Snail Theba pisana as indicator of soil contamination by trace elements: Potential exposure for animals and humans

Running title: Trace elements in a land snail

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Abstract

BACKGROUND: The ability of snails to accumulate trace elements (TE) is well known. We analyzed the Theba pisana snail as indicator of soil contamination by TE after a mine spill accident, to assess the exposure of animal and human consumption. Snails were collected in autumn and spring, when they are most active.

RESULTS: In general, TE in the soft tissues reached greater concentrations in the contaminated soils than in the non-contaminated soils, although significant differences were only found for As, Cd, Cu Fe and Hg. Cadmium content in tissues, with a maximum value of 10 mg kg⁻¹ (dry matter), was the most worrying result. Trace element concentrations in the snail bodies were still of concern for human consumption, As and Cd concentrations were sometimes higher than maximum concentration authorized in foodstuff. Generally, nutritional status of the contaminated snails was not altered: concentrations of the main nutrients (Ca, K, Mg, P and S) were similar to those of the non-contaminated snails.

CONCLUSIONS: Results reveal a potential risk for animal and human consumption of T. pisana. It seems thus advisable to avoid collecting this species for human consumption in the affected area. Periodic monitoring is recommended to assess potential risk evolution for animal consumption.

Keywords: cadmium, nutrients, mine spill, snails

1. Introduction

Anthropogenic activities, e.g. production and/or use of metallic compounds, smelting or the combustion of fossil fuels, are responsible for most of the trace element contamination occurring in soils.¹ In these contaminated soils remediation is needed to minimize their harmful effects on the trophic web. At present, cost-effective methods

such as stabilization by the application of organic or inorganic amendments are used extensively worldwide in order to minimize these effects.² Stabilization methods are often combined with phytostabilization, giving rise to the development of a vegetation cover which enhances the reduction of wind erosion, surface runoff or water percolation.^{3,4} This was the case of the Guadiamar Green Corridor, which was affected by a mine accident in the spring of 1998.⁵ In these contaminated soils the following restoration activities were carried out: i) cleaning-up of the affected area to remove the deposited sludge together with the plough layer of the soil, ii) application of organic and inorganic amendments and iii) afforestation with autochthonous Mediterranean species.⁶⁻⁸

To assess the efficiency of these restoration techniques soil analysis and bioassays with plants and microorganisms have been widely used.^{2,3} Although animals are less frequently used for this purpose, different animals species have been analysed at the Guadiamar Green Corridor (e.g., macroinvertebrates,⁹ geckos,¹⁰ lizards,¹¹ shrews,¹² fish,¹³ wetland birds,¹⁴ and even mane hair of horses¹⁵).

Snails are known to be sensitive to soil acidification and/or environmental pollution,¹⁶ and they are shown to easily accumulate pollutants, such as heavy metals.¹⁷ Land snails have therefore been suggested as sentinel organisms for biomonitoring.¹⁸ However, terrestrial snails have not been used for this purpose in the Guadiamar Green Corridor, despite the fact that they can be used to monitor the transfer of contaminants from different sources.¹⁹⁻²¹ Moreover, land snails have the peculiarity that they can be consumed not only by animals (thrushes, hedgehogs, carabid beetles²²) but also by humans.

Spain is, after France, the country where land snail human consumption is most frequent, especially in Andalusia (Southern Spain). Theba pisana (Müller 1774) is the

most consumed species in the South of Spain. Although since the 1980's most of the land snails for human consumption come from other countries, such as Morocco, part of these gastropods still comes from the Andalusian countryside.²³

After the Aznalcóllar accident, the Regional Administration purchased the affected lands and implemented the Guadiamar Green Corridor programme. The goal was to provide a continuous vegetation belt for wildlife to migrate along the Guadiamar River basin between the Doñana National Park in the South and the Sierra Morena mountains in the North.²⁴ In the Guadiamar Green Corridor, all agricultural and livestock activity was banned. However, although illegal, snail gathering for human consumption is a common practice in villages adjacent to the Green Corridor.

