| Phyton (Horn, Austria) | **Vol. 47** | Fasc. 1–2 | 123–132 | 19. 12. 2007 |
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# Fasciation Phenomena and Mineral Balance in *Spartium junceum* L.

By

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With 3 figures

Received October 5, 2005

Accepted January 25, 2007

Key words: Fasciation, mineral balance, chlorophylls. – *Spartium junceum* L., *Fabaceae*.

## Summary

REBOREDO F. & SILVARES C. 2007. Fasciation phenomena and mineral balance in *Spartium junceum* L. – Phyton (Horn, Austria) 47 (1–2): 123–132, with 3 figures. – English with German summary.

The present study reports stem fasciation in *Spartium junceum* L. (*Fabaceae-Genisteae*) for the first time. The plants were collected in a restricted area of the Lisboa-Oporto freeway in Portugal. In abnormal plants, undifferentiated cell masses of variable size forming a cockscomb predominate, whereas flattened stems with forms varying between ribbon-like structures with emergent leaflets to structures more similar to normal plants were scarce. Both abnormal plants had trichomes, which are absent in normal plants. Abnormal plants also showed markedly decreased concentrations of $Ca^{2+}$, $Mg^{2+}$ and chlorophyll, with $Ca^{2+}$ and $Mg^{2+}$ being strongly positively correlated ($P \leq 0.05$). Fasciation did not affect the levels of $Fe^{2+}$ in abnormal plants.

## Zusammenfassung

REBOREDO F. & SILVARES C. 2007. Fasziationsphänomene und Mineralstoffgleichgewicht in *Spartium junceum* L. – Phyton (Horn, Austria) 47 (1–2): 123–132, 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

In der vorliegenden Studie wird zum ersten Mal über Fasziation in *Spartium junceum* L. (*Fabaceae-Genisteae*) berichtet. Die Pflanzen wurden in einem be-

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grenzten Gebiet entlang der Autobahn Lissabon-Porto in Portugal gesammelt. Die Abnormalitäten traten entweder als undifferenzierte Zellmassen, die eine Hahnenkammstruktur bildeten, oder, seltener, als bandartige Strukturen mit Blättchen, die der Normalform eher ähnlich waren, in Erscheinung. Beide abnormen Formen besaßen Trichome, welche an normalen Pflanzen nicht vorkommen. Abnorme Formen wiesen niedrigere Gehalte an $Ca^{2+}$, $Mg^{2+}$ und Chlorophyll auf, wobei $Ca^{2+}$ und $Mg^{2+}$ stark positiv miteinander korreliert waren ($P \leq 0.05$). Die Fasziation hatte keinen Effekt auf die $Fe^{2+}$ Gehalte.

## Introduction

Fasciation (development of flattened organs, usually stems) is a natural phenomenon caused mainly by the pathogenic agent *Rhodococcus fascians*. It occurs in different species, such as *Glycine max* (Tang & Skorupska 1997) and *Arabidopsis* (Medford & al. 1992). Recently, phytoplasmas belonging to the aster yellows group were identified in *Lilium* sp. with flattened stems (Poncarová-Voráčková & al. 1998, Bertaccini & al. 2005). The appearance of fasciation, under in vitro conditions, was also observed in *Betula pendula* and *Prunus avium* (Iliev & al. 2003, Kitin & al. 2005).

The observation of morphological changes in the stems of *Spartium junceum* in restricted areas of the Lisbon-Oporto freeway ($A_1$), as well as the brief characterization of the different fasciated plants, were reported by Reboredo 1994a and were the first report on fasciation of *Spartium junceum*.

The ($A_1$) is the second most important freeway in Portugal, linking the capital (Lisbon) to Porto. The daily traffic volume registered in the 1$^{st}$ semester of 2003 was 36416 cars day$^{-1}$ (Brisa 2003). Close to this freeway, hydrocarbons associated with the use of petroleum derivatives were evident in the *Spartium* samples collected in the district of Oliveira do Bairro (Alves & al. 1999). Lead (Pb) related to vehicle exhaust may also have effects on these plants.

