Evaluation of pastures for horses grazing on soils polluted by trace elements

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Abstract

Pasture established on polluted soil may pose a risk to grazing livestock creating a requirement for mechanical management which may affect biodiversity and expend energy. The risk associated with managing pasture by grazing horses (non-edible livestock) is being assessed in the Guadiamar Valley (SW Spain), where soils are polluted with trace elements following a major pollution incident. Soil pollution does not affect biomass production or floristic composition of pasture, although both variables influence trace element accumulation in herbage. Element concentrations in herbage are below maximum tolerable limits for horses. Faecal analysis showed regulated absorption of essential elements, while non-essential elements seemed preferentially excreted. Elemental content of horse hair did not differ in animals from polluted and control pastures. If pastures are managed by grazing, periodic monitoring is recommended in view of the long-term chronic trace element exposure in these systems.

Keywords: Pasture composition; trace element ingestion; horses’ hair; horses’ faeces.

1. Introduction

Mineral content of plants eaten by herbivores can be strongly influenced by the soils and geology on which they are located (Sutton et al. 2002). Accumulation of potentially toxic trace elements in grazing animals may occur on soils that are naturally rich in metals or polluted by human activities as occurred following the release of metal-rich sludge when a tailings dam burst at Aznalcóllar in SW Spain (Grimalt and Macpherson 1999).

This accident (April 1998) affected an area of 4286 ha along the Agrio and Guadiamar River valleys. To mitigate the environmental impact of this accident, a large-scale restoration project was launched, including the compulsory purchase of the land (formerly devoted to crops and pastures) and the designation of a public nature reserve: ‘Green Corridor’ (CMA 2003). This project was one of the largest soil remediation operations in Europe (Domínguez et al. 2008). However, despite the clean-up and partial restoration of soils, the affected zone is still polluted with trace metals, but with an irregular distribution pattern (Cabrera et al. 2008).

Partial restoration of soil fertility helped in the establishment of herbaceous vegetation cover, based on autochthonous ruderal species well adapted to the local conditions and apparently unaffected by comparatively high trace element content in soil. The area was also planted with native trees and shrubs and these, together with the herbaceous cover, contribute to the sustainable phytostabilisation of the polluted soils, as their root systems physically bind the soil and prevent excessive re-entrainment (Pulford and Watson 2003).

Grazing of livestock was forbidden when the Green Corridor was created. As a result, the vigorous and healthy herbaceous cover competes with planted woody species for water and nutrients. In addition, the desiccated remains of the herbaceous vegetation present a fire hazard during summer droughts. Mechanical control of these herbaceous species is expensive, may affect biodiversity and generates greenhouse gas emissions. For these reasons the possibility of grazing with horses (non human-edible livestock) is being currently considered by the regional Government as a benign and sustainable management tool for control of the herbaceous cover.

In the context of changing agricultural and conservation policies in Europe, free-ranging herbivores are being more widely used to achieve conservation objectives. Cattle, sheep and horses are used to maintain open grasslands and marshes and their associated fauna (Menard et al. 2002). There is limited data available concerning the use of grazing horses for herb control in remediation programmes (Menard et al. 2002). Currently, horses are the only livestock found along the study area (Guadiamar basin). Despite the ban on grazing in the Green Corridor, there are numerous horses grazing illegally at present. In consequence, there is a clear need to assess risks associated with grazing these pastures. The objective of this paper is to evaluate the potential ingestion of As, Cd, Cu, Fe, Mn, Pb, Tl and Zn from pasture by horses and to assess the possibility of using grazing as a management tool for herbaceous vegetation in the Green Corridor. We analyzed the floristic composition of pastures, trace element concentrations in the dominant herbs, and trace element concentrations in horse faeces and hair. Soil-to-plant uptake relationships will be reported in a subsequent paper.

2. Material and Methods

2.1 Study area and Sampling sites

The Guadiamar River Valley, in SW Spain (37º 30-13’ N, 6º 13’ W), 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. Average annual temperature is 19 ºC (min. 9 ºC in January, max. 27 ºC in July) and annual average rainfall is 484 mm. Soils of 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 (see general description of the accident in Grimalt et al. 1999). The resulting flood inundated 55 km^2 of the basin southward towards the Doñana National Park. The affected soils, mostly under agricultural production, were contaminated with high concentrations of As, Cd, Cu, Pb, Tl and Zn (Cabrera et al. 1999). After the accident, an emergency cleanup removed the sludge and the first 0-10 cm of soil and organic matter and calcium-rich amendments were added with the aim of immobilizing trace elements and improving soil fertility (CMA 2003). Revegetation started in 1999, after the purchase of affected lands by the Regional Administration. At present, the plant cover is composed by native shrubs and trees planted at different densities, and a dense matrix of ruderal herbs, methods for the control of which are currently under consideration.

Sampling was carried out in the spring and autumn 2007. Samples of pasture and horse faeces and hair were collected at 8 sites along the Guadiamar Green Corridor. Sites 1 to 7 were affected by the spill and still present different levels of residual pollution and site 8 was not affected by the spill and is used as the control site for this study. To evaluate soil pollution, we used the pollution load index (PLI, as defined by Tomlinson et al. 1980). This index is based on the value of the concentration factor (CF) of each metal in the soil. The CF is the ratio obtained by dividing the concentration of each metal in the soil by background values (As, Cd, Cu, Pb and Zn) of soils in the Guadiamar valley. For each sampling site, PLI was calculated as the *nth* root of the product of the obtained nCF. Values of PLI ~ 1 indicate metal loads near background level, while values > 1 indicate soil pollution (Cabrera et al. 1999). The pH values, As and Pb (main soil pollutants) total concentrations and PLI values of the studied soils are shown in Table 1.

2.2 Sampling of pasture, horse faeces and hair

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. Pasture vegetation was sampled by collecting all the aboveground biomass found in a 25 x 25 cm quadrant. Pasture height and species present were noted before biomass sampling. The percentage of the plant cover in each quadrant was visually estimated, as well as the percentage of the cover that corresponded to each species ("relative plant cover"). Twenty (1 ha) or ten (1/2 ha) samples were taken at each site, each sampling quadrant being at least 30 m distant from the previously sampled replicate. A total of 100 samples of pasture were taken along the Corridor. All plant species were included in herbage analysis, despite the known preferential feeding of horses on grasses (Menard et al. 2002).

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).

