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Seasonal and temporal evolution of nutrient composition of pastures grown on remediated and non remediated soils affected by trace element contamination (Guadiamar Valley, SW Spain)

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Abstract

Elevated trace element concentrations in soils can affect the solubility and uptake of essential elements, resulting in nutrient deficiencies in plant tissues. The present paper deals with nutrient composition of pastures established on polluted and remediated soils (Green Corridor of the Guadiamar river Valley), in order to check the potential nutritional disorders that could derive from the soil pollution. In addition, nutrient composition of a representative grass, *Cynodon dactylon*, collected in 1999 and 2008 was compared in remediated and non-remediated sites of the polluted area. In general, nutrient concentrations of pastures were similar or even higher in polluted sites compared to 'control' sites. Therefore, the estimated potential ingestion of main nutrients by horses (the most abundant animals in the area) was also greater in the polluted and remediated soils and covered their nutritional requirements (more than 300 (N), 70 (S), 35 (P), 400 (K), 175 (Ca) and 30 (Mg) mg kg^{-1} body weight day $^{-1}$ in spring and autumn). Temporal evolution of nutrients and physiological ratios (N/S, Ca/P, K/Na, K/Ca+Mg) in *C. dactylon* showed a significant variation from 1999 to 2008, especially in the non-remediated area, leading to a recovery of the nutritional quality of this grass. The reasonable nutritional quality of pastures and the absence of negative interactions between nutrients and trace elements seem to indicate a stabilisation of soil pollutants in the affected area.

Additional key words: grasses, horse tolerance, mineral elements, nutrient ingestion, pasture evaluation.

Resumen

Evolución temporal y estacional de la composición nutricional de pastos de suelos recuperados y no recuperados afectados por contaminación con elementos traza (Valle del Guadiamar, SO de España)

Concentraciones elevadas de elementos traza en los suelos pueden interferir en la solubilidad y absorción de elementos esenciales originando deficiencias nutricionales en las plantas. En el presente trabajo se analiza el contenido de nutrientes en pastos de suelos contaminados, y recuperados, con elementos traza (Corredor Verde del Guadiamar), considerando posibles desórdenes nutricionales que pudieran derivarse de esta contaminación. Paralelamente, se han comparado los nutrientes de una gramínea representativa de la zona, *Cynodon dactylon,* recolectada en 1999 y 2008 en suelos contaminados, tanto recuperados como no recuperados. En general, el contenido de nutrientes de los pastos de las zonas contaminadas fue similar, o incluso más altos, que el de los pastos procedentes de una zona no contaminada. En consecuencia, la ingesta diaria de nutrientes estimada para caballos (animales más abundantes en la zona) resultó mayor en las zonas contaminadas, cubriendo satisfactoriamente las necesidades nutritivas de estos animales (más de 300 (N), 70 (S), 35 (P), 400 (K), 175 (Ca) y 30 (Mg) mg kg^{-1} peso corporal $día^{-1}$ en primavera y otoño). Los contenidos de nutrientes y relaciones fisiológicas (N/S, Ca/P, K/Na, K/Ca+Mg) de *C. dactylon* experimentaron una evolución significativa en el tiempo (1999 a 2008), especialmente en los suelos contaminados no recuperados, reflejando una recuperación de la calidad nutritiva de esta gramínea. La razonable calidad nutricional de los pastos analizados y la ausencia de interacciones negativas entre nutrientes esenciales y elementos traza puede ser reflejo de la estabilización a medio plazo de la contaminación de la zona.

Palabras clave adicionales: elementos minerales, evaluación de pastos, gramíneas, ingestión de nutrientes, tolerancia de caballos.

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Abbreviations used: ARC (Agricultural Research Council), BW (body weight), MTL (maximum tolerable level), NRC (National Research Council), PLI (pollution load index).

Introduction

Soils of degraded mining areas usually contain high concentrations of trace elements that may be problematic for vegetation and animals. Although most trace elements are required in small amounts by living organisms for their normal physiological processes, high accumulation is toxic to most life forms (Saxena *et al.*, 1999). The problems associated with trace element toxicity are further aggravated by their persistence in the environment. Periodical monitoring of their effects on ecosystem health is thus of concern. The transfer through the soil-plant-primary consumers may change with time, which should be considered when planning the management of polluted areas.

In April 1998, a mine accident occurred in SW Spain (Aznalcóllar, Seville) affecting 4286 ha of the Agrio and Guadiamar Valleys (Grimalt and Macpherson, 1999). After remediation and soil clean-up operations, a 'Green Corridor' was established (CMA, 2003). Subsequently the grazing of livestock was forbidden despite the remediation of these soils. As a consequence of this prohibition, vigorous and healthy herbaceous cover is growing in this area and competes with planted woody species for water and nutrients. Mechanical control of these herbaceous species is expensive and may affect biodiversity and generates greenhouse gas emissions. For these reasons, the possibility of grazing with horses (livestock not intended for human consumption) is currently being considered by the regional government as a benign and sustainable management tool for control of the herbaceous cover (Madejón *et al.*, 2009). Thus, there is a clear need to assess not only the risks associated with grazing these pastures, but also the influence of the pollution level on the main nutrient content of the herbage. As pointed out by Bargagli (1998), trace element toxicity due to continuous ingestion of contaminated diets by animals may be ameliorated or exacerbated, depending on the concentration of other elements, such as nutrients, in the diet. For example, marginal deficiencies of essential nutrients (Zn, Fe and Ca) can enhance Cd absorption by animals (Reeves and Chaney, 2008). It is well known that elevated concentrations of trace elements in soil can affect the uptake of essential elements by plants in several ways, resulting in nutrient deficiencies. Elements of similar characteristics compete for exchange sites in the soil matrix and in plant roots. For example, competitive interactions occur between As and P (Lambkin and Alloway, 2003), Tl and K (Kwan and Smith, 1991), Cd and Ca (Perfus-Barbeoch *et al.*, 2002), and Ni and Mg (Robinson *et al.*, 1999). Trace element contamination can also affect nutrient cycling by altering microbial activity and composition in soils (*e.g.,* symbiotic mycorrhizal fungi; Pennanen *et al.*, 1998; Hartley-Whitaker *et al.*, 2000; Tuomela *et al.*, 2005).