The present paper deals with the analysis of the Theba pisana, the most common land snail in the Guadiamar Green Corridor, twelve years after the accident in order to i) assess the efficacy of the trace elements stabilization measures taken in the area, using T. pisana snails as a potential indicator of the soil-plant system contamination, ii) assess the exposure of T. pisana snails for primary consumers and iii) finally, assess the potential exposure for human consumption.

2. Materials and Methods

2.1 Snail sampling

Theba pisana snails were collected along different sites in the Green Corridor in the spring and autumn of 2010. The sampling points inside the Green Corridor (from 37° 28' 10''N, 6°12'10''W to 37°19'21'', 6°15'18''W) were three zones with different grades of contamination and a non-contaminated area used as a control. Snails were

collected inside an area of about 1 ha in each sampling point. A minimum of 100-150 specimens of T. pisana per point were collected in order to obtain enough biomass for analysis (four replicates of about 30-40 individuals each per site). For clarity and simplicity results of the snails collected in the contaminated areas are treated jointly, performing the mean values of the three contaminated samplings points (n=12 for contaminated area and n=4 for control area).

2.2 Snail preparation

Snails were transported to the laboratory in coolers; a sub-sample (spring and autumn samplings) was immediately frozen at -80°C after sampling (without fasting), which represents the real situation as they would be consumed by predators. This sample would also serve to assess the use of snails as a bio-indicator of soil contamination.

Another sub-sample (spring and autumn samplings) was purged by starvation for 48h and subsequently subjected to three washings with deionised water (about five minutes each, to remove soil particles), according the procedure followed by most local consumers. Finally, the purged snails were frozen. This technique avoids an overestimation of metal bioavailability derived from the presence of metal-contaminated soil particles in the animal's gut.²⁵ These samples represent the situation as they would be consumed by humans.

The whole soft tissue (i.e. foot + viscera) of each snail (both purged or not purged) was removed from the shell for analysis. Shells were analysed separately. Although whenever possible it is advisable to analyze the main tissues or organs separately, such as hepatopancreas, gonads, kidneys and intestine,²⁶ we have selected

the whole body (soft tissues and foot) as this corresponds to potential consumption by humans and predators.

The whole soft tissue (foot + viscera) was oven-dried at 70°C to a constant weight to obtain dry biomass and then ground using a manual agate grinder (to avoid biomass loss). The same procedure was followed for shell preparation.

The snail fresh weight (complete animal) was calculated by weighing three batches of 25 snails at each sampling point. The weight of the whole soft tissue of each snail was also determined immediately after defrosting.

2.3 Chemical analysis

The whole soft tissues were digested by wet oxidation with concentrated HNO₃ under pressure in a microwave digester (Microwave Laboratory Station Milestone ETHOS 900, Milestone s.r.l., Sorisole, Italy). Three consecutive steps (5 min. each) of power (250 W, 450 W and 600 W) were applied. Shells were digested with a mixture 3:1 (v/v) of conc. HCl and HNO₃ ('aqua regia'). To avoid the effervescence of the shell biomass, rich in CaCO₃, HCl was first added, and after 30 minutes conc. HNO₃ was added. Analysis of nutrients (Ca, K, Mg, Na, P, S) and trace elements (As, Cd, Cu, Fe, Mn, Pb, Zn) in the digests of soft tissues and shells were performed by ICP-OES (inductively coupled plasma spectrophotometry) using a simultaneous spectrometer Varian ICP 720-ES with axially viewed plasma.

For the analysis of Hg in soft tissue, 1 ml of the digested samples diluted to 10 ml, was treated with 0.1 ml of potassium bromate/bromide reagent, and then after 1 hour with 0.01 ml of a solution of hydroxylamine chloride (13% w/v). These extracts

were then analyzed by an Atomic Fluorescence Spectrometer (Mercury plus Analytik Jena model).

The accuracy and precision of the analytical methods was assessed by routine analyses of the reference material mussel tissue ERM-CE278. Recovery rates for reference samples were between 90 and 100%.

2.4. Soil sampling and analysis

Soil samples were taken at 0-10 cm depth by a cylindrical auger (Ø 2.5 cm) (four replicates per each sampling point). Soil samples were air dried, crushed and sieved through a 2-mm sieve, and then ground to <60 µm. Pseudo-total trace element concentrations in soil samples (<60 µm) were determined by ICP-OES after aqua regia digestion in a microwave oven.

