by sewage-sludge fertilizer (*López-Díaz et al.*, 2007) could have produced a dilution effect. This could explain the reduction of heavy-metal concentration in the plants. In other studies, in which the pastures consisted of other unsown species, as well as *Holcus mollis*, in-plant heavy-metal concentration was increased significantly by sewage-sludge application (*Mosquera-Losada et al.*, 2001).

The percentage of extracted heavy metals from the pasture of this experiment, compared to those applied with sewage sludge, was very low in Zn and Cu (0.9%–2.2% and 0.7%–1.7%), and these values decreased as the sewage-sludge dose was increased. The lack of response of in-plant Zn and Cu to the mineral fertilizer could be explained by the fact that this fertilizer contains low amounts of heavy metals. The maximum Zn and Cu in-forage concentration established by *NRC* (1980) for bovines (500 and 100 mg kg<sup>-1</sup>, respectively), ovine (300 and 25 mg kg<sup>-1</sup>), and equines (500 and 800 mg kg<sup>-1</sup>) were never exceeded, which indicated that the pasture of this experiment is adequate for animal consumption. In general, Zn and Cu maintenance requirements of goats (45 and 7 mg kg<sup>-1</sup>, respectively; *Lamand*, 1981), horses (40 and 9 mg kg<sup>-1</sup>; *NRC*, 1989), bovines (20 and 4 mg kg<sup>-1</sup>), and ovine (35 and 5 mg kg<sup>-1</sup>) were not often reached. In these cases, supplements of these elements to the animals would be recommended if their nourishment was derived solely from these pastures.

Therefore, in very acidic soils with high SOM concentration, the application of sewage sludge increased availability of some heavy metals, such as Zn and Cu, but not their in-plant concentration. This is due to the increase of grass species, such as *Dactylis glomerata* L., that does not accumulate these elements and is also due to the dilution effect caused by an improvement in pasture production. Moreover, in this experiment, the initial level of in-plant (13–18 mg Zn kg<sup>-1</sup> and 2.1–4 mg Cu kg<sup>-1</sup>) and in-soil heavy metal was very low. Hence, the in-plant levels obtained were very low and often did not fulfill the livestock needs of Zn and Cu.

## 5 Conclusions

Liming did not vary Zn and Cu availability. However, sewage sludge did increase Zn and Cu availability, although total in-soil Zn was only increased in November 2000. Liming had very little effect on the concentrations of Zn and Cu in plants. The sewage-sludge treatments scarcely varied the in-forage proportion of Zn (until November 1999) or the Cu. Sewage-sludge application increased the in-plant proportion of Zn in some samplings of the second and third year, due to the sewage-sludge residual effect. In all cases, the forage obtained was not toxic for animal consumption. These results indicate that policy with respect to sewage-sludge application as a fertilizer must regulate both heavy-metal availability and total in-soil metal concentration, because, in this way, environmental risk could be better evaluated. Finally, the use of sewage sludge as a fertilizer produces a higher production and quality of pasture than mineral fertilizers in sandy-clay-loam soils, while maintaining adequate heavy-metal values for animal feeding with respect to Zn and Cu concentration.

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