The risk associated with grazing pastures on moderately polluted areas (Guadiamar river Valley), attending their concentrations of potentially toxic trace element, was evaluated in a previous paper (Madejón *et al.*, 2009). The present paper deals with the nutrient composition of these pastures that were affected by the spill of Aznalcóllar accident, in order to check the potential nutritional disorders that could derive from the trace elements presence. The evolution of the nutrient composition, and some trace elements, of a representative grass of the area, *Cynodon dactylon*, is also considered, by comparing their values in the year 1999 (18 months after the disaster; Madejón *et al.*, 2001, 2002) and 2008 (10 years after the disaster, this paper).

Material and methods

Study area

The Guadiamar River Valley, in south-western Spain, lies inside the Iberian Pyrite Belt, the largest massive sulphide province in Western Europe. The area has a semi-arid Mediterranean climate with mild rainy winters and warm dry summers. The average annual temperature is 19°C (min. 9°C in January, max. 27°C in July), and the annual average rainfall is 484 mm. Soils in the Guadiamar floodplain are mostly neutral or slightly alkaline, with the exception of some terraces (on the northern right bank), which have low pH. Soil texture varies from loamy sand to silty clay (Cabrera *et al.*, 1999). In 1998, the failure of a large mine tailing dam at Aznalcóllar (Seville) released about 6 M m^3 of trace element-polluted sludge into the Guadiamar River (Grimalt and MacPherson, 1999). The affected soils, mostly under agricultural production, were contaminated with high concentrations of As, Cd, Cu, Pb, Tl and Zn (Cabrera *et al.*, 1999). Revegetation, composed of native shrubs and trees planted at different densities and a dense matrix of ruderal herbs, started in 1999, after the purchase of affected lands by the Regional Administration.

Soil sampling

Sampling was carried out in the spring and autumn of 2007. Samples of soil were collected at eight sites along the Guadiamar Green Corridor (Fig. 1). Three soil samples were taken at each sampling site at 0-25 cm depth using a spiral auger. Plots of 1 ha (sites 3 and 7) or 0.5 ha (sites 1, 2, 4, 5, 6 and 8) were delineated. Plot size depended on plant diversity. Sites 1 to 7 were affected by the spill and still present different levels of residual pollution; site 8 was not affected by the spill and is used as the control site for this study. The location, general properties, and As and Pb concentrations from each sampling site are shown in Table 1. Other trace element concentrations ranged between (mg kg^{-1}): 0.69-2.44 for Cd, 62-195 for Cu, 0.40-1.33 for Tl and 325-622 for Zn. Main types of soils along the Green Corridor are: Xerofluvents, Xerochrepts and Haploxeralfs (Murillo *et al.*, 2005).

Pasture sampling

Sampling was carried out in the spring and autumn of 2007. Pasture vegetation was sampled by collecting all of the aboveground biomass found in a 25 × 25 cm quadrant. Pasture height, plant cover and the species present were noted before plant sampling. The most frequent species belong to the Poaceae (especially *Bromus* spp. and *Agrostis poureetii* Willd.), Fabaceae (*Medicago polymorpha* L.) and Asteraceae (*Senecio* spp.) families. Members of the Poaceae family accounted for the highest proportion of herbaceous cover, especially during autumn, when, on average, 77% of the plant cover at each sampling site consisted of grass species. The most frequent species in the pastures are *Avena sterilis, Bromus lanceolatus, Coleostephus myconis, Senecio lividus, Medicago polymorpha* (Madejón *et al.*, 2009).

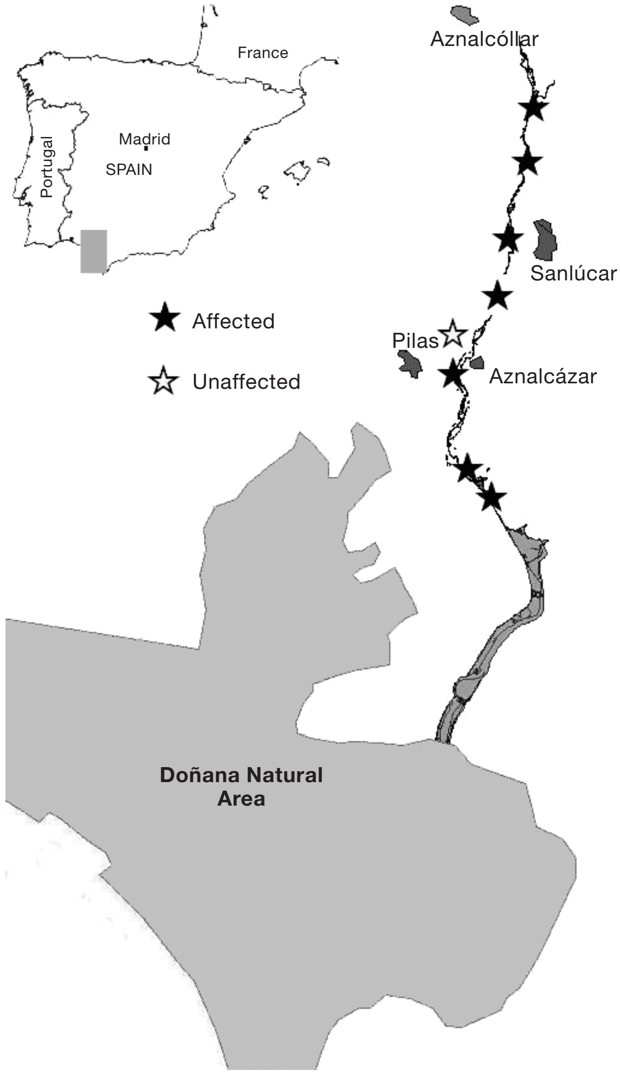

Figure 1. Map of the study area. Sampling sites are indicated by stars.