2.5 Statistical analysis

Mean and standard errors (SE) were determined for all variables. Normality of the data was tested prior to analysis and, when necessary, variables were transformed logarithmically. A Student's t-test was used to assess differences in the concentration of mineral elements in the snails collected from the non-contaminated and contaminated soils. The non-parametric Mann-Whitney-U test was used when necessary. A significance level of $P < 0.05$ was used throughout the study. All analyses were performed with SPSS 15.0

3. Results and Discussion

Different authors have reported consistent stabilization of the soil contamination in the Guadiamar river basin in the years after the mine accident.^{6,8} After restoration measures mean values of total trace elements were very variable and remained above background values. These anomalies are due to remains of sludge left on the soil and buried during restoration operations, resulting in an irregular distribution of trace elements in surface soils along the Guadiamar river basin.⁶ Table 1 shows that mean values of trace element concentrations in soils of the sampling sites were somewhat lower than those of affected soils through the Green Corridor, especially in the case of Cd and Pb.

Nevertheless, trace elements availability in the soils of the Green Corridor was found to be moderate,²⁴ which allows for a varied plant diet moderate in trace element concentration for most herbivores.¹⁵ Varied macro-fauna inhabits the Corridor at present, and different species of snails were identified: genus Cernuella, Cochlicella, Cornu, Otala, Rumina, Xerosecta, besides T. pisana (O.F. Müller 1774). These species were found in contaminated and non-contaminated sites. Compared to a previous study carried out close to the Green Corridor,²⁷ only two species were not observed: Ferussacia follicula (Gmelin 1791) and Xerotricha conspurcata (Draparnaud 1801). These data indicate the re-colonization and stabilization of the affected area.

The plant diet suitability, derived from a low concentration of potentially toxic trace elements, may have influenced the fact that there were not significant differences between the weight of T. pisana snails from non-contaminated and contaminated soils (1.51 ± 0.03 g and 1.43 ± 0.01 g, respectively). Under heavy soil pollution, snail weights are usually consistently lower than those from unpolluted areas.²⁸

Terrestrial snails are considered as bioindicators of trace element environmental contamination, because of their capacity to accumulate and store these elements in their tissues.^{19,29} In fact, even under moderate soil contamination, soil ingestion and adherence could increase metal levels in the snails' bodies.³⁰ We therefore studied T. pisana, the most common land snail in the contaminated area of the Guadiamar Green Corridor, as a potential bioindicator of the residual contamination by trace elements still present in this zone.

3.1. Trace element concentrations in unpurged T. pisana

In general, there were not significant differences between trace element concentrations in snails sampled in the three contaminated sites due to the internal variability within each site. Concentrations of the most mobile elements Cd and Zn ranged into, approximately 4-10 (Cd) and 100-200 (Zn) mg kg⁻¹ in autumn and 2-6 (Cd) and 70-250 (Zn) mg kg⁻¹ in spring. Concentrations of trace elements in snails may be very variable even in those collected in the same area.²⁶ Consequently, and for simplicity, the results in contaminated soils were processed jointly and compared with those in the non-contaminated soil.

Trace element concentrations in the frozen T. pisana soft tissues are shown in Figure 1. Significant seasonal differences for both contaminated and non-contaminated samples were found for As (greater values in spring), Cd and Cu (greater values in autumn). According to our results, it seems that there was not a uniform, definite seasonal trend for the presence of trace elements in the body of this snail. Other authors also found no seasonal variation in the As concentration in the snail Cantareus aspersus, although higher mortality was detected in summer.⁴ As pointed out for these authors,

the mechanism by which environmental conditions may influence snail response to As (and possibly to other toxicants) remains unclear. Further research in this context is thus advisable.