Horse faeces (three samples per site) were collected at each sampling site, except site 6, where none were found. Horse hair was collected from the mane of horses that had been grazing freely, if illegally, along the Green Corridor, for two years (n= 7). Hair and faeces were also collected from horses grazing on similar pasture in the vicinity of the Green Corridor, outside of the spill-affected area (n= 5). It was not always possible to take hair at each sampling point, as for faeces, because horses move freely.

Plant material was oven-dried at 70º C to constant weight, weighed to obtain dry biomass and directly ground without prior washing (to study the actual pollution of trace elements in the grass and their possible toxic impact through consumption as forage); dry biomass was passed through a 500 μm stainless-steel sieve.

Faeces were washed (for approximately 10 s) with a 0.1 N HCl solution, then with distilled water, and finally oven-dried at 70 ºC to constant weight, ground and passed through a 500μm stainless-steel sieve. The hair samples were washed in deionised water to remove dust and superficial contamination. This was followed by sequential washing with acetone, and distilled water. The washed hair samples were oven-dried for 24h at 70 ºC, then cut into lengths of less than 0.5 cm.

2.3 Analytical methods

Plant material was analysed for N by Kjeldahl digestion. Total protein in plants was calculated by multiplying Kjeldhal N content by 6.25. Plant material, faeces and horse hair were digested by wet oxidation with concentrated HNO_3 under pressure in a microwave digester. Three consecutive steps (5 min. each) of power (250 W, 450 W and 600 W) were applied. Analysis of mineral nutrients and Fe in the digests was performed by ICP-OES (inductively coupled plasma spectrophotometry; Thermo Jarrell Ash Corporation). Analysis of trace elements (As, Cd, Cu, Mn, Pb, Tl and Zn) was performed by ICP-MS (inductively coupled plasma-mass spectroscopy; Perkin Elmer, Sciex-Elan 5000), using an internal standard (Rh) and multielement standard solutions for calibration. The accuracy and precision of the analytical method was assessed by routine analyses of the following reference samples (NCS DC73350, China National Analysis Center for Iron & Steel, 2004, leaves of poplar, and BCR reference material no. 279, sea lettuce), and hair (BCR reference material no. 397, human hair). Recovery rates for reference plant samples were between 90% and 110% and for reference hair samples between 90% and 100%.

2.4 Data analysis

To assess the abundance of the different species compounding of pastures we calculated the relative frequency of each species (Table 2). This value indicates the number of observations of a given species in the whole sample set (N = 100 sample units), given as a fraction of unity. The relative plant cover (%) of different plant groups (families) was also calculated (Table 3).

Mean and standard deviation (SD) were determined for all variables. Normality of the data was tested prior to analysis and, when necessary, variables were transformed logarithmically. 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. Significant differences in Tables and Figures are marked by letters or asterisks.

Principal Component Analyses (PCA) were performed to explore the patterns of variation of trace element accumulation in the different pastures. Variables introduced into the PCAs were trace element concentrations, pasture production (biomass per area unit) and relative percentage of cover of Poaceae, Fabaceae and Asteraceae (main species composing the pastures) in each sample. General Linear Models (GLM) were used to analyse the possible effects of sampling site, productivity and plant composition (predictor variables) on the concentration of trace elements in the pasture (dependent variables). The variables were log-transformed or arcsin-transformed (those variables expressed as percentage) prior to these analyses. All analyses were performed with Spss 15.0.

3. Results and discussion

3.1. Biomass production and plant composition

The productivity of pastures in the study area showed significant differences between sites (only in spring) and between seasons (Figure 1). In spring, mean values of biomass production for each sampling site ranged from 200 to 700 g m^{-2}. In some cases there was considerable within-site variability, as indicated by a high standard deviation, especially at sites 4, 6 and 8 (Figure 1). These sites showed the highest biomass production, reaching up to 2217 g m^{-2}. In autumn, mean values for each site were below 350 g m^{-2} and there were no significant differences in biomass production between spill-affected and unaffected sites. The lack of effect of residual pollution on pasture productivity was reflected by the fact that the highest biomass production (site, 4, Figure 1) corresponded to the most polluted site (PLI 11.2, Table 1). This may be because bioavailability of trace elements in these soils is low, with little influence over plant establishment and subsequent growth. Previous studies have shown that, in field conditions found in the Guadiamar Green Corridor, pH is the most important factor controlling the trace element bioavailability and that, in general, the bioavailable concentrations are low where pH values exceed 5.0, as found in the soils considered here (Domínguez et al. unpublished).

The total number of species identified in the sampled pastures was 39 in spring and 23 in autumn (Table 2). The most frequent species belong to the Poaceae (especially *Bromus* spp. and *Agrostis pouretii*), Fabaceae (*Medicago polymorpha*) and

Asteraceae family (*Senecio* spp.). 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 (Table 3).

3.2. Trace element concentrations and nutritional value of pastures

The majority of trace element concentrations (As, Cd, Cu and Zn) were below the maximum tolerable levels (MTL) for horses reported by NRC (2005) (Figure 2). These are based on indices of animal health (levels in brackets were derived from interspecies extrapolation). Excessive elemental content of herbage was only found at sites 1 and 2 (close to the mine) in autumnal sampling, where concentrations of Pb and especially Fe were excessive, possibly due to contamination of herbage with soil (Figure 2). High levels of Fe in soil-contaminated pastures for other areas of SW Spain, not affected by a mine spill, were reported by Murillo et al. (1985). Soil contamination of fodder appears to be an important source of iron for horses, especially for foals (Brommer and Oldruitenborgh-Oosterbaan 2001). In general, it has been demonstrated that young horses have a high susceptibility to trace elements, especially in case of lead (Schmitt et al., 1971; Casteel2001).

In the case of Pb, although the MTL has been established as 10 mg kg^{-1}, its tolerance could be greater under adequate Ca levels in the diet (NRC, 2005), as is found in these pastures (see below).

There is no published information on the MTL for Tl. According to Hapke (1984), ‘safe’ Tl concentrations in pasture are > 0.5 mg kg^{-1} dry wt. In this study, Tl levels are, in general, far lower than this value and also lower than the safe value for the trophic web (2.5 mg kg^{-1} dry matter, Makridis and Amberger 1996).