Table 1. Soil sample location, pH, As and Pb concentrations and PLI[1] (pollution load index) values

Site	Latitude/Longitude	pH	Sand (%)	Silt (%)	Clay (%)	As (mg kg^{-1})	Pb (mg kg^{-1})	PLI
1	37°28’09.8”, 6°12’42.0”	7.6 ± 0.18	51	39	10	56.2 ± 20.1	128 ± 25.7	5.45
2	37°27’41.4”, 6°12’42.0”	8.3 ± 0.0	48	36	10	87.6 ± 42.0	148 ± 69.3	7.38
3	37°25’45.0”, 6°13’05.0”	8.2 ± 0.48	61	28	11	145 ± 85.6	290 ± 185	8.90
4	37°23’13.5”, 6°13’38.0”	7.0 ± 0.90	58	34	8	147 ± 9.88	263 ± 35.7	11.18
5	37°22’39.5”, 6°13’45.2”	8.2 ± 0.11	51	33	16	44.0 ± 14.1	62.8 ± 34.8	2.31
6	37°17’25.7”, 6°15’46.2”	8.1 ± 0.13	50	37	12	62.2 ± 9.95	128 ± 16.1	7.49
7	37°14’27.0”, 6°15’22.0”	8.0 ± 0.07	52	40	8	93.3 ± 32.2	188 ± 79.6	9.90
8	37°19’21.5”, 6°15’17.8”	7.8 ± 0.33	42	39	19	21.5 ± 2.53	14.3 ± 1.93	0.73

[1] PLI values according to Cabrera *et al.* (1999).

Twenty (1 ha) or ten (1/2 ha) samples were taken at each site, each sampling quadrant being at least 30 m away from the previously sampled replicate. A total of 100 samples of pasture were taken along the Green Corridor. All plant species were included in herbage analysis, despite the known preferential feeding of horses on grasses (Menard *et al.*, 2002). Representative species of Poaceae, Asteraceae and Fabaceae were also sampled in the spring. The identification of the plant species was checked in the laboratory; most autumnal species had to be grown under greenhouse conditions before they were sufficiently mature for final identification. Nomenclature follows that of Castroviejo (1986-2005).

Cynodon dactylon sampling

This grass was sampled in 1999 (18 months after the accident) and also in 2008 (10 years after the accident) in a fenced plot established for research purposes in which the polluted soil was not cleaned up or amended (concentrations of As and Pb extracted by «aqua regia» and determinated by ICP-OES were 929 mg kg^{-1} for As and 2,300 mg kg^{-1} for Pb; Madejón *et al.*, 2002). This site will be referred throughout the paper as the «non-remediated» site. This scenario represents the situation that would be present across the entire affected area if the restoration program had not been completed. Three samples of the *Cynodon dactylon* grass were collected from patches inside the fenced plot. This grass species was also sampled along the affected and remediated area of the Green Corridor (sites 1 to 7) in both years.

Laboratory analysis

Plant material was oven-dried at 70°C to constant weight, weighed to obtain dry biomass and directly ground and passed through a 500-mm stainless steel sieve without prior washing. Total N was determined by Kjeldahl digestion (Hesse, 1971), total protein in the plants was calculated by multiplying Kjeldahl N content by 6.25 (ARC, 1965). Plant material was digested by wet oxidation with concentrated HNO_3 under pressure in a microwave digester (Jones and Case, 1990). Three consecutive steps (5 min each) of power (250 W, 450 W and 600 W) were applied, and then these extracts were diluted with water of 18 mΩ deoinised quality. The analysis of mineral nutrients in the digests was performed by inductively coupled plasma-optical emission spectrophotometry (ICP-OES; Thermo Jarrel Ash corporation). The analysis of As and Pb was performed by inductively coupled plasma-mass spectroscopy (ICP-MS; Perkin Elemer, Sciex-Elan 5000). The accuracy and precision of the analytical method were assessed by routine analyses of a reference sample (NCS DC73350, China National Analysis Centre for Iron and Steel, 2004, leaves of poplar). Recovery rates for reference plant samples were between 90 and 110%.

Data analysis

Mean and standard error (SE) were determined for all variables. Normality of the data was tested prior to analysis, and, when necessary, variables were log-transformed. A Student *t*-test was used to assess significant differences between affected and unaffected sites. One way ANOVAs were used to analyse the differences in the biomass productivity and trace element concentration among sites. Significant statistical differences of all variables between sites were established by Tukey's test. If, after logarithmic transformation, the data did not fit a normal distribution, the non-parametric Kruskal-Wallis analysis of ranks was used.

Results

Pasture biomass

There was a negligible effect of the residual soil pollution on pasture productivity, as reflected by the fact that the highest spring biomass production was found in one of the most polluted site and by the fact that there were no significant differences in biomass production between spill-affected and unaffected sites at the two sampling times (Table 2).

Nutrient concentrations, physiological ratios of pastures, and the potential ingestion of nutrients by horses

Nutrient mean concentrations of pastures were rarely lower in polluted sites when compared to the control site (Fig. 2). In general, nutrient contents tended to be higher, although not always significant, in pastures

Table 2. Spring and autumn biomass production (g m^{-2}, mean ± SE) at each study site

Site	Spring	Autumn
1	204 ± 98^{c}	152 ± 58^{a}
2	249 ± 69^{bc}	171 ± 133^{a}
3	252 ± 152^{c}	137 ± 109^{a}
4	728 ± 641^{a}	195 ± 150^{a}
5	467 ± 294^{ab}	174 ± 167^{a}
6	592 ± 269^{a}	160 ± 87^{a}
7	479 ± 287^{ab}	323 ± 443^{a}
8	*558 ± 325^{ab}*	*194 ± 126^{a}*

Values in the same column (season) with the same letter do not differ significantly ($p < 0.05$, Tukey's test).

affected by the spill, with exceptions (*e.g.,* protein contents in sites 5 and 6 in spring: 1.1-fold lower in site 5 and 1.3-fold lower in site 6 than in the control soil; Fig. 2). The concentrations of Ca were significantly greater in some pastures from the polluted soils in autumn. Magnesium concentrations were similar or even higher (although differences were not significant) than those of the control. Autumnal concentrations of protein, S and K were significantly greater in pastures from all of the polluted sites than from the control site, as well as S (spring) and P (autumn) in some polluted sites (Fig. 2).