As expected, snails collected in contaminated soils reached greater concentrations of trace elements than those collected in the non-contaminated soils, although significant differences were only found for As, Cd, Cu (both samplings) and Hg and Fe (autumn sampling). As previously reported,^{31,32} at present Cd is the most worrying trace element present in the Green Corridor, due to its toxicity and mobility. Nevertheless, concentrations of Cd in snails in the contaminated area (mean values of 4.03 mg kg⁻¹ in spring and 6.41 mg kg⁻¹ in autumn) are far lower than those reported for other land snail species under different conditions.^{30,33} This fact suggests that, despite Cd mobility, an important grade of stabilization has been reached in this remediated zone, although the Cd contamination of snails is still consistent. In soils with low contamination of Cd, very low concentrations of this element have been reported in this species (eg. range of 0.004-0.008 µg g⁻¹ dry weight).³⁴ This confirms the high variability in the concentration of Cd in snails.

Biological indicators can be defined as species which reflect the condition or state of the environment in which they thrive.³⁵ In this context, T. pisana could be a suitable indicator for As, Cu and especially Cd under the current Green Corridor soil conditions, although further study is still needed in this regard.

Iron, Pb and Zn concentrations in snails collected in contaminated soils were slightly greater than those in non-contaminated soils (differences were not significant, except for Fe in autumn), indicating their low suitability as indices for soil contamination, under our environmental conditions. In some cases, Pb in snails has been reported as a suitable indicator, e.g. to evaluate road transport,³⁶ which seems not be the

case. Despite the fact that Mn was not a pollutant element in the sludge,³⁷ its concentration tended to increase in the snails from the contaminated soils, a fact also reported for many plant species (a potential snail diet) in the Green Corridor.^{15,38}

3.2 Risk for animal consumption

The Green Corridor is very close to the Doñana National Park, a wintering area for many European birds. For that reason it is important to evaluate the risk of consumption of snails, especially for avifauna in this area.

As pointed out before, the most worrying concentration for animal risk was found for Cd, with a maximum value of 10 mg kg⁻¹ in snails from contaminated soils (Figure 1). Although dietary levels of 10 mg kg⁻¹ (dry matter) can be tolerated chronically by poultry, these levels can result in unacceptable levels of Cd in the kidneys, liver and, in some cases, in animals' muscles.³⁹ Lower values in the diet are thus desirable. A MTL (Maximum Tolerable Level) of 0.5 mg kg⁻¹ had been recommended for livestock in general, although other authors argued that Cd toxicity levels for wildlife have been exaggerated.⁴⁰

Standardized experiments in the laboratory are necessary for approaching ecological risk assessment (ERA) by estimating critical effect levels from concentration-response relationships for survival/reproduction.⁴¹ Many studies have reported the comparatively higher Cd accumulation in the soft tissues of snails, compared to other trace elements.²⁹ They have also shown a high tolerance of snails to Cd, which represents a negative connotation from a toxicological point of view making periodical monitoring necessary, not only for this species but also for other molluscs in the area.

In this study, maximum concentrations of Cd, and other metals such as Cu, Pb and Zn, in the whole body of T. pisana were low (Figure 1). However, comparison with other areas is not always possible due to the mentioned extreme variability of trace element concentrations in molluscs²⁶. For example, for Cd a range of 3 – 68 mg kg⁻¹ has been reported in literature for the snail Cepaea nemoralis from different zones.⁴²

Concentrations of As were always higher in snails sampled in contaminated soils than in the non-contaminated soil, especially in spring (Figure 1). However, these values were always below 4 mg kg⁻¹ in the soft tissues and they may be considered as moderate values. The maximum tolerable level for domestic animals is 30 mg kg⁻¹ dry weight,³⁹ although other sources set a level for animal feeding of 2-4 mg kg⁻¹ dry weight.⁴³ Arsenic mean value of 20 mg kg⁻¹ has been detected in snails exposed to stabilized and untreated As-contaminated soils (120 mg kg⁻¹ in soil).⁴ However these authors observed that after amendment addition As transfer soil-snail was significantly reduced. Although very high values of Pb in molluscs from heavy polluted sites have been reported in literature, e.g. 40 mg Pb kg⁻¹ in soft tissues of H. aspersa,⁴⁴ 365 mg Pb kg⁻¹ in Cepaea nemoralis from smelters and mines,⁴⁵ concentrations in T. pisana from the contaminated soils of the Green Corridor were always below 2-3 mg kg⁻¹, low values far from the maximum levels tolerated by chicken (30 mg kg⁻¹).⁴⁶