Cadmium is mobile in plants and may become concentrated in leaves; therefore grazing animals may potentially receive the highest exposure to this element. Although chronic dietary levels of 10 mg kg^{-1} dry matter can be tolerated by non-ruminants, these levels can result in unacceptable levels of Cd in kidneys, livers and, in some cases, in muscle (NRC 2005) taking to account animal and human health. Lower values in the diet are thus desirable (e.g., those in Figure 2). An MTL of 0.5 mg kg^{-1} dry wt has been recommended for livestock (Chaney 1989), although Beyer (2000) argues that Cd toxicity levels for wildlife have been exaggerated. Marginal deficiencies of essential nutrients (Zn, Fe and Ca) can enhance Cd absorption by animals (Reeves and Chaney 2008). Such deficiencies are not present in these pastures (Figure 2 and Table 4).

In general, apart from other factors (very young, old, reproducing, sick, exposed to stressful environments), animals consuming nutritional imbalanced diets may be more sensitive to toxicoses (NRC 2005). Apart from the Ca/P ratio, which is comparatively high, the pastures in the study area have a reasonable nutritive value (Table 4). Protein contents of the pasture would satisfy horse requirements, typically about 1 g crude protein kg^{-1} weight day^{-1}. Concentrations of S, P, Ca and Mg do not exceed MTL levels for horses (0.5%, 1%, 2% and 0.8% respectively, NRC 2005). Only K content is greater than the MTL of 1%, although this is a conservatively safe maximum tolerable level for non-ruminants; the NRC (2005) set the maximum tolerable amount of K at 3 % for both ruminant and non-ruminant species.

Ratios of Ca/P measured in autumn forage appear excessive for non-ruminants, although this ratio was lower in spring, when there is a longer grazing period (Table 4). Horses feed on forage that is usually low in phosphorus (Jordan et al. 1975). According to data reported by Schryver et al. (1983), the high Ca/P ratio in the pasture was corroborated by the comparatively high ratio Ca/P in faeces collected in the affected soils: 2.25 ± 1.47 (see section 3.5).

3.3. Plant composition and trace element accumulation

Principal Component Analyses (PCA) of the studied plant variables revealed that the floristic composition of the pastures had some influence on patterns of trace element accumulation, especially in the spring growing season. Application of PCA to spring samples identified three factors with eigenvalues > 1, explaining 68 % of the total variability (Table 5). The first factor was the most important one; it was positively related to the potentially toxic element concentrations, and negatively related to the dry biomass and the grass composition of the samples. The second factor related Cd concentrations and the relative abundance of Asteraceae species. The third factor showed a slight relationship between Tl concentrations and the relative abundance of Fabaceae. These relationships can be observed in Figure 3a. Pasture biomass was associated with the presence of a high proportion of grasses; both variables were situated in the negative side of the Factor 1-axis. Therefore, it is possible that samples with a high grass cover tend to show lower trace element concentrations, due to a dilution effect caused by high biomass production of these species. The ‘dilution effect’ of elements due to biomass increase has been profusely considered in literature (e.g. Jarrel and Beverly, 1981). In our study, this effect must be considered not only as a plant physiological phenomenon but also as a mechanical phenomenon, reducing the herbage exposure to trace element (soil contamination of pastures) (Healy, 1973). Sampling units with a high proportion of Asteraceae tended to have higher Cd concentration. These patterns were not so clear for autumn sampling, due to reduced biomass production and species diversity (Table 5). Nevertheless, biomass production and trace element concentration were still in opposite sides of the Factor 1- axis (Figure 3b) for this sampling period. Apart from the dilution effect, grasses can accumulate comparatively less trace elements than other species (e.g. Asteraceae). Considering only pasture samples that had a grass cover ranging between 90-100 %, autumnal concentrations of As, Fe and Pb at site 1, and Cd at site 4, decrease to values of 1.50, 1980, 5.50 and 0.90 mg kg^{-1}, respectively, compared to those shown in Figure 2. This could be important, given that horses feed preferentially on graminaceous patches.

The General Linear Models applied to the trace element concentrations confirmed that increased biomass production negatively affected the accumulation of most of these elements, and that this dilution effect was more significant in spring (Table 6). In these samples, all trace element concentrations, except Tl, were significantly influenced by either biomass production or the proportion of grass cover. The sampling site also significantly influenced trace element accumulation. In contrast, for the autumn pastures, sampling site was the only significant factor, with the exception of Pb concentrations, for which biomass production was also a significant predictor (Table 6). Therefore grazing on poor grown or partial covered pastures may increase exposure to metals derived from comparatively greater concentration of trace elements in forage which may be due to the “dilution effect” as exposed above. Horses usually feed closer to the ground, using shorter grass than other animals (Menard et al. 2002; Fleurance et al. 2007).

3.4. Ingestion of trace elements by grazing horses

Animals' estimated daily intake of trace elements via food intake, both essential and non-essential, are shown in Table 7. These are based on an average pasture ingestion of 21 g dw per kg body weight (Aronson 1978; Liu 2003). Although other authors (Fleurance et al. 2001) consider that horses could ingest more than this amount, most available data in the literature are close to this ingestion value. For example, for 500 kg BW horses, Dulphy et al. (1995) reported an ingestion within a range of 20.3 (grasses) – 23.9 (alfalfa) g dm kg^{-1} BW day^{-1}.

Trace element intake in autumn was higher than in spring, due to the spring dilution of trace element content, attributable to better pasture growth (see section 3.3). In general, the ratios between daily element ingestions in affected sites and control sites (A/C, Table 7) were higher in autumn than in spring, apart from Zn.

High rates of Fe intake were predicted from our data, arising as a result of herbage contamination by the Fe-rich soil. However, iron toxicity is not a common problem in most domestic animals, probably because of the limited absorption and uptake of Fe when intakes are high (NRC 2005). To determine the effect of iron excess on liver function, adult ponies were given 50 mg Fe kg^{-1} BW day^{-1} (Pearson and Andreasen 2001). Treated individuals showed no adverse clinical signs or development of hepatic lesions. Maximum predicted ingestion from the present study was 20.9 mg Fe kg^{-1} BW day^{-1}; even if this was doubled, it would not exceed 50 mg Fe kg^{-1} BW day^{-1}.

Manganese is considered to be one of the least toxic of the essential elements (NRC 1980) and ingestion by horses at the Green Corridor is far below toxic thresholds. The main effect of chronic excess Zn intake is a reduced efficiency of Cu absorption (NRC 2005). However, the predicted ingestion level in the Green Corridor was always below that which could be considered excessive. In feeding experiments using horses, inclusion of 500 mg Zn kg^{-1} in the diet had no obvious effects on Cu metabolism (Hoyt et al. 1995).