The greatest increases in pasture concentrations compared to the control site were found for S, ranging from 1.9 to 2.9 times greater (autumn) and 1.4 to 2.6 (spring), followed by Ca (1.5-2.3 in autumn; Fig. 2).

Overall, lower N/S ratios were found in pastures from the polluted soils than in pastures from the control soils in both spring and autumn (Table 3), due to the significantly greater S concentrations of pastures from

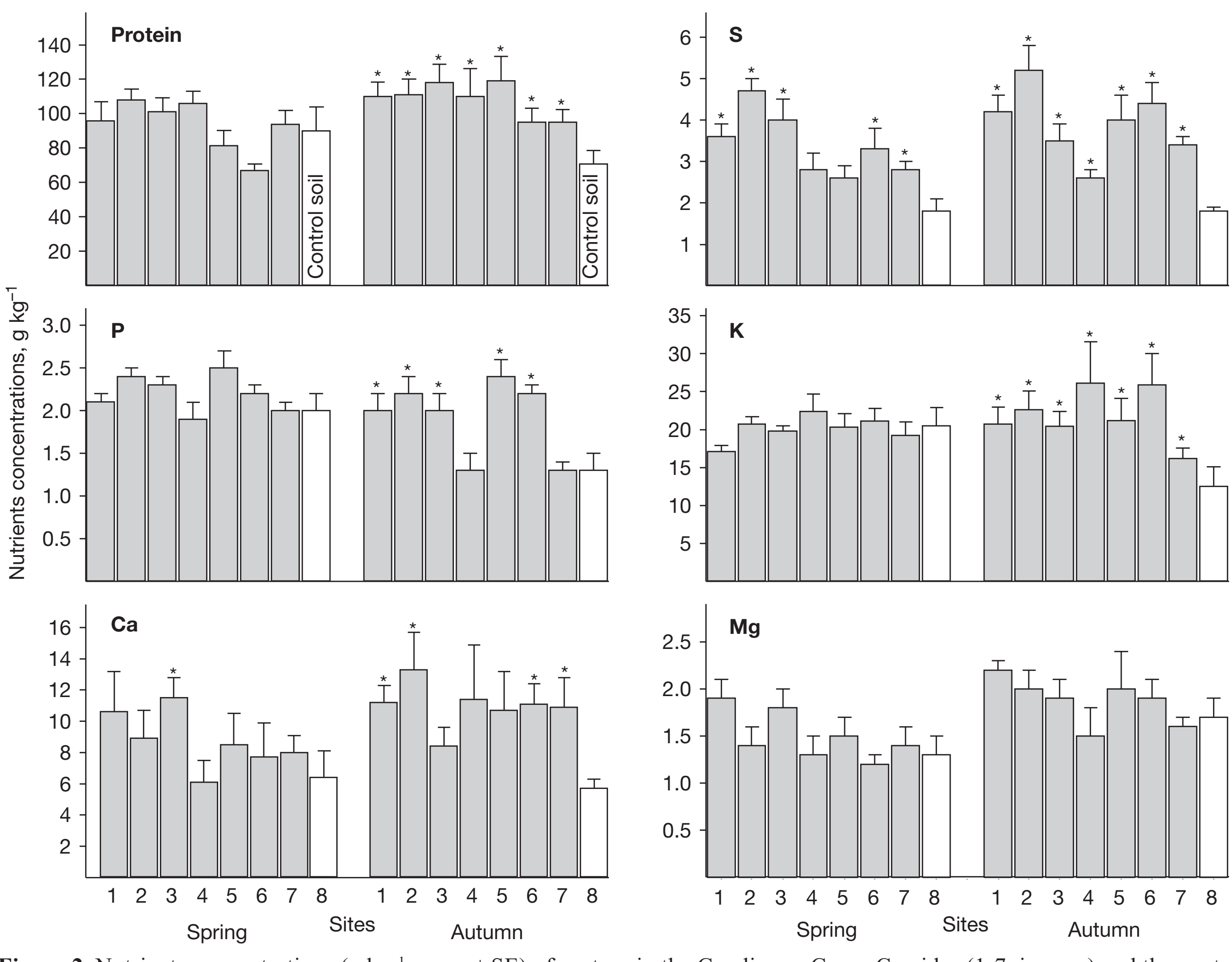

Figure 2. Nutrients concentrations (g kg^{-1}, mean ± SE) of pasture in the Guadiamar Green Corridor (1-7, in grey) and the control site (8, in white). Significant differences between each site and site 8 (Control) for each season are marked with an asterisk ($p < 0.01$, Student t-test).

Table 3. Seasonal variation in physiological ratios of pastures (mean values ± standard error in a dry matter basis) between spring and autumn

Sites	N/S		Ca/P		K/Na		K/Ca+Mg	
	Spring	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**
1	4.36 ± 0.43^{c}	4.34 ± 0.27^{bc}	4.86 ± 1.11^{a}	5.73 ± 0.40^{a}	23.4 ± 3.0^{ab}	28.2 ± 5.1^{ab}	0.85 ± 0.13^{a}	0.75 ± 0.10^{a}
2	3.91 ± 0.28^{c}	3.52 ± 0.20^{c}	3.80 ± 0.72^{a}	5.87 ± 0.77^{a}	38.4 ± 5.4^{a}	36.8 ± 5.5^{a}	1.19 ± 0.17^{a}	0.74 ± 0.05^{a}
3	4.74 ± 0.49^{c}	5.74 ± 0.55^{abc}	4.95 ± 0.54^{a}	4.05 ± 0.29^{a}	19.5 ± 3.0^{b}	24.8 ± 4.5^{ab}	0.89 ± 0.11^{a}	0.98 ± 0.07^{a}
4	6.89 ± 0.68^{ab}	6.82 ± 0.83^{a}	2.97 ± 0.37^{a}	7.75 ± 1.89^{a}	11.1 ± 3.8^{b}	9.46 ± 2.2^{b}	1.62 ± 0.17^{a}	1.20 ± 0.23^{a}
5	5.26 ± 0.69^{bc}	4.94 ± 0.35^{bc}	3.21 ± 0.62^{a}	4.15 ± 0.62^{a}	10.4 ± 2.5^{b}	13.5 ± 6.0^{b}	1.29 ± 0.25^{a}	0.91 ± 0.14^{a}
6	3.55 ± 0.23^{c}	3.86 ± 0.58^{bc}	3.22 ± 0.76^{a}	5.20 ± 0.64^{a}	14.4 ± 1.9^{b}	16.6 ± 4.8^{b}	1.53 ± 0.22^{a}	1.00 ± 0.15^{a}
7	5.15 ± 0.32^{bc}	4.58 ± 0.32^{bc}	4.14 ± 0.76^{a}	9.09 ± 2.22^{a}	12.7 ± 2.3^{b}	10.1 ± 1.8^{b}	1.19 ± 0.13^{a}	0.85 ± 0.11^{a}
8	8.17 ± 0.53^{a}	6.50 ± 0.80^{ab}	3.14 ± 0.78^{a}	8.30 ± 1.80^{a}	14.0 ± 2.5^{b}	8.30 ± 1.8^{b}	1.63 ± 0.27^{a}	0.77 ± 0.15^{a}
Adequate nutritional values[1,2]	*10*[1]		*0.5-2*[1]		*5-8*[2]		*< 1.8*[1]	

