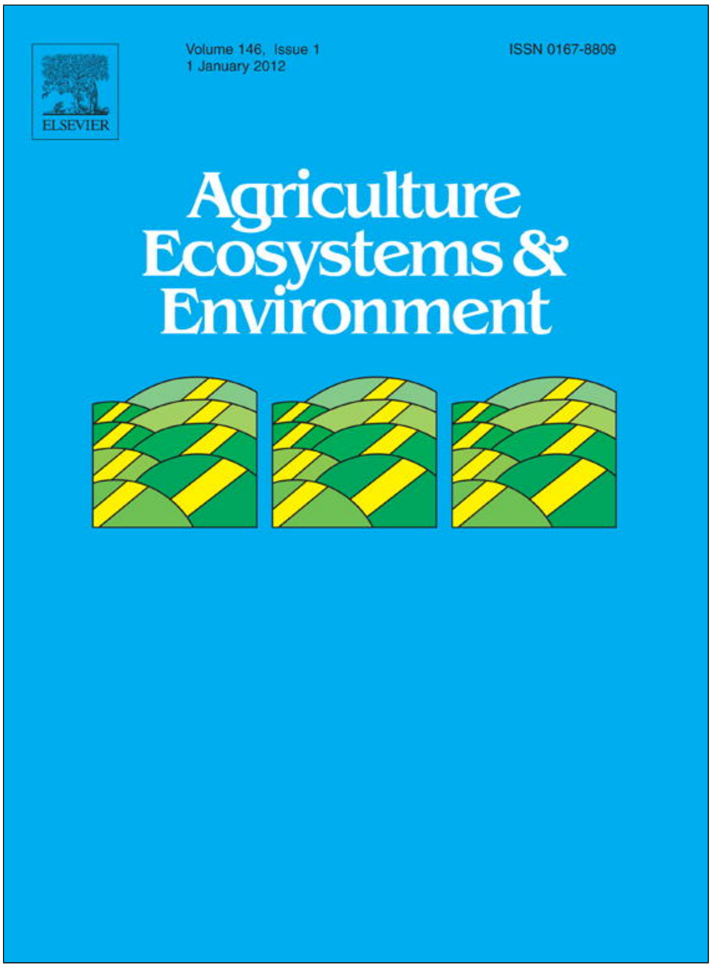
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Pasture and soil zinc evolution in forest and agriculture soils of Northwest Spain three years after fertilisation with sewage sludge

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ABSTRACT

The main problem with the agricultural use of sewage sludge is the higher heavy metal (mainly zinc) concentrations in the sludge than in the soil. In Spain, R.D. 1310/1990 and European Directive 86/278 limit the total heavy metal concentration in soil but not the changes in heavy metal availability, which directly affect plant absorption and thereby represent a possible risk to human health. The heavy metal availability in soil depends on different factors (pH, soil organic matter, and weather) and the type of soil (agriculture or forest). This study evaluate the effects of two types of soils (forest and agriculture), two types of vegetation (natural and sown), and two types of fertilisation (sludge fertilisation and mineral fertilisation, with a no fertiliser control) in afforested and treeless pastures and in sown and unsown forestlands of Northwest Spain on the total and available Zn concentration in soil and the concentration of Zn in grasslands. The experimental design was completely randomised with nine treatments and three replicates. The fertilisation with sewage sludge increased the total, Mehlich 3 and sward Zn concentrations in forest and agriculture soils, and the levels of Zn were lower in the forest than in the agriculture soils probably because of the low soil pH in the former which probably limited the mineralisation of the sludge in the forest soil. Therefore, there is still a potential source of Zn in forest soils than in agriculture soils, when the incorporation conditions improve. This makes advisable to extend the period without grazing after sewage sludge inputs in forest than agriculture soils to avoid direct Zn intake by animals through the soil consumption. The effect of the application of sewage sludge on sward Zn concentrations was more relevant in unsown pastures of forest soils than in agriculture soils due to the different absorption capacity of unsown compared with sown species. Zinc inputs from sewage sludge to soils did not cause harmful effects on plants or animals. However, the management of heavy metal availability must be included by policy makers in further directives to better evaluate environmental risk. Moreover, Zn maintenance requirements of animals like goats, horses, bovines and ovine were not often reached, which may make advisable to provide Zn supplement to animals if their nourishment is derived solely from these pastures.

1. Introduction

Silvopastoral systems are a type of agroforestry system in which trees and pasture production are combined, and these systems are currently promoted by the EU (Council Regulation 1698/2005 (EU, 2005)). In the Galician silvopastoral systems, the productivity (understory and tree) can be limited by low soil fertility due to acidity (Zas and Alonso, 2002). As compared to the exclusively agricultural systems, the use of sewage sludge as a fertiliser can modify the productivity of different components of the silvopastoral system and simultaneously reduce the risk of heavy metal leaching because the tree roots reach a greater soil depth and volume than pasture roots and therefore take up metals (Rigueiro-Rodríguez et al., 2008).

Pinus radiata (D. Don) is a tree species that is currently used in silvopastoral systems in temperate areas, such as Australia, New Zealand and Chile (Hawke, 1991; Knowles, 1991; Benavides et al., 2009), because of its fast growth. This species is widely used in the Atlantic biogeographic region of Europe (mostly north of Spain and west of France) in both forestry and farm grassland soils. The benefits of using sewage sludge as fertiliser in silvopastoral systems with *P. radiata* (D. Don) in agriculture and forest soils have already been shown in the different areas of the Atlantic biogeographic region of Spain (Mosquera-Losada et al., 2001, 2009, 2010b; Egiarte et al., 2009).

Nitrogen fertilisation is one of the simplest tools to enhance crop production. Nitrogen fertilisation can be performed with mineral or organic forms of nitrogen, but the recent increases in inorganic fertiliser prices along with increasing environmental concerns have

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reduced the use of inorganic nitrogen fertilisers in the EU (EFMA, 2009). Moreover, inorganic fertilisers are currently being replaced by organic fertilisers, such as sewage sludge, because of the lower cost of this nitrogen resource and the promotion or using sewage sludge by the EU (European Directive 86/278 (EU, 1986)) and Spanish regulations (R.D. 1310/1990 (BOE, 1990)). Sewage sludge has been used as a fertiliser in agriculture for many years because it eliminates waste and reduces environmental pollution whilst imparting organic matter and macronutrients, particularly N and P, to the soil (Beltran et al., 2002; Cogger et al., 2004; Mosquera-Losada et al., 2010a). However, to use this residue as a fertiliser, its heavy metal concentration, which is higher than that normally found in soils (Smith, 1996), must taken be into consideration. In Europe (European Directive 86/278 (EU, 1986)) and Spain (R.D. 1310/1990 (BOE, 1990)), there are regulations that limit the total heavy metal concentration in soil and sewage sludge to minimise the harmful effects of sewage sludge fertilisation on soil, vegetation, animals and human health. National regulations also establishes periods without grazing after direct sewage sludge inputs, which varies between countries (Smith, 1996).