In general, higher A/C ratios (ingestion values at affected sites/ingestion values at control site), were found for non essential elements (Table 7). Although As was one of the elements that caused social alarm due to its toxicity, the predicted ingestion levels do not seem high for horses. According to NRC (2005), a daily food intake of 2.66-4.00 mg As kg^{-1} BW in horses did not produce any discernible injury. This ingestion was 70 to 100-fold greater than the maximum predicted values found in our study (autumn, Table 7).

In the case of Cd, ingestion levels in the Green Corridor could be tolerable. The maximum predicted Cd intake from food found in this study (Table 7) was much lower (110-fold) than lethal ingestion levels of 1.1 mg Cd kg^{-1} BW day^{-1} reported by Liu (2005) for horses. Lead has been incriminated as a cause of accidental poisoning in domestic animals more than any other element, particularly in cattle, sheep and foals and horses (Liu 2003; Schmitt et al., 1971). This element is often present 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). This value is 17-fold greater than maximum predicted values found in the present study (Table 7). Palacios et al. (2002) found negative effects in horses that received a diet which resulted in a dose of between 2.4-99.5 mg Pb kg^{-1} BW day^{-1}, and Liu (2005) found lethal effects in horses at 6 mg Pb kg^{-1} BW day^{-1} (about 60-fold greater than maximum predicted values found in this study).

Thallium is more toxic to mammals than Cd, Pb, or even Hg (Nriagu 1998), but there is very little published information concerning lethal ingestion rates for grazing animals. Frerking et al. (1990) reported non-fatal Tl poisoning in cattle at an ingestion rate of 0.75 mg Tl kg^{-1} BW day^{-1} over a 6-week ingestion period. This value is 750-fold higher than maximum ingestion values predicted from the data presented above (Table 7). Konermann et al. (1982) found no injury in pigs that received a daily intake of either 0.05 or 0.1 mg Tl kg^{-1} BW day^{-1}. Therefore, a daily ingestion of Tl predicted to be approximately 0.001 mg kg^{-1} BW in the affected areas seems to be tolerable for horses and other animals (Table 7).

3.5. Trace elements in horse hair and faeces

The elemental composition of animal excrement can reflect changes in diet and the level of metal contamination in the diet. The concentrations of some minerals in excreta can be greater than those found in feed (NRC 2005). Our results show this for non-essential elements in horse faeces (Table 8). Concentrations of As were 2.7-fold greater in faeces than in autumnal pasture, 1.6-fold greater for Cd, 3.0-fold greater for Pb and 2.6-fold greater for Zn. The A/C ratios were also greater for non essential elements than those of essential elements (Table 8).

In mammals, the absorption mechanisms for essential metals are controlled by homeostatic or homeorhetic mechanisms (Wilkinson et al. 2003), that could also be utilised for other non-essential metals, although, in general, non-essential elements are often characterised by low absorption rates. For example, the transport of Cd from the mucosa to the bloodstream is much less (about 1%) than that of essential metals such as Zn (up to 50%). Cadmium absorbed into mucosal cells is bound to cell membranes and returns to the gastrointestinal tract following the desquamation of these cells. In contrast, Zn is retained and may be released as required, depending on the body burden (Wilkinson et al. 2003). Mammals convert inorganic As into methylated metabolites which are rapidly excreted; hence the carry-over of As compounds from feeds to edible tissues of mammalian species is very low (Kan and Meijer 2007). Analysis of horse faeces seems to show a preferential excretion of non-essential trace elements compared to essential metals (Table 8). The lowest A/C ratio in faeces was found for Cu; excessive accumulation of this essential element could be controlled by metallothioneins in animal tissues (Yin at al. 2008).

Hair has often been proposed for biomonitoring environmental contaminants but suffers from problems with external contamination. This fibre is metabolically very active during its growth and its composition is highly influenced by the health and nutritional status of the individual before it leaves the epidermis (Hasan et al. 2004). Thus, long-term exposure to heavy metals can be readily identified by hair. Trace element content of mane hair of horses has been used to assess diseases, metabolic disorders and nutritional status, because sampling and storage of hair is straightforward when compared to other biological materials (Asano et al. 2002).

Higher As levels have been reported in the wool of sheep exposed to dietary arsenic (Raab et al. 2002). Ward and Savage (1994) investigated exposure of horses to several toxic heavy metals, including Cd and Pb, from vehicle emissions using hair analysis. They detected increases in Pb and Cd concentrations in equine hair and blood, with a significant correlation between blood and hair levels of Pb. According to Combs et al. (1983) little Cd is incorporated into hair, making this tissue a poor indicator of exposure. In the present study, despite greater concentrations of Cd in hair of horses from the affected areas than in control horses, accumulation seems rather low in both cases (<0.1 mg kg^{-1}).

Element ratios in the hair of horses grazing in the Green Corridor and horses from outside this area are shown in Table 9. Copper and Zn had the lowest ratios, similar to the ingestion values and faecal ratios for these elements. Iron is abundant in soils, so despite the rigorous hair washing procedure, the high A/C ratio for Fe could indicate some degree of soil contamination; the highest ratio was found for Tl.

In general, trace elements in hair of ‘control horses’ were in the same range as those reported for healthy horses (Table 9). In comparison with horses from the Green Corridor, the greatest difference was in Fe content. This could be explained by greater exposure to Fe-rich soil in their diet, compared to the race horses studied by Asano et al. (2002). In the case of non-essential elements, values were always in the same range as reported for healthy horses (no data was found for Tl), and even lower in the case of As. Although there was no attempt to correlate concentrations in horse hair to a measure of toxicity in horses, results from hair seem to indicate an acceptable situation for grazing in the Corridor.

4. Conclusions

Biomass production of the pasture appears to be unaffected by soil pollution in the Guadiamar Green Corridor. Trace element concentrations in pasture plants were below the maximum tolerable level for horses, although in autumn concentrations were higher than in spring, due to a dilution effect of greater biomass production in the latter season. The floristic composition of pastures had some influence on patterns of elemental accumulation. Pastures composed mostly of grasses had lower trace element concentrations, since these species produced the greatest biomass. Asteraceae species tended to show higher concentrations of other elements, such as Cd. Therefore, grazing should preferably occur in areas and during periods where high biomass grasses are dominant. Grazing on regenerating pastures in autumn may increase exposure to metals derived from comparatively greater concentrations of trace elements in, and soil adhering to, herbage, especially in the case of Pb and Fe.