Ratio values followed by the same letter in the same column do not differ significantly ($p < 0.01$, Tukey's test). [1] ARC (1965). [2] Georgievskii (1982).

the polluted soils, especially in the spring. There were no differences between pastures from polluted and unpolluted soils for Ca/P, K/Na and K/Ca+Mg ratios, except for the K/Na ratio found at the sites closer to the mine (site 2), which were more degraded and probably more impoverished in Na (Table 3).

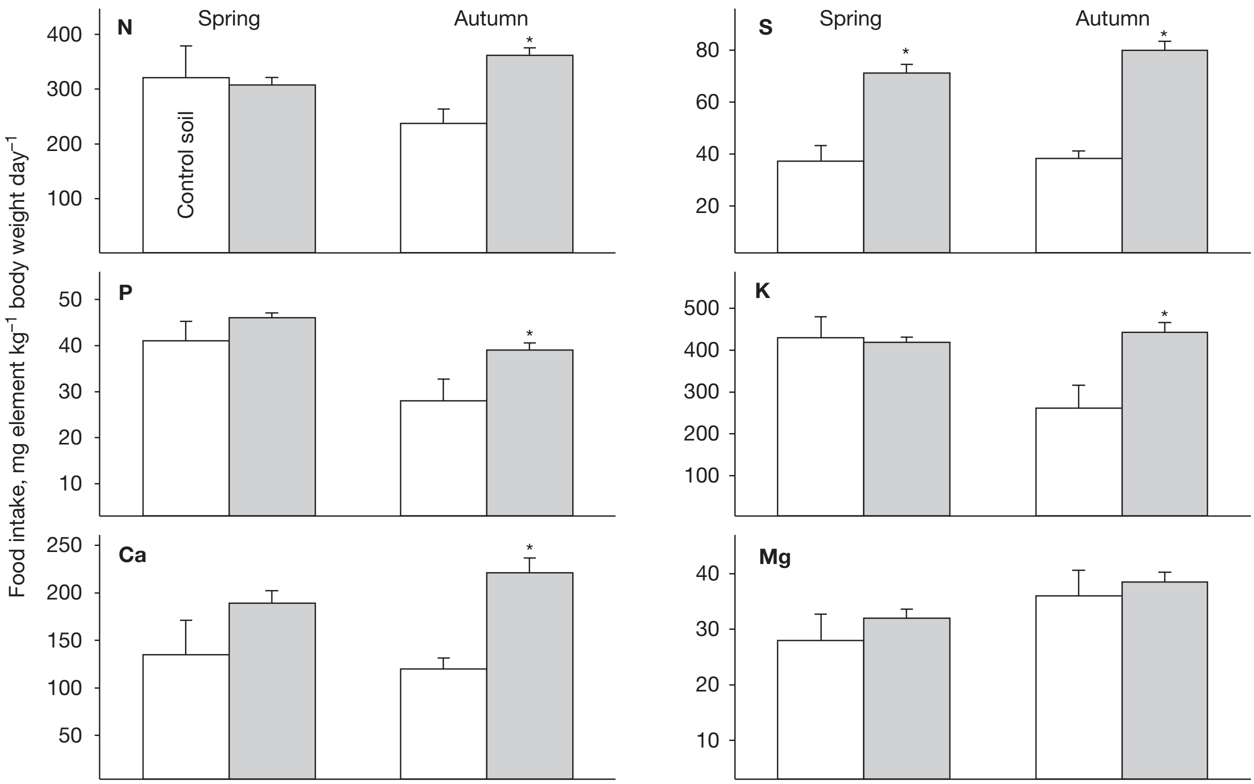

Figure 3. Daily predicted intake of nutrients (mean values ± SE) by consumption of pasture growing on affected (grey columns) and control soils (white columns). Estimated food intake for horses in mg element kg^{-1} body weight day^{-1} (data based on a daily food intake of 21 g of plant dry weight kg^{-1} of body weight, Aronson, 1978). Significant differences between affected soils and control soils for each season are marked with an asterisk ($p < 0.01$, Student t-test).

Table 4. Nutrient (g 100 g^{-1}), and As and Pb (mg kg^{-1}) concentrations in *Cynodon dactylon* plants growing in remediated and non-remediated (sludge covered) soils in 1999 and 2008 (mean values ± standard error, dry matter)

Site	Year	N	P	K	Ca	Mg	S	As	Pb
Remediated (n = 21)	1999	2.31 ± 0.49	0.17 ± 0.04	1.32 ± 0.27	0.94 ± 0.29*	0.25 ± 0.02*	0.72 ± 0.14*	20.3 ± 13.6*	38.4 ± 26.3*
	2008	1.79 ± 0.38	0.25 ± 0.07	1.60 ± 0.35	0.38 ± 0.06	0.12 ± 0.02	0.43 ± 0.07	1.31 ± 2.34	0.97 ± 0.94
Non-remediated (n = 3)	1999	1.11 ± 0.11	0.11 ± 0.04*	0.85 ± 0.33*	1.19 ± 0.22*	0.34 ± 0.06*	1.51 ± 0.31*	168 ± 73.1*	270 ± 113*
	2008	2.60 ± 0.27	0.19 ± 0.02	1.99 ± 0.16	0.36 ± 0.02	0.14 ± 0.01	0.48 ± 0.02	5.49 ± 3.1	20.1 ± 12.8

Significant differences between the studied years are marked with * ($p < 0.01$, Tukey's test).