The low values of the less mobile elements (As and Pb) in the snail body (Figure 1) could be related, among other reasons, with the moderate concentrations of these elements in plants in the studied area.^{7,47}

Copper, Fe, Mn and Zn are essential micronutrients for plants and animals, but at high concentration can be toxic. Despite the fact that in the Green Corridor soil Cu availability and transfer to plant tissues is low,⁴⁸ the T. pisana snail accumulates ca. 200 mg kg⁻¹ dry weight of this element (Figure 2), values far greater than the required

nutritional range for most poultry (3 – 11 mg kg⁻¹ dry weight). Nevertheless, these levels are harmless for most tolerant species, because most adult birds absorb only 5-10% of the ingested Cu from their diet.^{49, 50}

Concentrations of Fe in snails from the contaminated sites were also higher than the nutritional requirements for poultry (50 – 80 mg kg⁻¹; Figure 1), although they never surpassed the tolerance threshold (1000 mg kg⁻¹ of Fe for chicken) which may induce toxic effects.⁴⁷ Absorption of Fe is also limited in most animals.

Concentrations of Mn were greater than the required nutritional range for poultry (30 – 70 mg kg⁻¹)⁵⁰ in spring, although harmless due to the low toxicity of this element. Zinc was also higher than the required nutritional range for poultry (30 – 70 mg kg⁻¹),⁵⁰ but lower than the recommended threshold level (<178 mg kg⁻¹) to prevent marginal sub-lethal effects, and far lower than the level (<2000 mg kg⁻¹) to prevent the death of chicks and ducklings.⁵¹

Mercury is one of the most toxic elements when released into the environment, whose negative ecological and health effects are well known.⁵² Although Hg was not one of the most abundant elements in the spill,⁶ it was important to study its presence in soils, plants and animals in the affected area. Significant differences between snails from non-contaminated and contaminated soils were only found in the autumn sampling (Figure 1). In general, all the values found were low and smaller than the MTL for both inorganic and organic Hg in the diet (MTL for poultry: inorganic 0.2 mg kg⁻¹ and organic 1.0 mg kg⁻¹).³⁹ On a fresh weight basis, they were even lower than normal contents in some foods in UK (e.g. 0.054 mg kg⁻¹ fish fresh weight).⁵³

It seems that the moderate trace element concentrations in the area did not alter the nutritional level of the snails.⁷ Main nutrients were similar in snails from contaminated and non-contaminated soils (Table 2), and, except for Ca (greater values

in autumn, $p < 0.05$) and K (greater values in spring, $p < 0.05$), similar in both samplings. Potassium and S concentrations were in general greater than the MTL for poultry, Phosphorous concentrations were close to the MTL and Ca concentrations were within the range of MTL (Table 2). Concentrations of Mg were slightly smaller than the MTL (Table 2). Marginal deficiencies of essential nutrients can enhance Cd absorption by animals.⁵⁴ Such deficiencies were not present in the studied snails.

Shell analysis is also advisable since many birds need an extra Ca supplement for developing eggs and proper growth of nestling skeleton. Part of the shell may be ingested by birds when consuming snails because it can be an important Ca source for these purposes.^{28,55} The high Ca concentration in shells from contaminated and non contaminated soils (about 340 g kg^{-1}) may not only accomplish this purpose but also counteract the potential harmful action of toxic metals. Trace element contents in shells (Table 3) were much lower than those in soft tissues, and far smaller than the corresponding MTLs.

3.3 Risk for human consumption

Theba pisana is the most consumed snail by humans in South Spain.²⁷ People are very fond of collecting snails in the countryside, including the Green Corridor, despite the fact that any activity related to animal and human nutrition was forbidden in the area.

For this kind of snail, a common practice before cooking is the starvation (at least for one day, in order to remove soil particles from the gut) followed by washing to remove the adhered soil. For a better evaluation of potential human exposure this practice was also carried out in the laboratory before the analysis.