Predicted values of daily elemental intake for horses, in spring and autumn, seem to be much lower than critical values reported to induce toxicity. In general, faecal analyses showed that absorption of essential elements is regulated by homeostatic mechanisms which control their accumulation. However, non-essential elements tend to be preferentially excreted by horses. Finally, trace elements in horse hair from the Green Corridor were in the same range as reported for healthy horses, therefore there appears to be no evidence that supports a toxic risk to horses grazing this region. The use of horses as management tools for the long-term restoration of this ecosystem should be further investigated, but any long-term strategy should incorporate systematic monitoring of both forage and animals.

Acknowledgements

We acknowledge the Regional Ministry of Environment (Junta de Andalucía) for supporting this study and the Spanish Ministry of Education for a PFU grant awarded to M.T. Domínguez. Dr. P Madejón thanks her Ramón y Cajal contract financed by the Spanish Ministry of Innovation and Science. We also thank Olga Cazalla (Centre for Scientific Instrumentation, University of Granada) for the ICP-MS measurements, Dr. Teodoro Marañón for his comments on the manuscript and José María Alegre for his help in different stages of the study.

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Figure captions

Figure 1. Spring and autumn biomass production (mean + SD) at each study site. Asterisks mark significant differences between seasons, within each sampling site (* $p < 0.05$, ** $p < 0.01$, ** $p < 0.001$). Letters indicate differences among sites, during the spring season. For the autumn season there were no significant differences among sites.

Figure 2. Trace element concentrations (mean + SD) of pasture in the Guadiamar Green Corridor (1 – 7) and the control site (8). Significant differences between each site and site 8 (Control) for each season are marked with * (spring) or ** (autumn).

Figure 3. Plot of the two first factors extracted by a Principal Component Analyses of the pasture characteristics and trace element concentrations, applied to the spring (a) and to the autumn (b) samples. The percentage of variance explained by each factor is indicated in their respective axes. Factor-variable correlations are shown in Table 5. Biomass production (BM, g m-2) and relative cover (%) of Poaceae (POA), Fabaceae (FAB) and Asteraceae (AST) species.

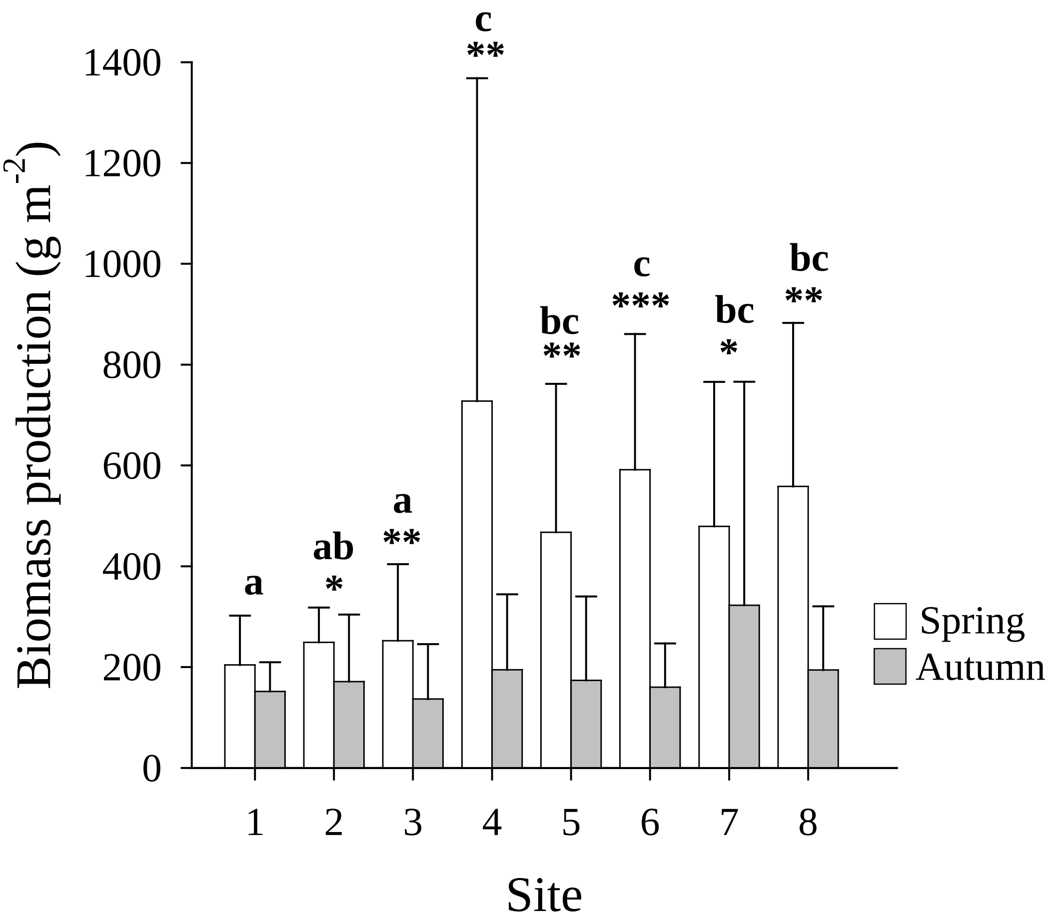
Biomass production (g m-2)
1400
1200
1000
800
600
400
200
0
a
ab
*
a
**
c
**
bc
**
c
***
bc
*
bc
**
Spring
Autumn
1
2
3
4
5
6
7
8
Site