Although the directives to use sewage sludge as a fertiliser are exclusively based on the total concentration of heavy metals (EU, 1986; BOE, 1990), some authors have claimed that the availability of the heavy metal should be included as a criterion in addition to the pH level (Antoniadis et al., 2008). Heavy metal availability is usually correlated with plant heavy metal uptake and thereby provides information regarding the potential risk for the heavy metals to reach humans, which would cause illnesses through the trophic chain (Alirzayeva et al., 2006; García-Sánchez et al., 2008). Heavy metal availability in soil depends on the soil pH and other factors, such as the parent material, heavy metal concentration, soil organic matter content and weather (Smith, 1996; Mosquera-Losada et al., 2011). Therefore, heavy metal availability can be modified by the type of soil or the previous use of the soil (agriculture or forest) where sewage sludge has been applied. Forest soils usually have higher organic matter contents than agriculture soils (Romanyà et al., 2007), and organic matter controls the dynamics of metal transfer to plants and limits metal availability (McBride et al., 1997).

Zinc is one of the most abundant heavy metals in sewage sludge (Antoniadis, 2008; Mosquera-Losada et al., 2010a). Although Zn is essential for both plants and animals, it may become toxic at high concentrations (Ngole and Ekosse, 2009). Zinc is known to be amongst the most mobile heavy metals in the soil/plant system (Sauerbeck, 1991), mainly in acid soils like forests (Mosquera-Losada et al., 2009), where may be limiting to fulfil plant or animal needs of Zn. However, Zn phytotoxicity is rarely reported (He et al., 1995). Moreover, Zn is the element in sewage sludge identified as the main concern in relation to potential impacts on soil microbial activity (Smith, 2009), which may have a negative effect on the fertility of soil (De Vries et al., 2004). Furthermore, elevated inputs of sewage sludge may increase the leaching losses of Zn to ground and surface waters, thus affecting drinking water quality and aquatic organisms, respectively (De Vries et al., 2004). Therefore, when sewage sludge is used as a fertiliser, it is important to be aware of its effects on the concentrations of Zn in soil and plants. The hypothesis of this paper is that Zn movement and availability in the upper layer of the forest soils is higher than in agriculture soils due to the higher soil acidity of forest soils, which usually modify absorption by plants. The second hypothesis is based on land use practice: Zn absorption by trees cause a lower levels of Zn in pasture developed in forest soils when compared with grasslands without trees. The aim of this study was to evaluate the total and available Zn concentration in soil and the concentration of Zn in the pasture response to mineral or municipal sewage sludge inputs in grasslands, forestlands and silvopastoral systems developed under *P. radiata* (D. Don) establishment in agriculture and forest soils.

2. Materials and methods

2.1. Characteristics of the study site

The experiment was initiated in December 2006 through the installation of 27 pots in the town of Piugos (Lugo, Galicia, NW Spain, European Atlantic Biogeographic Region) at an altitude of 470 m above sea level. Fig. 1 shows the monthly mean precipitation and temperature values for 2007, 2008 and 2009 in addition to the normal mean precipitation and temperature values of the study area. The total annual rainfall was 658, 1000 and 873 mm in 2007, 2008 and 2009, respectively. In addition, the rainfall registered in the spring of 2007, 2008 and 2009 was 440, 605 and 368 mm, respectively. In general, these years were drier than the mean normal year (998 mm) for the study area. However, the mean monthly precipitation in 2007 was higher than the mean normal precipitation from June to August, which reduced the traditional drought period in 2008 and 2009 and limited pasture growth. The annual mean temperature was 12 °C.

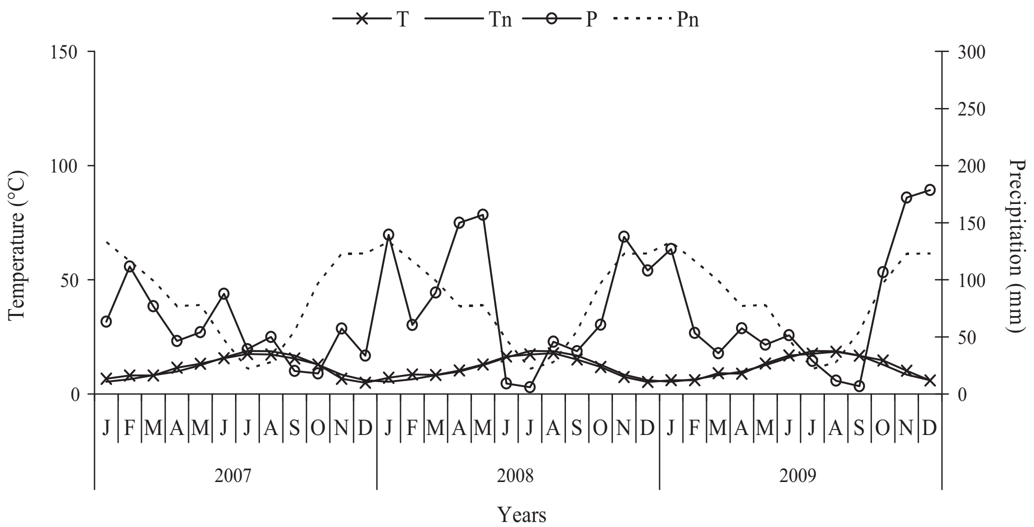

Fig. 1. Mean monthly precipitation and mean monthly temperature data in 2007, 2008, and 2009 and mean normal data for the study area. *T*: mean monthly temperature (°C), Tn: mean normal temperature, *P*: mean monthly precipitation (mm), and Pn: mean normal precipitation.

Table 1
Heavy metal concentrations[a] in the agriculture soil and in the forest soil at the beginning of the experiment and legal limits established by European Directive 86/278 and Spain R.D. 1310/1990.

Soil	Heavy metal concentrations (mg kg^{-1})					
	Cd	Cu	Cr	Ni	Pb	Zn
Initial agriculture soil	0.1	1	0.9	–	17.7	28.8
Initial forest soil	0.9	7.8	2	–	–	32.5
Spanish legal limits	1–3	50–210	100–150	30–112	50–300	150–450

–, Element concentration below detection limit of the technique used in its determination.
[a] Limits depend on soil pH (minimum, soil pH < 7; maximum, soil pH > 7).