Trace element concentrations in the snail bodies were still of concern for human consumption (Figure 2). Arsenic and Cd concentrations were sometimes higher than maximum concentration authorized in foodstuff. Arsenic is found in most food and rarely exceeds $1 \text{ mg kg}^{-1} \text{ dw}$, except in seafood. In the UK the maximum permitted level is 1 mg kg^{-1} in unspecified food, although no distinction is drawn between organic and inorganic forms.⁵³ In this study As content in snails could be a problem in spring, when levels were between 0.52 to 2.68 mg kg^{-1} , but not in autumn when values were between 0.10 to 0.50 mg kg^{-1} . In the case of Pb, the Commission Regulation (EC) No 1881/2006 establishes Pb maximum levels of 1.5 mg kg^{-1} fresh weight for bivalve molluscs and 1.0 mg kg^{-1} fresh weight for cephalous.⁵⁶ On a fresh weigh basis, the mean value of Pb found in this study was 0.64 mg kg^{-1} with a maximum value of 1.26 mg kg^{-1} , close to the established limit.

As pointed out before, Cd is still one of the most concerning trace elements in the area,³² and has been described as one of the most dangerous trace elements in food,⁵³ although it is usually found at low levels in it ($0.07 \text{ mg kg}^{-1} \text{ fw}$ in offal, $0.02 \text{ mg kg}^{-1} \text{ fw}$ in fish and $0.001 \text{ mg kg}^{-1} \text{ fw}$ in meat). The Commission Regulation (EC) No 1881/2006 establishes Cd maximum levels of 1 mg kg^{-1} fresh weight for bivalve molluscs and cephalous.⁵⁶ On a fresh weight basis we found a mean value ca. 0.60 mg kg^{-1} with a maximum value of 0.78 mg kg^{-1} . Although, these values were lower than the established maximum level, it is much higher than normal levels in different foods. As for As, maximum values of Cd were recorded in spring, when snails are preferably consumed. The high Ca level in purged snails (up to $20 \text{ g kg}^{-1} \text{ dw}$, data not shown) represents a beneficial feature from a toxicological point of view.^{26, 54}

Despite its toxicity, under the current situation of the Green Corridor, the problem of Hg is less than that of As, Pb and Cd. The Commission Regulation (EC) No

1881/2006 establishes Hg maximum levels of 1 mg kg⁻¹ fresh weight.⁵⁶ This limit is far greater than Hg values in purged snails (Figure 2).

Maximum values of essential nutrients such as Cu and Mn (about 25 mg kg⁻¹ fresh weight) and Fe (about 80 mg kg⁻¹ fresh weight) do not represent a serious problem for human health considering their low absorption and toxicity. However, the criteria to establish tolerable levels of trace elements for human food are ample, which means that all these approaches should be viewed with caution, especially in regard to the most toxic metals. Considering the mentioned variability of metal concentration in molluscs, the results reported here for some potentially toxic trace elements (Cd in particular) make advisable a periodic, more extensive monitoring of snails in the Green Corridor.

4. Conclusions

Twelve years after a spill accident at SW Spain (Guadiamar Green Corridor at present) the presence and the composition of the snail Theba pisana in the affected soils seem to indicate a consistent stabilization of the trace element contamination in the area. However, there is still moderate soil contamination along the Green Corridor that caused elevated trace element concentrations in the soft tissues of this snail, especially of Cd (mean value of 6.41 mg kg⁻¹ with a maximum value of 10 mg kg⁻¹ dry matter, in unpurged snails), that could represent a potential exposure for animal consumption. Trace element concentrations in the snail bodies were also of concern for human consumption, especially that of Cd, although those of other elements, such as As, Pb and Hg, concentrations were close to the critical levels for consumption. Results of this study show that it would be advisable to avoid snail consumption by humans, as this would prevent unnecessary risks. It is not possible to control consumption by animals,

which reinforces the need for periodical monitoring to assess the evolution of the potential risk, especially, the numerous avifauna inhabiting the Doñana National Park, a Biosphere Reserve and European Community Special Protection Area, located close to the affected area.