Since nutrient concentrations were on average higher in affected areas than in the control site, the potential ingestion of main nutrients by horses could also be greater at these areas. Predicted ingestion values were significantly higher for all nutrients except Mg in autumn and for S in both spring and autumn as a consequence of the greater S content of the pastures in the affected area (containing high levels of S in soils). Pasture contamination with adhered soil could also contribute to these values (Fig. 3).

Temporal variation of element concentration and physiological ratios in *Cynodon dactylon* plants in remediated and non-remediated soils

In general, all nutrients (N, P, K, Ca, Mg and S) and the toxic elements (As and Pb) in *Cynodon dactylon* plants showed significant temporal variations, especially in the case of As and Pb (Table 4).

Nitrogen behaviour was different depending on field sites. In the remediated soils, N content decreased 1.3-fold from 1999 to 2008, whereas in the non-remediated soils, N concentrations increased 2.3-fold, indicating a slow, but consistent, recovery of this particular area.

The concentrations of P and K increased in time for both sites, especially in the non-remediated site, where values were around 2-fold greater in 2008 compared to 1999. On the contrary, concentrations of Ca, Mg and S decreased significantly in time (Table 4). The greatest decreases were found in the non-remediated soils for Ca and S, a more than 3-fold difference between values in 1999 and 2008, which seems to indicate a natural attenuation along the affected area.

The most remarkable temporal variation was shown by the potentially toxic trace elements As and Pb, corroborating the recovery of the area. From 1999 to 2008, the decrease of As was 16-fold in the remediated soils and 31-fold in non-remediated soil. In the case of Pb, the greater decrease corresponded to the remediated soils, 40-fold lower in 2008 than in 1999 (13-fold lower in the non-remediated soil, Table 4).

The physiological ratios of *C. dactylon* also showed significant variations from 1999 to 2008, especially in the non-remediated site (Table 5). In this soil, the N/S ratio increased 7-fold from 1999 to 2008, K/Na increased 4-fold, and K/Ca+Mg increased 6-fold, while the Ca/P ratio decreased more than 6- fold. All of these variations show a substantial recovery of the pasture quality according to the adequate values reported in literature for these ratios (Table 4).

Table 5. Physiological ratios of *Cynodon dactylon* plants growing in remediated and non-remediated (sludge covered) soils in 1999 and 2008 (mean values ± standard error, dry matter)

Site	Year	N/S	Ca/P	K/Na	K/Ca + Mg
Remediated (n = 21)	1999	3.26 ± 0.26*	5.64 ± 0.66*	4.99 ± 0.58*	0.53 ± 0.06*
	2008	4.20 ± 0.15	1.64 ± 0.13	14.9 ± 1.51	1.45 ± 0.11
Non-remediated (n = 3)	1999	0.76 ± 0.10*	12.5 ± 4.0*	3.13 ± 1.29*	0.27 ± 0.09*
	2008	5.41 ± 0.43	1.90 ± 0.12	12.0 ± 0.59	1.70 ± 0.13
Adequate values[1,2]		*10*[1]	*0.5-2*[1]	*5-8*[2]	*< 1.8*[1]

[1] ARC (1965). [2] Georgievskii (1982). Significant differences between the studied years are marked with * ($p < 0.01$, Student t-test).

The potential ingestion of nutrients, and especially of As and Pb, by animals grazing *Cynodon* changed deeply from 1999 to 2008. The potential ingestion of S, Ca and Mg would be significantly greater in 1999 in both remediated and non-remediated soils. The ingestion of P and K would be greater in 2008 in both soils, although the differences were not always significant (Fig. 4), while N did not show a definite pattern. The most remarkable variations were associated with the trace elements As and Pb, whose concentrations decreased by 16- (remediated) and 39- (non-remediated) fold in case of As and 31- and 13-fold for Pb, corroborating the recovery of the area.

Discussion

In general, concentrations of the analysed nutrients covered horse and livestock requirements (Frape, 1986; NRC, 2005). Besides those individuals that are very young, old, reproducing, sick, or exposed to stressful environments, healthy animals consuming nutritionally imbalanced diets may be more sensitive to toxicoses (NRC, 2005). Considering a pasture ingestion by horses of 21 g dw kg^{-1} body weight, pastures should have a minimum protein content of 50 g kg^{-1} dry matter to satisfy horse requirements for proteins, typically about 1 g crude protein kg^{-1} weight day^{-1} (Frape, 1986; NRC,