The pots used in this experiment were cylindrical (approximately 2 m^3 in size with a height of 144 cm and width of 134.5 cm) and were filled with soils. Fifteen pots were filled with agriculture soil from Sarria (Lugo, Galicia, NW Spain), and 12 pots were filled with forest soil from Bascuas (Condesmo, Lugo, Galicia, NW Spain). The soils were excavated to 1 m depth, then mixed and packed into the pots and trying to simulate the use of forest and agriculture soils to recover lands in the area. The soils were Gleyic Umbrisols (FAO classification) and Umbrept Inceptisols (USDA system). Initial agriculture soil analyses showed a loamy sand soil texture (83.5% sand, 12.3% silt and 4.2% clay) with an acidic water pH (5.5) (Faithfull, 2002) and low soil organic matter (SOM; 36.3 g kg^{-1}) (Kowalenko, 2001). Moreover, the forest soil analyses showed a sandy loam soil texture (71.7% sand, 19.5% silt and 8.9% clay) with an acidic water pH (5.2) (Faithfull, 2002) and a higher SOM content (72 g kg^{-1}) than the agriculture soils (Kowalenko, 2001). All heavy metal concentrations in both agriculture and forest soils (Table 1) were below the maximum thresholds for the use of sewage sludge as a fertiliser as specified by EU Directive 86/278/CEE (EU, 1986) and Spanish legislation under R.D. 1310/1990 (BOE, 1990).

2.2. Experimental design

The experimental design was completely randomised with nine treatments and three replicates. The treatments followed a design that consisted of a fractional factorial design of a 2p fully factorial with "p" being equal to four factors (two levels per factor). The treatments were chosen because they were the most traditional practices in the area of study (agriculture soil without trees, forest soil without pastures and silvopastoral systems) in forest and agriculture lands. The following treatments were used: (1) agriculture soil + pasture sowing (*Agriculture + P*); (2) agriculture soil + pasture sowing + sewage sludge (*Agriculture + PS*); (3) agriculture soil + pasture sowing + mineral fertiliser (*Agriculture + PM*); (4) agriculture soil + pasture sowing + sewage sludge + tree (*Agriculture + PST*); (5) agriculture soil + pasture sowing + mineral fertiliser + tree (*Agriculture + PMT*); (6) forest soil + sewage sludge + tree (*Forest + ST*); (7) forest soil + mineral fertiliser + tree (*Forest + MT*); (8) forest soil + pasture sowing + sewage sludge + tree (*Forest + PST*); and (9) forest soil + pasture sowing + mineral fertiliser + tree (*Forest + PMT*). The following procedure was used:

- Pasture sowing (P) – the pasture was sown with a mixture of *Dactylis glomerata* L. var. Artabro (12.5 kg ha^{-1}), *Lolium perenne* L. var. Brigantia (12.5 kg ha^{-1}) and *Trifolium repens* L. var. Huia (4 kg ha^{-1}) in December 2006.
- Tree (T) – a one-year-old *P. radiata* (D. Don) tree was planted in January 2007.
- Sewage sludge (S) – an anaerobically digested sludge with an input of 320 kg total N ha^{-1} was applied to the surface in December 2006.
- Mineral (M) – in the Agriculture + PM, Agriculture + PMT, Forest + MT and Forest + PMT treatments, 500 kg ha^{-1} of 8% N:24% P_2O_5:16% K_2O was applied at the beginning of the year in 2007, 2008 and 2009 and 40 kg of N ha^{-1} as calcium ammonium nitrate (26% N) was applied after each harvest.

2.3. Sewage sludge

Anaerobically digested sludge was collected from the municipal waste treatment plant of Lugo. A calculation of the required amount of sludge was conducted according to the percentage of total N and dry matter content in the sludge (EPA, 1994), taking into account that approximately 25% of the total N from anaerobically digested sewage sludge is available in the first year after application as indicated by EPA (1994). EU Directive 86/278/CEE (EU, 1986) and Spanish regulation R.D. 1310/1990 (BOE, 1990) regarding heavy metal concentrations in the application of sewage sludge to soil were also considered. The composition of the sewage sludge applied is summarised in Table 2. Initial soil analyses showed a concentration of 79.2 kg Zn ha^{-1} in the agriculture soils and 89.4 kg Zn ha^{-1} in the forest soils, meanwhile the fertilisation with sewage sludge implied inputs of 11.4 kg Zn ha^{-1} into the agriculture and forest soils.

2.4. Field samplings and laboratory determinations

As described in R.D. 1310/1990 (BOE, 1990), soil samples were collected down to a depth of 25 cm in March 2008, January 2009 and January 2010. When samples were taken, there was not a sewage sludge layer, because the residue was already incorporated. In the laboratory, the soil samples were air-dried, passed through a 2-mm sieve, and ground with an agate mortar. Soil pH was determined in water (1:2.5) (Faithfull, 2002), and the total Zn concentration was analysed with the VARIAN 220FS spectrophotometer using atomic absorption (VARIAN, 1989) after nitric acid digestion in a CEM MDS-2000 microwave (CEM, 1994). The available Zn was measured after

Table 2
Chemical properties of the sewage sludge applied and legal limits[a] established by European Directive 86/278 and Spain R.D. 1310/1990.

Parameters	Anaerobic sludge	Spanish legal limits
Dry matter (%)	20.5	
pH	7.5	
N (g kg^{-1})	35	
P (g kg^{-1})	17.8	
K (g kg^{-1})	3.5	
Ca (g kg^{-1})	27.1	
Mg (g kg^{-1})	8.4	
Na (g kg^{-1})	1.5	
Fe (g kg^{-1})	17.9	
Cr (mg kg^{-1})	39.4	1000–1500
Cu (mg kg^{-1})	143	1000–1750
Ni (mg kg^{-1})	29.4	300–400
Zn (mg kg^{-1})	1250	2500–4000
Cd (mg kg^{-1})	0.7	20–40
Pb (mg kg^{-1})	84.4	750–1200
Mn (mg kg^{-1})	6.1	

[a] Limits depend on soil pH (minimum: soil pH < 7, maximum: soil pH > 7).

extraction with Mehlich 3 (Mehlich, 1985) with a VARIAN 220FS spectrophotometer using atomic absorption (VARIAN, 1989).

To estimate the sward Zn content, two samples (0.3 m × 0.3 m) of pasture were randomly taken from each pot with an electric hand clipper at a height of 2.5 cm at the following months: May, June and August 2007; May and July 2008; and May and June 2009 (autumn data were not used in this study and only were showed the data of spring affected by the treatments). The samples were labelled and transported to the laboratory where the samples were dried at 60 °C for 72 h for the chemical analyses. The sward Zn contents were determined with a VARIAN 220FS spectrophotometer using atomic absorption (VARIAN, 1989) after nitric acid digestion in a CEM MDS-2000 microwave (CEM, 1994). All analyses were performed and compared with reference samples.