Figure 1

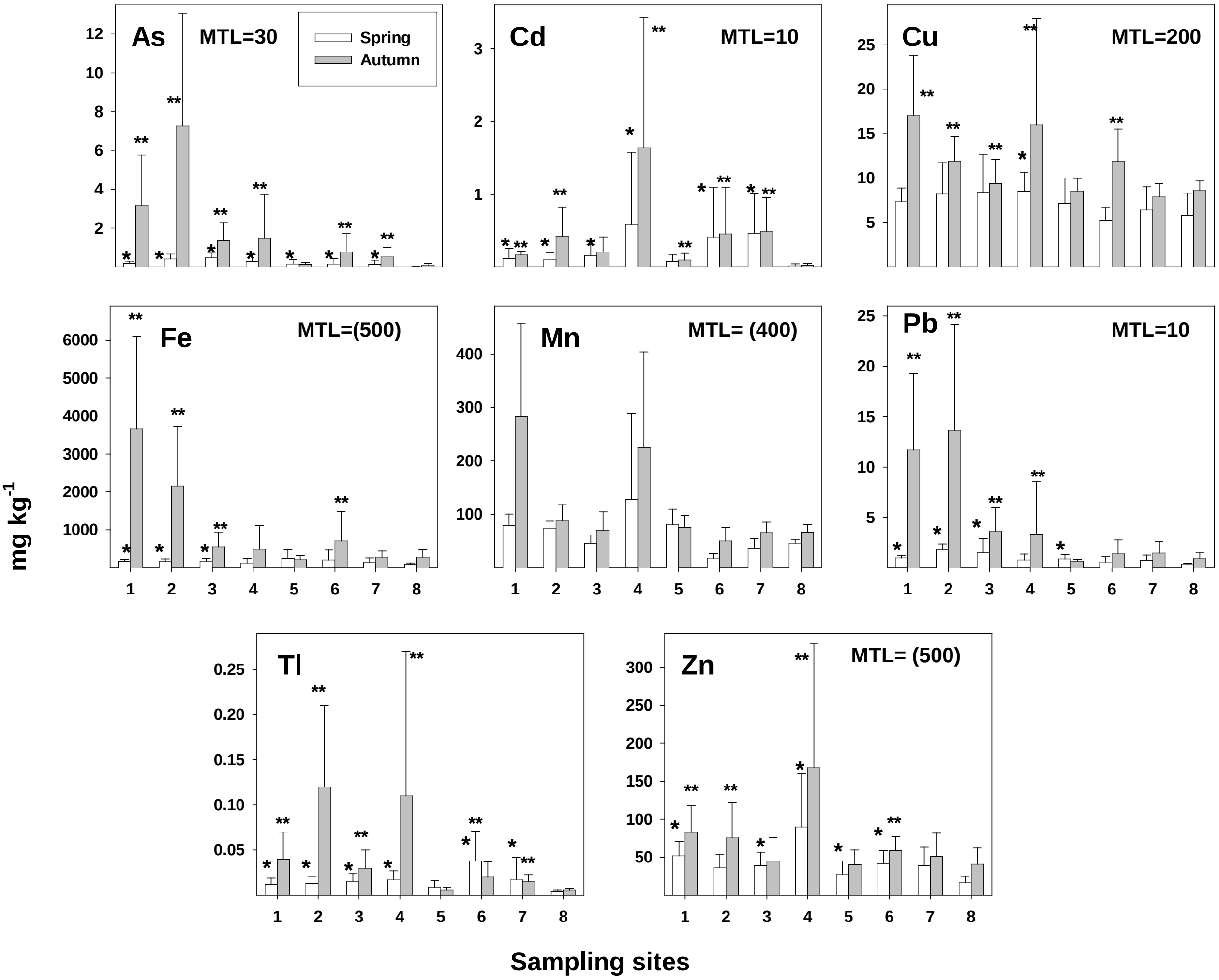
As
MTL=30
Spring
Autumn
Cd
MTL=10
Cu
MTL=200
Fe
MTL=(500)
Mn
MTL= (400)
Pb
MTL=10
Tl
Zn
MTL= (500)
mg kg^{-1}
Sampling sites
1 2 3 4 5 6 7 8
2 4 6 8 10 12
1 2 3
5 10 15 20 25
1000 2000 3000 4000 5000 6000
100 200 300 400
5 10 15 20 25
0.05 0.10 0.15 0.20 0.25
50 100 150 200 250 300
*
**

Figure 2

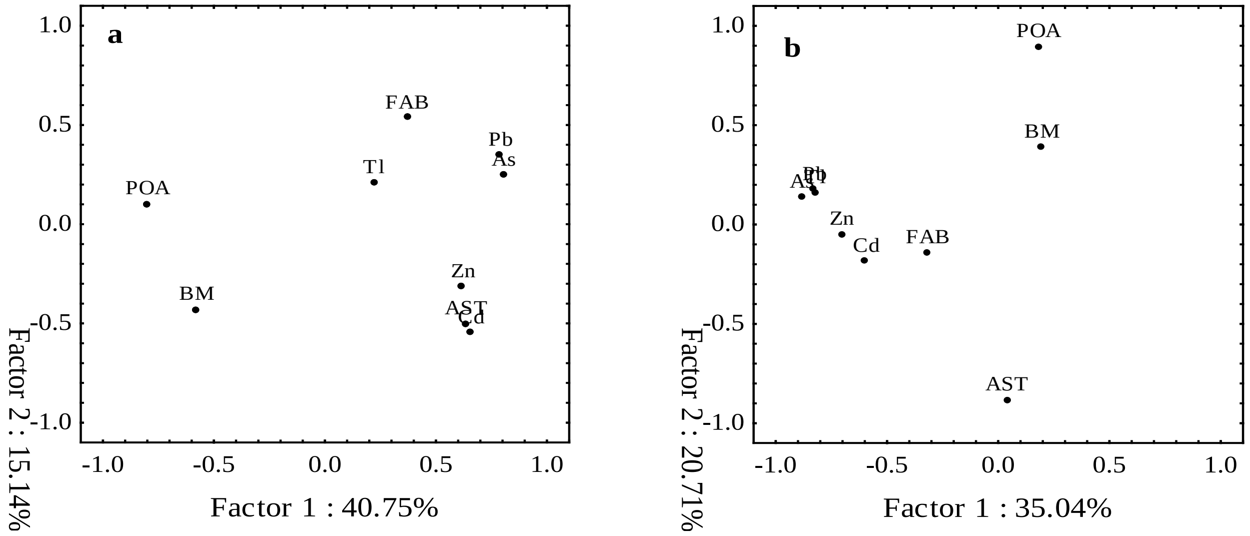
a
1.0
0.5
0.0
-0.5
-1.0
FAB
Pb
As
Tl
POA
Zn
BM
AST
Cd
-1.0
-0.5
0.0
0.5
1.0
Factor 1 : 40.75%
Factor 2 : 15.14%
b
1.0
0.5
0.0
-0.5
-1.0
POA
BM
As
Pb
Tl
Zn
Cd
FAB
AST
-1.0
-0.5
0.0
0.5
1.0
Factor 1 : 35.04%
Factor 2 : 20.71%

Figure 3

Table 1. pH, As and Pb concentrations and PLI (Pollution Load Index) values calculated for the 8 sampled soils.