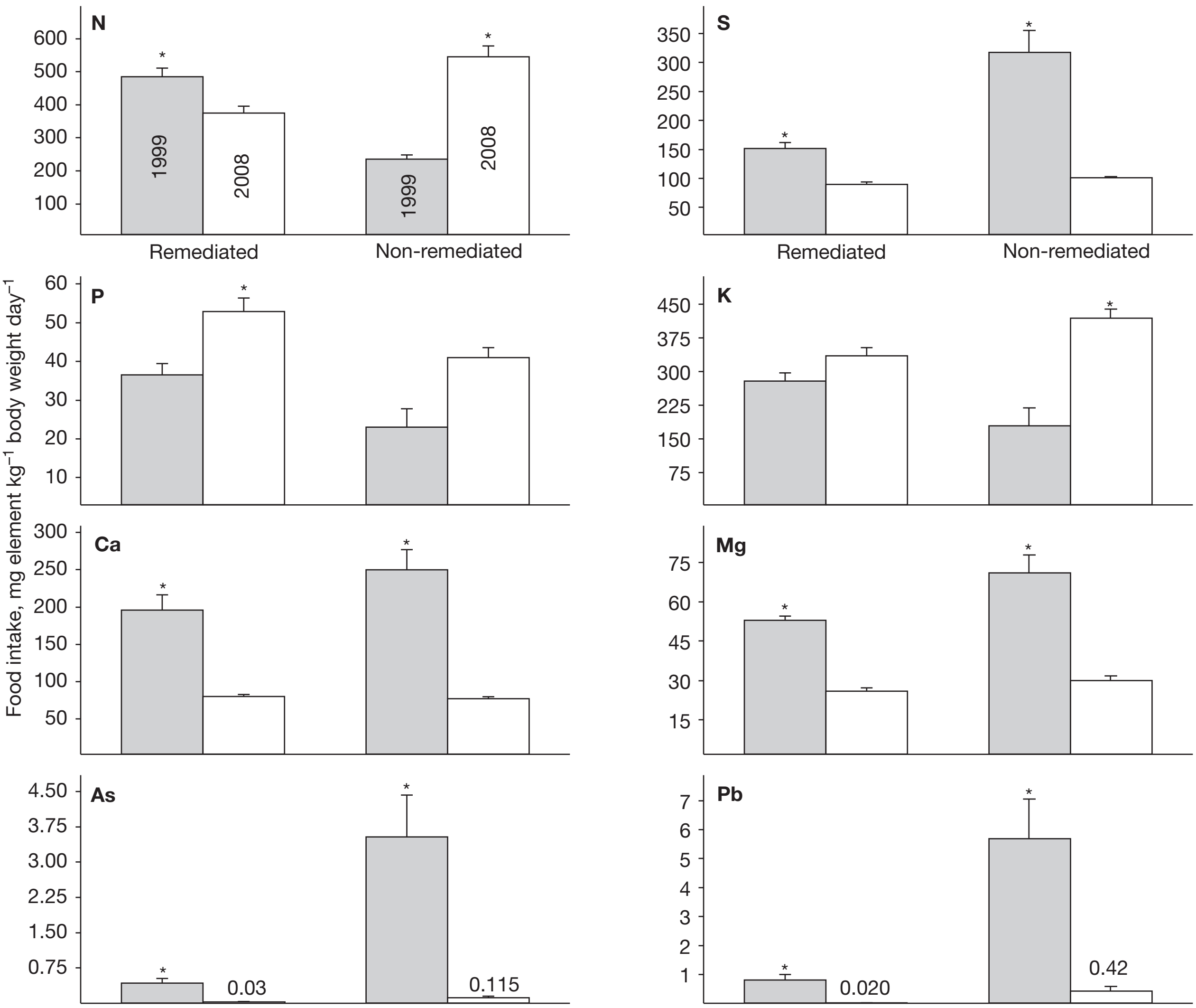

Figure 4. Daily predicted intake on nutrients (mean values ± SE) by consumption of the grass *Cynodon dactylon* growing on remediated and non-remediated soils for the years 1999 (grey columns) and 2008 (white columns). Estimated food intake for horses in mg element kg^{-1} body weight day^{-1} (data based on a daily food intake of 21 g of plant dry weight kg^{-1} of body weight, Aronson, 1978). Significant differences between on remediated and non-remediated soils for each year are marked with an asterisk ($p < 0.01$, Student t-test).

2005). This requirement would be satisfied by the studied pastures, both from polluted and unpolluted sites, even if the ingestion rates were doubled. Therefore, the residual soil pollution did not affect the protein content of the pastures, despite the fact that some metals (*e.g.,* Cd, up to 3.20 mg kg^{-1} in the affected soils and 0.05 mg kg^{-1} in the control soil) can limit uptake of N-NO_3, of K, Ca and Mg, reducing their concentrations in plant tissues (Fodor *et al.*, 1995; Boussama *et al.*, 1999; Hagemeyer, 1999; Stolt and Oscarson, 2002). Also, microorganisms are reported to be sensitive to trace elements (*e.g.,* Tl up to 2.93 mg kg^{-1} in the affected soils and 0.22 mg kg^{-1} in the control soil), and the inhibition of bacteria-induced nitrate formation may have an impact on N uptake by plants (Kabata-Pendias and Pendias, 1992). However, the overall situation at the affected area seems to be adequate for plant N nutrition (Fig. 3).

As expected, S concentrations of the pasture were greater in the affected soils than in the control soil due to the S richness of the sludge remaining in the affected soils, exceeding the dietary recommended level of 1.8 g kg^{-1} (Georgievskii, 1982). The maximum tolerable level (MTL) of 5 g kg^{-1} (NRC, 2005) was not exceeded, although S concentrations at site 2 were close to this maximum value (Fig. 2). However, horses in the Green Corridor move freely and usually graze at different sites. Potassium was another abundant element at both the affected and unaffected areas, exceeding the adequate range of 2-10 g kg^{-1} for most livestock and even the MTL for horses. However, this is a conservatively safe MTL for non-ruminants; the NRC (2005) set the MTL of K at 30 g kg^{-1} for both ruminant and non-ruminants species (this value is only exceeded in site 4 in autumn; Fig. 2).

Calcium and Mg content in the pastures from both the affected and unaffected areas can also satisfy the dietary requirements of horses (an adequate range of 2.8-4.5 g kg^{-1} for Ca and an adequate value of 2 g kg^{-1} for Mg). In any case, Ca and Mg concentrations exceed the corresponding MTL for horses (20 and 8 g kg^{-1}, respectively) despite the fact that the concentrations of both elements were quite high in the whole area (into the range of 6-13 for Ca and 1.2-2.2 g kg^{-1} for Mg). This is an expected situation noting the dominance of calcareous soils in the Guadiamar river basin (Domínguez *et al.*, 2008). Also, this is a positive feature for horses' nutrition, and livestock in general, since Ca may have a protective action against metal toxicity (Carbonell *et al.*, 1998). However, excessive Ca in the diet can reduce the availability of other minerals, such as P and Zn, especially if they are marginally adequate.

The richness of Ca in most soils may limit plant P uptake by P-soil fixation. In fact, in most cases the P concentration of the pasture was below the adequate range for horses of 2-3 g kg^{-1}, and well below the MTL of 10 g kg^{-1} (NRC, 2005; Fig. 3). Forage that feeds horses is usually low in phosphorus (Jordan *et al.*, 1975); in our case, the autumnal Ca/P ratio was excessively high for non-ruminants (even at the control site), although this ratio decreased in spring, which is a longer grazing period (Table 3).