2.5. Statistical analysis

Soil variables were analysed with repeated measures ANOVA (proc glm procedure) according to the following model: $Y_{ijk} = \mu + A_i + T_j + TA_{ji} + B_k + \varepsilon_{ijk}$. The following variables were analysed: Pasture + Tree treatments (*Agriculture + PMT*, *Agriculture + PST*, *Forest + PMT* and *Forest + PST* treatments); forest soils (*Forest + PMT*, *Forest + PST*, *Forest + ST* and *Forest + MT* treatments); agriculture soils (*Agriculture + PMT*, *Agriculture + PST*, *Agriculture + PM* and *Agriculture + PS* treatments); and agriculture treatments only with pasture (*Agriculture + P*, *Agriculture + PM* and *Agriculture + PS* treatments). In the repeated measures ANOVA, the variables were represented as follows: Y_{ijk} is the studied variable; μ is the variable mean; A_i is the year I; T_j is the treatment *j*; TA_{ji} is the treatment year interaction; B_k is the block *k* and ε_{ijk} is the error.

The data obtained from the soil and pasture variables were also analysed with three two-way ANOVAs (proc glm procedure) according to the following model: $Y_{ij} = \mu + F_i + T_j + \varepsilon_{ij}$. The first ANOVA was performed to discern the effects of soil type (agriculture *vs.* forest) with mineral and sludge fertilisation in the Pasture + Tree treatment (silvopastoral systems) with two levels of fertilisation (F; sludge and mineral) and two types of soil (T; forest and agriculture) and their interactions (soil × fertilisation; *Agriculture + PMT*, *Agriculture + PST*, *Forest + PMT* and *Forest + PST* treatments). In the first ANOVA, the variables were represented as follows: Y_{ij} is the studied variable; μ is the variable mean; F_i is the fertiliser factor *i*; T_j is the soil type factor *j* and ε_{ij} is the error. The second two-way ANOVA was performed to discern the effects of two levels of pasture vegetation (S; sown and unsown pasture) with two levels of fertilisation (F; sludge and mineral) and their interactions (sown × fertilisation) on forest soil (*Forest + PMT*, *Forest + PST*, *Forest + ST* and *Forest + MT* treatments). In the second ANOVA, the variables were represented as follows: Y_{ij} is the studied variable; μ is the variable mean; F_i is the fertiliser factor *i*; T_j is the vegetation factor *j* and ε_{ij} is the error. The third two-way ANOVA was performed to discern the effects of two levels of tree plantation (T; tree and no tree plantation) with two levels of fertilisation (F; sludge and mineral) and their interactions (silvo × fertilisation) on agriculture soil (*Agriculture + PMT*, *Agriculture + PST*, *Agriculture + PM* and *Agriculture + PS* treatments). In the third ANOVA, the variables were represented as follows: Y_{ij} is the studied variable; μ is the variable mean; F_i is the fertiliser factor *i*; T_j is the vegetation factor *j* and ε_{ij} is the error. Finally, a one-way ANOVA of one factor with three levels of fertilisation (F) (no fertilisation, NF; mineral, M; and sludge, S) was used to discern the effects of fertilisation on agriculture soil with herbaceous vegetation (*Agriculture + P*, *Agriculture + PM* and *Agriculture + PS* treatments).

The LSD test was used for subsequent pairwise comparisons ($p < 0.05$; $a = 0.05$) if the ANOVA was significant. The statistical software package SAS (2001) was used for all analyses.

3. Results

3.1. Year effect

Table 3 shows that, in all established treatments, water soil pH was lower in 2010 than in 2008, meanwhile the total Zn in the soils was higher in 2009 and 2010 than in 2008 and the amount of Zn extracted by Mehlich 3 was not modified throughout the period of the study.

3.2. Land use practices

The soil pH was significantly affected by the type of soil ($p < 0.001$) in the *Pasture + Tree* treatments in 2008 (agriculture = 6.33 *vs.* forest = 5.83), 2009 (agriculture = 5.93 *vs.* forest = 5.43) and 2010 (agriculture = 6.00 *vs.* forest = 5.57) (Fig. 2). On the other hand, when agriculture and forest soil were compared it was also observed a significant effect of the type of soil ($p < 0.01$) (agriculture = 35.2 mg kg^{-1} total Zn *vs.* forest = 29.0 mg kg^{-1} total Zn) and the type of fertilisation ($p < 0.05$) (sludge = 34.1 mg kg^{-1} total Zn *vs.* mineral = 30.1 mg kg^{-1} total Zn) on the total soil levels

Table 3
Water soil pH, total Zn (mg kg^{-1} of dry weight soil) and amount of Zn extracted by Mehlich 3 (mg kg^{-1}) in Pasture + Tree treatments, in Forest soils, in agriculture soils and in no forested agriculture soils in 2008, 2009 and 2010.[a]

	Parameter	Year			Year effect	SEM
		2008	2009	2010		
Pasture + Tree	Water pH	6.09 a	5.68 b	5.79 b	***	0.05
	Total Zn (mg kg^{-1})	32.1 c	41.1 b	44.2 a	***	1.42
	Zn Mehlich 3 (mg kg^{-1})	1.83	2.16	1.67	ns	0.15
Forest soils	Water pH	5.87 a	5.43 b	5.59 b	***	0.04
	Total Zn (mg kg^{-1})	30.2 b	38.0 a	42.4 a	***	1.4
	Zn Mehlich 3 (mg kg^{-1})	1.41	1.68	1.23	ns	0.11
Agriculture soils	Water pH	6.31 a	5.88 c	5.98 b	***	0.04
	Total Zn (mg kg^{-1})	35.3 c	42.6 b	50.1 a	***	1.26
	Zn Mehlich 3 (mg kg^{-1})	2.43	3.03	3.10	ns	0.27
Agriculture soils: Pasture	Water pH	6.32 a	5.87 b	5.88 b	***	0.05
	Total Zn (mg kg^{-1})	34.9 c	41.3 b	51.3 a	**	1.62
	Zn Mehlich 3 (mg kg^{-1})	2.38	3.19	3.30	ns	0.34

ns, not-significant; SEM, mean standard error.
[a] Different letters indicate significant differences between years.
** $p < 0.01$.
*** $p < 0.001$.