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1 Table 1. Trace element concentrations of the soils of the sampling sites (extracted by ‘aqua regia’, mg kg⁻¹ on a dry matter basis; mean values ±
2 SE). In affected soils, the mean values and ranges of the three sampling sites are presented. Mean values of the affected soils and ranges of
3 background concentrations through the ‘Green Corridor’ (‘GC’) are also shown

Soil	Concentration	As	Cd	Cu	Pb	Zn
Control	Mean ± SE	21.5 ± 1.13	0.05 ± 0.01	9.37 ± 0.86	14.3 ± 0.86	40.7 ± 2.00
	Minimum	18.9	0.037	7.72	12.4	35.5
	Maximum	24.8	0.07	12.6	16.6	47.7
Affected	Mean ± SE	92.1 ± 9.10	1.29 ± 0.14	99.7 ± 8.66	117 ± 18.6	348 ± 29.3
	Minimum	44.0	0.47	66.8	106	202
	Maximum	300	3.90	200	634	611

Affected soils of the ‘GC’ (mean SD)*		153 ± 121	4.44 ± 1.91	155 ± 93.0	321 ± 293	462 ± 253
Background values (‘GC’, ranges)**		8.37 - 38.5	0.12 - 1.06	12.3 - 85.0	19.5 - 86.3	53.9 - 271

4 *From Cabrera et al.⁶

5 ** From Cabrera et al.³⁷

6

7 Table 2. Nutrient concentrations (g kg^{-1} dry matter) in soft tissues of the unpurged snails (mean
 8 values $\pm$ SE) in spring and autumn. Significant differences between snails from non contaminated
 9 (NC) and contaminated soils (C) for each season are marked with an asterisk ($p < 0.05$).

Sampling	Soil	Ca	K	Mg	P	S
Spring	NC	19.0 ± 2.30	7.10 ± 0.40	$3.80 \pm 0.20^*$	8.90 ± 0.20	4.90 ± 0.10
	C	23.0 ± 2.50	7.20 ± 0.50	3.20 ± 0.10	9.40 ± 0.80	6.20 ± 0.20
Autumn	NC	33.0 ± 6.10	8.60 ± 0.20	3.90 ± 0.30	10.3 ± 0.30	5.30 ± 0.10
	C	39.5 ± 6.30	8.30 ± 0.10	3.80 ± 0.10	10.0 ± 0.20	5.30 ± 0.20

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11

12 Table 3. Trace element concentrations in snail shells from non contaminated (NC) and contaminated (C) soils (mg kg⁻¹ dry
 13 matter, mean values $\pm$ SE). For each season, significant differences between snail shells from NC and C soils are marked with
 14 an asterisk ($p < 0.05$).

Sampling	Zone	As	Cd	Cu	Fe	Mn	Pb	Zn
Autumn	NC	0.25 $\pm$ 0.09	0.40 $\pm$ 0.20*	8.08 $\pm$ 3.06*	35.0 $\pm$ 3.9*	5.84 $\pm$ 0.60	0.01 $\pm$ 0.003*	18 $\pm$ 5.30
	C	0.57 $\pm$ 0.23	1.23 $\pm$ 0.23	32.0 $\pm$ 5.35	73.0 $\pm$ 11	52.0 $\pm$ 20.0	0.27 $\pm$ 0.05	25 $\pm$ 4.38
Spring	NC	0.37 $\pm$ 0.17	0.22 $\pm$ 0.03*	5.18 $\pm$ 0.33*	115 $\pm$ 17.9	13.7 $\pm$ 1.22	0.53 $\pm$ 0.17	9.03 $\pm$ 0.76*
	C	0.81 $\pm$ 0.13	1.00 $\pm$ 0.09	18.7 $\pm$ 1.82	204 $\pm$ 49.3	143 $\pm$ 5.06	1.53 $\pm$ 0.37	39.6 $\pm$ 2.36
MTL ^a		30.0	10.0	250	500	2000	10.0	500

15 ^aMTL:Maximum Tolerable Levels for poultry (NRC,2005)³⁹

Figure 1. Trace element concentrations (mg kg⁻¹, dry matter) in soft tissues of unpurged snails (mean values $\pm$ SE) in spring and autumn. In each season, significant differences between non contaminated (NC) and contaminated soil (C) are marked with an asterisk ($p < 0.05$).

1

2 Figure 2. Trace element concentrations (mg kg⁻¹, dry matter) in soft tissues of the
3 purged snails (mean values $\pm$ SE) in spring and autumn. In each season, significant
4 differences between snails from non contaminated (NC) and contaminated (C) soils are
5 marked with an asterisk (p < 0.05)