The N/S ratio was lower at the affected sites due to the S enrichment of soils and plants in these zones. Thus, in affected soils, this ratio was lower than 10, the optimum value. The relatively high K/Na ratio indicated a shortage of Na in the pastures at the northern sampling sites (sites 1 and 2), but not at the other sites. Considering that horses move freely along the area, a Na deficiency in the animals, undesirable from a reproductive point of view (Georgievskii, 1982), is unlikely. The K/Ca + Mg ratio was reasonably well equilibrated in all the sampling sites.

The results of this work show that residual pollution (Madejón *et al.*, 2009) is still present in the affected soils of the Green Corridor natural reserve but does not influence the nutritional quality of the pastures ingested by horses. The greater nutrient concentrations at the polluted sites, when so, could not be attributed to a concentration effect derived from smaller plant biomass performance in these sites (Jarrel and Beverly, 1981), according to the plant biomass results.

This reasonable nutritional quality could counteract the potential toxicity of the trace elements (Reeves and Chaney, 2008). However, diets based on grasses, which are the predominant species in most of the analysed pastures, seem not to pose a risk of trace element toxicity for grazing animals in our study area (Madejón *et al.*, 2009).

Horses are selective feeders that can promote the existence of overgrazed areas since their tooth structure is suited to grazing close to the ground, thus limiting the regrowth of selected species. This can promote the growth of other less palatable plants, such as Asteraceae. It would be desirable that other less selective grazers, such as cattle and donkeys, be rotated through the pasture to clean up the «leftovers» that horses avoid. For such a complete rotational program, monitoring not only the grasses but also the «leftovers» would be necessary. Other groups of plants (*e.g.,* Asteraceae) have comparatively high transfer coefficients for most trace elements (unpublished). Plantaginaceae can also reach high levels of trace elements in other polluted areas in the South Spain (Chopin and Alloway, 2007).

In general, the pasture composition reported in this work (based on grasses) seems to show a recovery of nutrient balance in the studied area, especially in the non-remediated soil. This was corroborated by the variation of the composition of *C. dactylon*, 10 years after the accident (Table 4). This species can be used as an example of the regeneration experienced by this particular area. The slight decrease of N in remediated soils could be related to the previous N content in the soil (before the accident, these soils were fertilised for agriculture). The lower content of S, As and Pb in 2008 was related to both the lower availability of these elements in soils (stabilisation) and lower soil deposition in plants, which was quite pronounced in the first years after the accident due to the soil clean-up operations (Madejón *et al.*, 2006).

In most cases, the potential ingestion of the main nutrients in the affected area is similar or even greater than those in the control area (Fig. 3). This is a positive feature in the case of elements such as Ca, as described above. This is also positive for P, since horses' diets are frequently deficient in P. Even in the case of S, a high ingestion could counteract a possible Cu toxicity by precipitation as sulphide (Georgievskii, 1982).

In the studied area, the Green Corridor, the stabilisation of soil pollutants could be indicated by the neutralisation of negative interactions between nutrients and essential elements and by the increase of the nutritional quality of the pastures. For example, the physiological ratios in *Cynodon* plants from the non-remediated soil were much more equilibrated in 2008 than in 1999 (Table 5). The potential ingestion of N (non-remediated soil) and P (remediated) and K (non-remediated) significantly increased from 1999 to 2008. In contrast, the potential ingestion of S, Ca and Mg decreased, possibly due to a lesser effect of the residual sludge in 2008. The sludge can experience periodical oxidation-reductions, accomplished by soil acidification and a partial sulphide and carbonate solubilisation, which makes S, Ca and Mg more available for plants. Thus, the consistent reduction of S, Ca and Mg concentrations (Table 4) may be indicative of a stabilisation of the residual soil pollution of the area. Examples of natural recovery of very polluted areas have also been reported by Lepp and Madejón (2007) and Madejón and Lepp (2007) for other latitudes.

Consequently, the ingestion of most toxic trace elements, such as As and Pb, in the non-remediated plot sharply decreased in 2008 to levels that could be tolerated by horses (Table 4). In the non-remediated plot, the effect of the sludge was especially marked in As and Pb uptake; consequently the decrease of their concentrations in time plants was more evident. According to the NRC (2005), a daily As intake of 2.66-4.00 mg kg^{-1} BW by horses does not produce any discernible injury; this range is 23-35-fold greater than the concentration of 0.115 mg kg^{-1} found in our study site in 2008 (Fig. 4). Lead deserves special attention since Pb has been reported as a cause of accidental poisoning in domestic animals more than any other element, particularly in cattle, sheep and horses (Liu, 2003). This element is often present together with Cd, and their effects could be additive. A minimum cumulative fatal dosage of 1.7 mg Pb kg^{-1} BW day^{-1} has been reported for horses (Palacios *et al.*, 2002). These authors also found negative effects in horses that received a diet that resulted in a Pb accumulation of 2.4-99.5 mg Pb kg^{-1} body weight (BW) day^{-1}. Liu (2003) found lethal effects in horses at 6 mg Pb kg^{-1} BW day^{-1}, similar to the Pb accumulation that would be produced by the ingestion of *Cynodon dactylon* in 1999. In 2008, the estimated ingestion of Pb in the non-remediated site was 0.42 mg Pb kg^{-1} BW day^{-1}, 14 times lower than the lethal dose. In the remediated area, the potential ingestion would be 300 times lower, corroborating the recovery of the pastures in the affected area of the Guadiamar valley.

As conclusions, the residual pollution is still present in the affected soils of the 'Green Corridor' natural reserve and does not have any influence on the nutritional richness of the pastures feed by horses, which could counteract a potential toxicity of trace elements present in the herbage. Moreover, trace elements contents in plants diminished in time, as showed the change of As and Pb contents (two of the most abundant trace elements in the affected soils) in one of the most representative species (*C. dactylon*) growing in the studied area. All these results seem to confirm that grazing with horses may be an actual possibility to control the herbaceous cover which represents a sustainable management tool on this afforested area, although further periodical monitoring should be advisable.

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