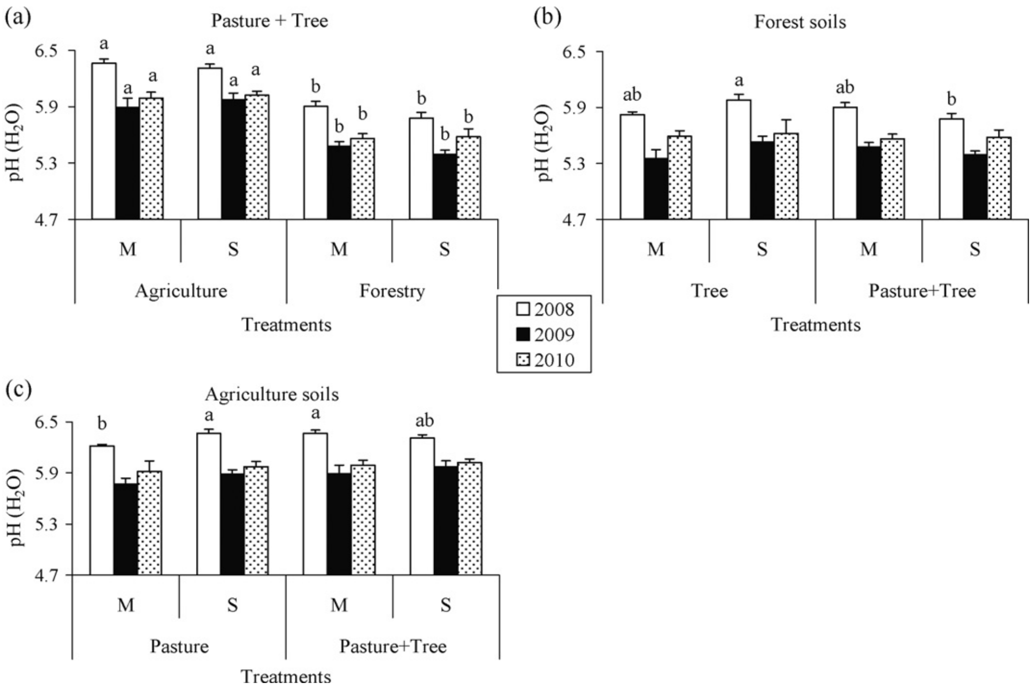

Fig. 2. Water soil pH under Pasture + Tree (a), forest soils (b) and agriculture soils (c) in 2008, 2009, and 2010. M, mineral; S, sludge. Different letters indicate significant differences amongst treatments within the same year. Vertical lines indicate mean standard error.

of Zn (Fig. 3) in 2008 and on soil levels of Mehlich 3 Zn in 2008 (soil effect, $p < 0.05$; fertilisation effect, $p < 0.01$) and 2010 (soil effect, $p < 0.001$; fertilisation effect, $p < 0.01$) (Fig. 4). As observed in the total soil levels of Zn, the concentration of Zn extracted by Mehlich 3 was significantly higher in agriculture soils than in forest soils and when sewage sludge was applied as compared to mineral fertiliser in 2008 (agriculture = 2.24 mg kg^{-1} Mehlich Zn *vs.* forest = 1.42 mg kg^{-1} Mehlich Zn; sludge = 2.45 mg kg^{-1} Mehlich Zn *vs.* mineral = 1.21 mg kg^{-1} Mehlich Zn) and 2010 (agriculture = 2.21 mg kg^{-1} Mehlich Zn *vs.* forest = 1.12 mg kg^{-1} Mehlich Zn; sludge = 1.95 mg kg^{-1} Mehlich Zn *vs.* mineral = 1.38 mg kg^{-1} Mehlich Zn). Moreover, it is apparent that the total Zn was significantly lower ($p < 0.05$) in the forest treatments fertilised with mineral (PMT) than in the other forest (PST) and agriculture (PMT and PST) treatments. The relative differences amongst treatments were higher when Zn extracted by Mehlich 3 was considered instead of total Zn. Finally, the concentration of Zn in the sward (Table 4) was significantly affected by the treatments in May 2007 (soil × fertilisation interaction effect, $p < 0.05$; fertilisation effect, $p < 0.05$), August 2007 (soil × fertilisation interaction effect, $p < 0.05$; fertilisation effect, $p < 0.01$), May 2008 (soil × fertilisation interaction effect, $p < 0.05$) and July 2008 (fertilisation effect, $p < 0.05$). In May 2007, August 2007 and May 2008, the concentration of Zn in the sward was lower in the forest soils fertilised with mineral (PMT) than in the other treatments applied to the agriculture (PMT and PST) and forest soils (PST). In July 2008, the concentration of Zn in the sward was higher when sewage sludge was applied compared to mineral fertiliser application (sludge = 20.8 mg kg^{-1} Zn *vs.* mineral = 17.1 mg kg^{-1} Zn). Similar to the other harvests (May 2007, August 2007 and May 2008), the mineral fertilisation of the forest soils (PMT) also significantly decreased the levels of Zn in the sward ($p < 0.05$) compared to the other treatments. Therefore, these results show that the higher levels of total and Mehlich 3 Zn in agriculture soils fertilised with sewage sludge than in forest soils which received mineral caused an increase of the concentration of Zn of the sward in the former.

3.3. Forest management

Within the *Forest soils*, the water soil pH was significantly affected by the interaction sown × fertilisation ($p < 0.05$) in 2008 (Fig. 2). In this type of soil, the soil pH was significantly increased when organic fertilisation was applied and trees were planted but without pasture sowing (ST) as compared to the same treatments fertilised with sludge and in which tree and pasture were established (PST). Regarding to the amount of total Zn in the soil,

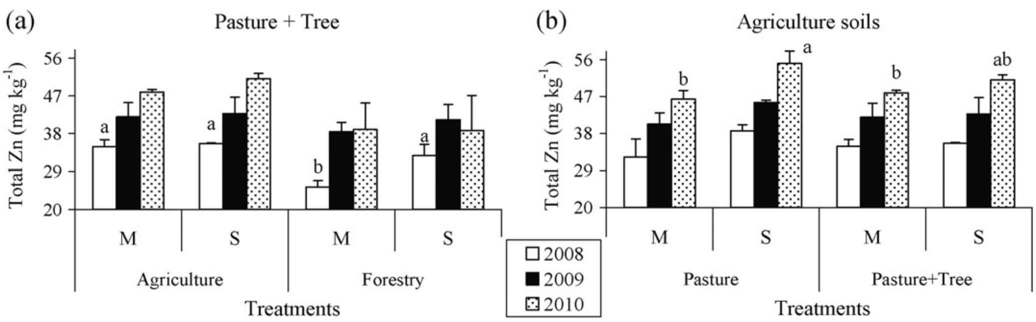

Fig. 3. Total Zn concentrations in soil (mg kg^{-1}) under Pasture + Tree (a) and agriculture soils (b) in 2008, 2009, and 2010. M, mineral; S, sludge. Different letters indicate significant differences amongst treatments within the same year. Vertical lines indicate mean standard error.