Site	Latitude/Longitude	pH	As (mg kg^{-1})	Pb (mg kg^{-1})	PLI
1	37º28’09.8’’, 6º12’42.0’’	7.6 ± 0.18 ab	56.2 ± 20.1 b	128 ± 25.7 bc	5.45
2	37º27’41.4’’, 6º12’42.0’’	8.3 ± 0.0 4b	87.6 ± 42.0 bcd	148 ± 69.3 bcde	7.38
3	37º25’45.0’’, 6º13’05.0’’	8.2 ± 0.48 b	145 ± 85.6 d	290 ± 185 cde	8.90
4	37º23’13.5’’, 6º13’38.0’’	7.0 ± 0.90 a	147 ± 9.88 d	263 ± 35.7 e	11.18
5	37º22’39.5’’, 6º13’45.2’’	8.2 ± 0.11 b	44.0 ± 14.1 c	62.8 ± 34.8 bc	2.31
6	37º17’25.7’’, 6º15’46.2’’	8.1 ± 0.13 b	62.2 ± 9.95 c	128 ± 16.1 cd	7.49
7	37º14’27.0’’, 6º15’22.0’’	8.0 ± 0.07 b	93.3 ± 32.2 cd	188 ± 79.6 de	9.90
8	37º19’21.5’’, 6º15’17.8’’	7.8 ± 0.33 ab	21.5 ± 2.53 a	14.3 ± 1.93 a	0.73

For each element, values followed by the same letter do not differ significantly ($p<0.05$)

Table 2. Species composition of the pastures (affected and control sites). The relative frequency of observations of each species (N = 100) during the spring and autumn sampling is indicated.

Family	Sp	Spring	Autumn	Family	Sp	Spring	Autumn
Poaceae	*Agrostis pourretii*	0.08	0.35	Fabaceae	*Biserrula pelecinus*	0.01	
	Avena barbata subsp. barbata	0.03			*Medicago doliata*	0.06	0.01
	Avena sterilis	0.1	0.05		*Medicago murex*	0.07	0.01
	Bromus diandrus	0.17	0.11		*Medicago polymorpha*	0.13	0.03
	Bromus lanceolatus	0.21			*Scorpiurus muricatus*	0.04	0.04
	Bromus madritensis	0.1			*Vicia villosa subsp. varia*	0.02	
	Hordeum marinum	0.06		Others	*Anagallis arvensis*	0.07	0.01
	Lamarckia aurea	0.03			*Beta vulgaris*	0.01	
	Lolium perenne	0.1			*Echium plantagineum*		0.05
	Phalaris minor	0.05	0.03		*Equisetum arvense*	0.01	0.03
	Piptatherum miliaceum	0.08	0.1		*Euphorbia helioscopia*	0.01	
	Andryala integrifolia	0.1			*Heliotropum europaeum*		
Asteraceae	*Carduus pycnocephalus*	0.02			*Hirchsfeldia incana*		
	Chrysanthemum coronarium	0.09			*Malva hispanica*	0.04	0.05
	Coleostephus myconis	0.21	0.12		*Plantago coronopus*	0.01	o
	Convulvulus arvensis	0.01	0.06		*Plantago lagopus*	0.05	o
	Conyza bonariensis	0.05			*Ranunculus trilobus*	0.03	o
	Cynodon dactylon	0.01	0.25		*Scirpoides holoschoenus*		0.02
	Galactites tomentosa	0.02			*Torilis arvensis*	0.04	0.07
	Lactuca serriola	0.01					
	Leontodon longirostris	0.09	0.02				
	Senecio lividus	0.22	0.01				
	Senecio vulgaris	0.14	0.16				
	Silybum marianum	0.03	0.02				
	Xanthium strumarium		0.01				

Table 3. Relative composition of plant groups in the sampling units (expressed by the relative percentage of cover, mean ± SE), per site and season.

Site	Spring				Autumn			
	Poaceae	Fabaceae	Asteraceae	Others	Poaceae	Fabaceae	Asteraceae	Others
1	72 ± 6	19 ± 6	8 ± 4	1 ± 1	61 ± 11	11 ± 7	24 ± 10	4 ± 1
2	58 ± 10	16 ± 9	10 ± 5	16 ± 9	63 ± 14	10 ± 9	3 ± 1	24 ± 13
3	40 ± 9	27 ± 7	27 ± 8	5 ± 4	85 ± 7	0	7 ± 5	8 ± 5
4	71 ± 12	6 ± 6	14 ± 10	8 ± 7	67 ± 15	0	9 ± 8	24 ± 13
5	58 ± 15	9 ± 9	31 ± 15	2 ± 1	77 ± 13	0	0	22 ±12
6	64 ± 14	5 ± 5	24 ± 13	8 ± 3	79 ± 13	0	20 ± 13	1 ± 1
7	54 ± 10	21 ± 8	24 ± 8	1 ± 1	82 ± 7	14 ± 7	4 ± 2	0
8	62 ± 15	0	18 ±12	20 ± 12	99 ± 1	0	0	0

Table 4. Seasonal differences in protein and nutrient concentrations (mean ± SD), and Ca/P ratios in pasture growing on affected (n=90) and non-affected soils (n=10)

Element	Season	Affected soils	Control soils
Proteins (%)	Spring	8.67 ± 4.00	9.54 ± 5.47
	Autumn	10.8 ± 3.87	6.41 ± 1.44
S (%)	Spring	0.34 ± 0.15	0.18 ± 0.09
	Autumn	0.37 ± 0.15	0.18 ± 0.04
P (%)	Spring	0.22 ± 0.05	0.20 ± 0.06
	Autumn	0.18 ± 0.07	0.12 ± 0.06
K (%)	Spring	2.00 ± 0.55	2.04 ± 0.76
	Autumn	2.09 ± 1.02	1.04 ± 0.54
Ca (%)	Spring	0.90 ± 0.61	0.64 ± 0.55
	Autumn	1.03 ± 0.70	0.57 ± 0.19
Mg (%)	Spring	0.15 ± 0.07	0.13 ± 0.07
	Autumn	0.18 ± 0.08	0.17 ± 0.07
Ca/P	Spring	4.02 ± 2.68	3.13 ± 2.46
	Autumn	6.11 ± 5.54	5.47 ± 1.96

Table 5. Results of the Principal Component Analyses (factor-variable correlations) applied to the trace element concentrations (mg kg^{-1}) and other characteristics of the studied pastures: biomass production (BM, g m^{-2}) and relative cover (%) of Poaceae (POA), Fabaceae (FAB) and Asteraceae (AST) species.