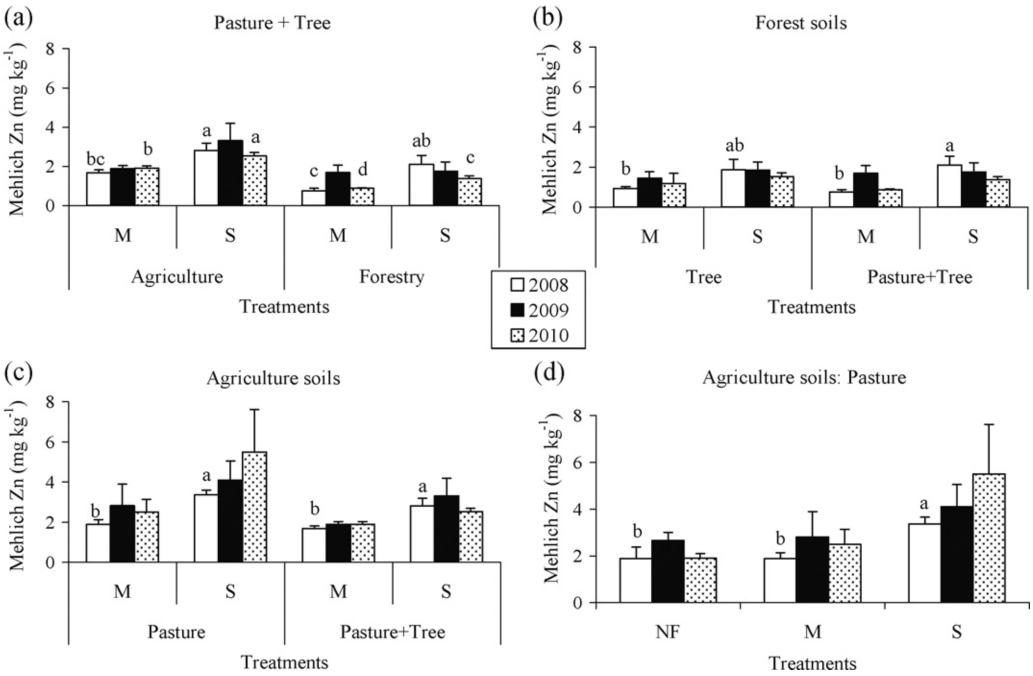

Fig. 4. Amount of Zn (mg kg^{-1}) extracted by Mehlich 3 under Pasture + Tree (a), in forest soils (b), in agriculture soils (c) and in non-forested agriculture soils (d) in 2008, 2009, and 2010. M, mineral; S, sludge; NF, no fertilisation. Different letters indicate significant differences amongst treatments within the same year. Vertical lines indicate mean standard error.

no differences appeared amongst treatments when *Forest soils* were taken into account ($p > 0.05$). However, the amount of Zn extracted by Mehlich 3 (Fig. 4) was significantly higher when the soil was treated with sludge rather than mineral fertiliser (sludge = 1.98 mg kg^{-1} Mehlich Zn *vs.* mineral = 0.84 mg kg^{-1} Mehlich Zn) ($p < 0.05$) in 2008. The positive effect of the sludge on the levels of Zn extracted by Mehlich was also observed on the concentration of Zn in the sward (Table 4) in the harvests of May 2007 (fertilisation effect, $p < 0.05$; sludge = 49.5 mg kg^{-1} Zn *vs.* mineral = 28.4 mg kg^{-1} Zn), May 2008 (fertilisation effect, $p < 0.05$; sludge = 27.7 mg kg^{-1} Zn *vs.* mineral = 22.4 mg kg^{-1} Zn) and July 2008 (fertilisation effect, $p < 0.05$; sludge = 23.5 mg kg^{-1} Zn *vs.* mineral = 17.7 mg kg^{-1} Zn). In these harvests, it was also found a significant effect of the sowing, being the levels of Zn in the sward higher in unsown than in sown pots in May 2007 (sown effect, $p < 0.05$; unsown = 51.7 mg kg^{-1} Zn *vs.* sown = 26.3 mg kg^{-1} Zn), May 2008 (sown effect, $p < 0.01$; unsown = 28.8 mg kg^{-1} Zn *vs.* sown = 21.3 mg kg^{-1} Zn) and July 2008 (sown effect, $p < 0.05$; unsown = 23.5 mg kg^{-1} Zn *vs.* sown = 17.6 mg kg^{-1} Zn). In August 2007, the forest × fertilisation interaction and the effect of the type of fertiliser ($p < 0.05$) were significant when the concentration of Zn in the sward was evaluated. The concentration of Zn in the sward was higher when the pots were fertilised with sludge and sown (PST) or unsown (ST) compared to mineral fertilisation of sown pots (PMT). Finally, the concentration of Zn in the sward was significantly higher ($p < 0.01$) in those pots without pasture sowing and fertilised with sewage sludge (ST) than in the other established treatments (MT, PMT and PST) in May 2007. Moreover, the sward concentration of Zn significantly ($p < 0.05$) decreased with mineral fertilisation and when the trees and pasture were established (PMT) compared to the other treatments (MT, ST, PMT and PST) in May and July 2008.

3.4. Agriculture management

Fig. 2 shows that water soil pH was significantly affected by treatments in 2008 (silvo × fertilisation interaction effect; $p < 0.05$) within *Agriculture soils*, which were all sown with pasture species. These results demonstrated that soil pH was higher in those soils without trees and fertilised with sludge (PS) and in soils with trees but fertilised with mineral (PMT) than in treeless soils fertilised with mineral (PM). Moreover, when the *Agriculture soils* were studied, it was also found that the soil concentration of total Zn ($p < 0.05$) (Fig. 3) and the amount of Zn extracted by Mehlich ($p < 0.01$) (Fig. 4) were significantly higher when sewage sludge was applied as compared to mineral fertiliser (sludge = 50.1 mg kg^{-1} total Zn *vs.* mineral = 47.1 mg kg^{-1} total Zn; sludge = 3.08 mg kg^{-1} Mehlich Zn *vs.* mineral = 1.77 mg kg^{-1} Mehlich Zn) in 2010 and 2008, respectively. In general, the positive effect of the application of sewage sludge on the levels of total and Mehlich 3 soil Zn was also observed on the concentration of Zn in the sward which was significantly modified by the silvo × fertilisation interaction ($p < 0.05$), being the levels of Zn in the sward lower in those pots planted with trees and fertilised with mineral (PMT) than in the other treatments (PM, PS and PST) in May 2008 (Table 4). On the other hand, no differences in water soil pH and in the amount of total Zn in soil appeared amongst treatments when treeless *Agriculture soils* were taken into account ($p > 0.05$). However, in these treeless *Agriculture soils*, the Zn extracted by Mehlich 3 in 2008 ($p < 0.05$) (Fig. 4) and the Zn concentration in the sward in August 2007 and May 2008 ($p < 0.05$) (Table 4) were significantly increased when sludge fertiliser (S) was applied when compared to mineral fertilisation (M) and no fertilisation (NF) treatments.

4. Discussion

4.1. Year effect

The total concentration of heavy metals in soil depends directly on the type of soil and indirectly on the pH level (Smith, 1996). In this study, the soil pH in 2010 was reduced in comparison to 2008, which may have been due to an increase in N mineralisation (the step in which NH_4^+ transforms into NO_3^- and H^+ is released into the soil solution media after NO_3^- leaching by rainfall)

Table 4
Concentrations of Zn in sward (mg kg^{-1}) under Pasture + Tree, in Forest soils, in agriculture soils and in no forested agriculture soils in those harvests that Zn was significantly affected by treatments in the years 2007, 2008 and 2009.[a]

	Plant Zinc (mg kg^{-1})																		
	Pasture + Tree					Forestry					Agriculture					Agriculture soils: Pasture			
	Agriculture		Forestry		SEM	Tree		Pasture + Tree		SEM	Pasture		Pasture + Tree		SEM	NF	M	S	SEM
	M	S	M	S		M	S	M	S		M	S	M	S					
May 2007	29 a	29.4 a	20.3 b	32.2 a	1.6	36.5 b	66.8 a	20.3 b	32.2 b	6.3									
August 2007	27.4 ab	31.2 a	19.3 b	34.8 a	2.1	27.9 ab	29.7 a	19.3 b	34.8 a	2.1						30.1 ab	24.5 b	33.3 a	1.6
May 2008	25.2 a	24 a	17.7 b	24.9 a	1.2	27.1 a	30.4 a	17.7 b	24.9 a	1.7	23.6 b	30.6 a	25.2 b	24 a	1.0	25.1 b	23.6 b	30.6 a	1.3
July 2008	19.7 a	20.8 a	14.5 b	20.9 a	1.0	20.8 a	26.2 a	14.5 a	20.9 a	1.5									

[a] M, mineral; S, sludge; NF, no fertilisation. Different letters indicate significant differences amongst treatments within each harvest.
SEM, mean standard error.

(Whitehead, 1995) caused by the higher temperatures and precipitations registered at the end of 2008 and 2009 compared with 2007 in addition to tree and pasture cation extractions from the soil (Mosquera-Losada et al., 2006). Moreover, N mineralisation, which could explain an increase of the sewage sludge incorporation into soil, may have promoted heavy metal release from the sludge to the soil. These reasons may explain why the total Zn in the soil increased in 2010 compared to 2008 in all treatments. The increase in total Zn in the soil was high when sewage sludge was applied because Zn was present in the sewage sludge in greater amounts than the background values in the untreated soil (Smith, 1996; Mosquera-Losada et al., 2010a). However, Rigueiro-Rodríguez et al. (2010) reported a reduction of the total Zn in soil over time in a three-year-old silvopastoral system developed with *Fraxinus excelsior* L. Soil pH (5.6), and the sewage sludge doses used in the *F. excelsior* L. study were similar to the present experiment. However, the proportion of sand in the *F. excelsior* L. experiment was almost 10 and 20% higher than the proportion of sand in the agriculture and forest soils in the present study, respectively, which may have favoured Zn leaching in the *F. excelsior* experiment. Previous studies have shown the important roles that clay minerals have in the immobilisation of heavy metals through their highly specific surface (Antoniadis, 2008; Zubillaga et al., 2008; Smith, 2009). In all treatments of this study, the soil concentration of total Zn was always less than the limits set by Spanish regulations for the use of sewage sludge in agriculture for acidic soils (150 mg kg^{-1}) (R.D. 1310/1990) (BOE, 1990). This trend may be explained by the fact that this experiment was located in an area without nearby pollution sources and that both soils had initially low levels of this element compared to the levels found by others including Antoniadis (2008) (62.58 mg kg^{-1} Zn), Egiarte et al. (2008) (48.5 mg kg^{-1} Zn) and McLaren et al. (2010) (62.8 mg kg^{-1} Zn). Regarding the concentration of available Zn in soil, many researchers have shown that the availability of this element may be increased in the long term as a consequence of soil acidification (White et al., 1997; Antoniadis and Alloway, 2001; Omil et al., 2007). In this study, however, the availability of Zn was not significantly modified by the different years of study, although the soil pH was reduced from the first to the last year of the experiment. This result may be due to the increased availability of Zn in soil with pH values lower than 5.5 and to extraction of the tree and pasture (Römkens and Salomons, 1998; Porta et al., 2003). In the present study, almost all the soil pH values were always higher than 5.5 in all treatments.