Variable	Spring			Autumn		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
As	0.81	0.24	-0.19	-0.88	0.14	-0.31
Cd	0.66	-0.55	0.25	-0.59	-0.19	0.63
Pb	0.79	0.35	-0.24	-0.83	0.17	-0.37
Tl	0.22	0.21	0.51	-0.82	0.16	0.15
Zn	0.62	-0.32	0.32	-0.70	-0.05	0.50
BM	-0.58	-0.43	0.42	0.20	0.39	0.03
POA	-0.80	0.10	-0.11	0.18	0.89	0.28
FAB	0.37	0.53	0.58	-0.32	-0.14	-0.65
AST	0.64	-0.51	-0.30	0.04	-0.89	0.15

Table 6. Results of the general linear models applied to the concentrations of trace elements in the pastures as dependent variables, with the site sampling, biomass production and the relative composition of Poaceae, Fabaceae and Asteraceae as predictor variables. Only predictors with a significant effect on each concentration are shown (n.s.= non-significant)

Element	Spring					Autumn				
	Factor	*F*	*p*	R^2 model	*p* model	Factor	*F*	*p*	R^2 model	*p* Model
As	Biomass	8.97	0.003	0.49	< 0.001	Site	19.16	< 0.001	0.61	< 0.001
	Site	5.81	< 0.001							
Cd	Asteraceae	9.68	0.002	0.47	< 0.001	Site	8.55	< 0.001	0.35	< 0.001
	Site	5.38	< 0.001							
Pb	Biomass	7.35	0.08	0.47	< 0.001	Biomass	4.36	0.039	0.63	< 0.001
	Site	3.45	0.003			Site	20.13	< 0.001		
Tl	n.s.	n.s.	n.s.	n.s.	ns	Site	7.20	< 0.001	0.32	< 0.001
Zn	Poaceae	4.00	0.048	0.45	< 0.001	Site	8.19	< 0.001	0.33	< 0.001
	Site	9.18	< 0.001							

Table 7. Daily predicted intake of trace elements (Mean values ± SD) by consumption of pasture growing on affected (n=90) and control (n=10) soils. 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 per kg of body weight). A/C is the ratio between values at affected and control sites.

Elements	Season	Soil	Cu	Fe	Mn	Zn
Essentials	Spring	Affected (A)	0.15 ± 0.06*	3.60 ± 2.96*	1.27 ± 1.31	0.94 ± 0.70
		Control (C)	0.12 ± 0.05	1.75 ± 0.92	0.97 ± 0.15	0.34 ± 0.18
		A/C	**1.25**	**2.06**	**1.31**	**2.76**
	Autumn	Affected (A)	0.23 ± 0.12*	20.9 ± 31.3*	2.32 ± 2.41*	1.44 ± 1.48
		Control (C)	0.18 ± 0.02	5.90 ± 3.53	1.40 ± 0.31	0.86 ± 0.45
		A/C	**1.28**	**3.54**	**1.66**	**1.67**
			As	Cd	Pb	Tl
Non essentials	Spring	Affected (A)	0.006 ± 0.006*	0.006 ± 0.01	0.02 ± 0.02*	0.001 ± 0.004*
		Control (C)	0.0004 ± 0.0005	0.0005 ± 0.0005	0.007 ± 0.002	0.0001 ± 0.00005
		A/C	**15.0**	**12.0**	**2.86**	**10.0**
	Autumn	Affected (A)	0.04 ± 0.06*	0.010 ± 0.02*	0.10 ± 0.14	0.001 ± 0.001*
		Control (C)	0.002 ± 0.002	0.0006 ± 0.0006	0.02 ± 0.01	0.0001 ± 0.00004
		A/C	**20.0**	**16.6**	**5.0**	**10.0**

Significant differences between soils are marked with an asterisk.

Table 8. Trace element concentrations (mg kg^{-1}, mean ± SD) in horses' faeces from affected and control sites. A/C is the ratio between values at affected and control sites.

Elements	Soil	Cu	Fe	Mn	Zn
Essential	Affected (A)	34.9 ± 23.9	1914 ± 774*	187 ± 64.5*	142 ± 60.3
	Control (C)	19.4 ± 8.81	914 ± 78.8	90.6 ± 6.75	68.7 ± 19.0
	A/C	**1.8**	**2.1**	**2.1**	**2.1**
		As	Cd	Pb	Tl
Non essential	Affected (A)	4.93 ± 3.69*	0.78 ± 0.57	13.8 ± 12.6	0.13 ± 0.11
	Control (C)	0.58 ± 0.08	0.15 ± 0.13	2.02 ± 0.37	0.02 ± 0.001
	A/C	**8.5**	**5.2**	**6.8**	**6.5**

Significant differences between sites are marked with an asterisk.

Table 9. Trace element concentrations (mg kg^{-1}, mean ± SD) in mane hair of horses grazing affected and control sites. A/C is the ratio between affected and control sites.

Elements	Soil	Cu	Fe	Mn	Zn
Essentials	Affected (A)	14.7 ± 5.05*	334 ± 151*	14.1 ± 7.53*	185 ± 23.8
	Control (C)	9.84 ± 0.71	21.7 ± 3.57	4.29 ± 1.04	208 ± 6.82
	A/C	**1.5**	**15.4**	**3.3**	**0.9**
Reference range[a]		*5.9 ± 1.0*	*22.4 ± 17.5*	*4.99 ± 4.25*	*101.7 ± 14.9*

		As	Pb	Tl
Non essentials	Affected (A)	0.85 ± 0.49*	1.34 ± 0.66*	0.015 ± 0.01*
	Control (C)	0.19 ± 0.12	0.37 ± 0.02	0.001 ± 0.001
	A/C	**4.47**	**3.62**	**15.0**
Reference range		*1.11 ± 0.17*	*0.77 ± 0.29*	-

Significant differences between sites are marked with an asterisk.

[a] Reference range of trace elements in mane hair of healthy race horses (Asano et al., 2002)