4.2. Land use practices

In this experiment, all soil studied variables (water pH and total and Mehlich 3 Zinc), were significantly higher in agriculture than in forest soils. The higher pH level in the agriculture soils may be explained by the recent history of liming and fertilisation of the agriculture soils, which causes the soil pH levels to be increased and available aluminium levels reduced. The lower pH in forest soils as compared to agriculture soils may have restricted the biological activity of the soil in the forest soils (Omil et al., 2007; Djukic et al., 2009). Restricted biological activity would have decreased the incorporation rate of the sewage sludge, thereby suggesting that the total and Mehlich 3 soil levels of Zn were lower in the forest soils than in the agriculture soils (López-Díaz et al., 2007). Therefore, there is still a potential source of Zn in forest soils than in agriculture soils, when the incorporation conditions improved, as happened in 2010. This makes advisable to harvest pasture instead of grazing to avoid direct zinc intake by animals through the soil consumption. When the effect of the fertilisation treatments (mineral and sludge) on soil pH in the agriculture and forest soils (*Pasture + Tree*) was evaluated, the soil pH was not modified because the doses of sewage sludge were not high enough to modify the soil pH due to the initial intermediate soil pH. The lack of response of soil pH to the organic fertilisation has been previously observed by Rigueiro-Rodríguez et al., 2000 in silvopastoral systems established under *P. radiata* (D. Don) with a soil pH value close to neutrality (6.8) and by Ferreiro-Domínguez et al. (2011) in silvopastoral systems established under *Quercus rubra* L. in agrarian soils with an acidic pH (5.2). In silvopastoral systems with *P. radiata* (D. Don), however, López-Díaz et al. (2007) found a positive response of soil pH to sewage sludge fertilisation. The change in soil pH observed by López-Díaz et al. (2007) may have been due to the lower initial soil pH (4.97) in comparison to the present study and the application of higher doses of sewage sludge compared to the doses used in the present study during three consecutive years. On the contrary, the concentration of total and Mehlich 3 Zn was higher when sewage sludge was applied compared to the application of mineral fertilisation in the *Pasture + Tree* treatments. The low response of Zn to mineral fertiliser may be explained by the fact that the total amount applied was much lower than amount applied with the sewage sludge treatments despite the heavy metal content of this mineral fertiliser (Verloo and Willaert, 1990). Moreover, Zn is the most abundant heavy metal in sewage sludge, and it has been considered by Spanish regulation R.D. 1310/1990 (BOE, 1990) as an application to be utilised in agriculture (Mosquera-Losada et al., 2010a). Many other studies have also shown an increase in Zn content in soil as a result of sewage sludge applications (Mosquera-Losada et al., 2001, 2009; Yuan, 2009; Rigueiro-Rodríguez et al., 2010). Importantly, the Mehlich method was more sensitive to the changes in the treated soil Zn levels. Therefore, the quantification of the sewage sludge inputs on Zn soil dynamics will be better evaluated with the Mehlich method than with estimation of the total concentration of Zn in soil. Finally, the concentrations of Zn in the sward developed in forest (13.5–66.8 mg kg^{-1} Zn) and agriculture soils (16.3–35.3 mg kg^{-1} Zn) were at the low end of the concentrations commonly found in swards (27–150 mg kg^{-1} Zn) and below the excessive and toxic levels (100 and 400 mg kg^{-1} Zn, respectively) for plants (Smith, 1996; Kabata-Pendias and Pendias, 2001). Moreover, the Zn concentration in the sward of forest soils was lower than the concentrations obtained by Mosquera-Losada et al. (2001) in a forest area fertilised with sewage sludge (55.72–123.65 mg kg^{-1} Zn). There are several reasons for the difference between the two studies. The experiment performed by Mosquera-Losada et al. (2001) was located in a more acidic soil (4.97) in which the Zn availability was higher compared to the present study, and the doses of sewage sludge used in the present study were lower than those used in the study by Mosquera-Losada et al. (2001). On the other hand, the levels of Zn in the sward developed in agriculture soils were similar to the levels found by Rigueiro-Rodríguez et al. (2010) (18.63–49.31 mg kg^{-1} Zn) in a silvopastoral system established in an agrarian soil fertilised with sewage sludge. This finding is highly relevant because sewage sludge input will improve the levels of plant microelements rather than causing detrimental effects. In general, the increase in heavy metal availability usually increases the heavy metal concentration in plants (Krebs et al., 1998). In this study, the lower availability of Zn in forest than in agriculture soils and when the mineral was applied compared with sewage resulted in a lower concentration of Zn in the sward in the Forest + PMT treatment than in the other treatments. With regard to animals, the maximum Zn in the forage concentrations established by NRC (1980) for bovines (500 mg kg^{-1} Zn), ovine (300 mg kg^{-1} Zn), and equines (500 mg kg^{-1} Zn) were never exceeded, which indicated that the pasture of this experiment was adequate for animal consumption. In general, Zn maintenance requirements of goats (45 mg kg^{-1} Zn) (Lamand, 1981), horses (40 mg kg^{-1} Zn) (NRC, 1989), bovines (20 mg kg^{-1} Zn) (NRC, 2000) and ovine (35 mg kg^{-1} Zn) (NRC, 1985) are not often reached. In these cases, supplements of these elements to the animals are recommended if their nourishment is derived solely from these pastures.

4.3. Forest management

Within the *Forest soils*, the sown pasture caused a reduction of soil pH when compared with unsown treatments in the pots fertilised with sewage sludge in 2008. The high proportion of *Agrostis capillaris* L. (63.5%) in the unsown treatment (ST) (Mosquera-Losada et al., 2010b) and *L. perenne* L. (52.06%) in the treatment sown with pasture (PST) (Mosquera-Losada et al., 2010b) may have explained the reduction of soil pH in the PST treatment compared to the ST treatment. *L. perenne* L. is a more extractive species than *A. capillaris* L. (Grime et al., 2007), which increases the cation soil extractions, thereby reducing the soil pH. Amongst other mechanisms, such as leaching and mineralisation, cation extraction increase is one of the main causes of soil acidification (Whitehead, 1995; Nillsson, 2004). In *Forest soils*, there were no appreciable significant differences as a result of the treatments of fertilisation or sowing on the concentration of total soil Zn, because most of the biological activity of the soil was greatly restricted by the low soil pH of the forest soils and by the low rainfall (Omil et al., 2007; Djukic et al., 2009). However, as observed in the *Pasture + Tree* treatments, the availability of Zn was increased in sewage sludge treated soils compared with mineral fertilisation in 2008. On the other hand, the amount of heavy metals removed by plants mainly depends on the species (Petruzzelli et al., 1994). In the present study, the concentration of Zn in the sward of the *Forest soils* was lower in the treatments sown with *D. glomerata* L., *L. perenne* L. and *T. repens* L. than in the unsown treatments. This result may be explained by the fact that sowing increased the proportion of monocot species in the pasture, which do not accumulate Zn (Mosquera-Losada et al., 2001, 2009). The differences in Zn absorption found in the current study have been previously described by Adarve et al. (1998), who observed that legume species accumulate a higher concentration of heavy metals than grasses.

4.4. Agriculture management

When only *Agriculture soils* were taken into account, the application of sludge increased the soil pH more than the application of minerals in treeless treatments. Moreover, the soil pH was also higher in the mineral treatments when trees were planted (PMT) compared to the treatments without tree planting (PM). These results may have occurred because the sludge contributed more Ca, Mg and micronutrients to the soil than the mineral fertiliser (Smith, 1996; López-Díaz et al., 2007; Mosquera-Losada et al., 2010a). Moreover, the higher pasture production found in treatments without trees (10.81 Mg ha^{-1}) compared to treatments with trees (8.92 Mg ha^{-1}) (Mosquera-Losada et al., 2010b) may have also caused an increase in cation extraction, thereby reducing the soil pH (Whitehead, 1995). As reported in *Pasture + Tree* treatments and when only *Forest soils* were studied, the higher concentration of Zn in the sewage sludge than in mineral implied an increase of the levels of total and Mehlich Zn in the soil which increased the concentration of Zn in the sward. Moreover, it was also found that in *Agriculture soils* with trees and mineral fertilisation (PMT), the concentration of Zn in the pasture was lower than in the treatment with mineral fertilisation but without trees (PM), which may have been due to tree extractions.

5. Conclusion

Fertilisation with sewage sludge increased the total Zn in the soil over time but never exceeded the limits set by Spanish regulations (150 mg kg^{-1}) and did not cause harmful effects on the plants.

The low incorporation rate derived from the low soil pH in forest compared to agriculture soils implied that the total and Mehlich 3 soil levels of Zn were lower in the forest soils, but a potential further increase of Zn in forest soils and direct consumption by animals are still probably. Therefore period of grazing between sludge inputs to forest soils should be longer than for agriculture soils. Sewage sludge inputs increased the total, Mehlich 3 and pasture Zn concentrations in forest and agriculture soils, and this effect was more relevant on unsown pastures of forest soils. Despite the higher concentrations of Zn in sewage sludge than in soil, these results indicate that the use of high quality sewage sludge at pasture establishments in silvopastoral systems is sustainable. However, policies with respect to the application of sewage sludge as a fertiliser must regulate both heavy metal availability and total heavy metal concentrations in soil because environmental risk is better evaluated in this way.

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