To address the question whether nutritional balance and photosynthetic pigments were affected in fasciated plants of *S. junceum*, we conducted the present study. Furthermore, Pb was also measured in both normal and abnormal plants.

## Material and Methods

*S. junceum* L. plants and soil samples from the top layer (0–2cm) were collected at five sites ("Stations") between the 33.5 and 35.0 km of the Lisboa-Oporto highway (no abnormal plants were detected before 33.5 km and after 35.0 km). The plants were washed with bidistilled water and separated according to their morphological changes and development stage – normal stems (N.S), normal flowering stems (N.F.S), flattened stems (F.S.) and crested harsh tissues on the top of the caulinar axis (cockscomb), henceforth called undifferentiated cell masses (U.C.M.).

Cations: Plants and soils were crushed in an agate mortar and dried at 70 °C to a constant weight. Plant material and soils were digested in the mixture $HNO_3$-$HClO_4$ (4:1) according to REBOREDO 1988, 1993, 1994b. For digestion, 1 g aliquots of dry soil and plant samples, respectively, were placed in a 100 ml borosilicate beaker with 10 ml of $HNO_3$ and allowed to evaporate until dry. The sample was digested again with 10 ml of $HNO_3$ and 5 ml of $HClO_4$ and allowed to evaporate until dry. The residue was dissolved in a 2% aqueous solution of HCl (w/v), filtered and diluted to a final volume of 50 ml. Ca, Fe, Mg, Mn and Pb were determined by atomic absorption spectrometry using a Perkin-Elmer model 403 (Perkin-Elmer, Norwalk, CT, USA) fitted with deuterium background corrector. The operating conditions were those recommended by the manufacturer. The Pb soluble levels were monitored by shaking one gram of each plant sample at 50 °C in 50 ml of bidistilled water for 12 h. The supernatant was carefully removed and filtered through a Whatman GF/C fiber glass filter (Whatman, Maidstone, England).

Pigments: Each of the 0.5 g samples of fresh stem was crushed in an agate mortar with pure acetone and then filtered. The remaining extract was centrifuged at 7.826 x g for 5 min. Absorbances of chlorophyll a, chlorophyll b and carotenoids were measured at 661.6, 644.8 and 470 nm, respectively, using a Shimadzu UV-160 (Shimadzu Scientific Instruments Inc., Columbia, USA) and the pigment concentrations were calculated according to LICHTENTHALER 1987.

Electron microscopy: Scanning electron microscopy studies were performed at 20 KV using a Jeol 330A (Jeol, Tokyo, Japan) on small pieces of fresh plant material previously sputtered with gold.

Statistics: An analysis of variance was performed and the average values were then compared using the Tukey's test (SOKAL & ROHLF 1994). An adjustment of data through a linear regression was performed and the determination coefficient ($R^2$) determined.

## Results and Discussion

Fasciated plants are neither dwarfed, nor is the flower production affected (Fig. 1A). Rarely, flattened stems (Figs. 1B and 1C) were observed with structures quite similar to the normal ones (Fig. 1B) to ribbon-like structures with emergent leaflets (Fig. 1C). The latter with the emergent leaflets also located at the top – are consistent with the observation of flattened stems in *Prunus avium*, with densely lanceolate leaves at the top (KITIN & al. 2005). The larger diameter of the *Prunus avium* flattened stems ranged from 5 to 12 mm (KITIN & al. 2005), while *S. junceum* flattened stems ranged from 0.4 to 0.9 cm in width (REBOREDO 1994a).

The scarcity of flattened stems contrasts with the frequent observation of undifferentiated cell masses of variable size on the top of the caulinar axis forming a cockscomb in Stations 2 to 5 (Fig. 1D). Only rarely we observed "witches brooms" (Fig. 2A).

In normal stems, the numerous rows of aligned stomata are clearly visible because trichomes are absent (Fig. 2B). This contrasts with the trichomes of flattened stems and undifferentiated cell masses (Figs. 2C and 2D).

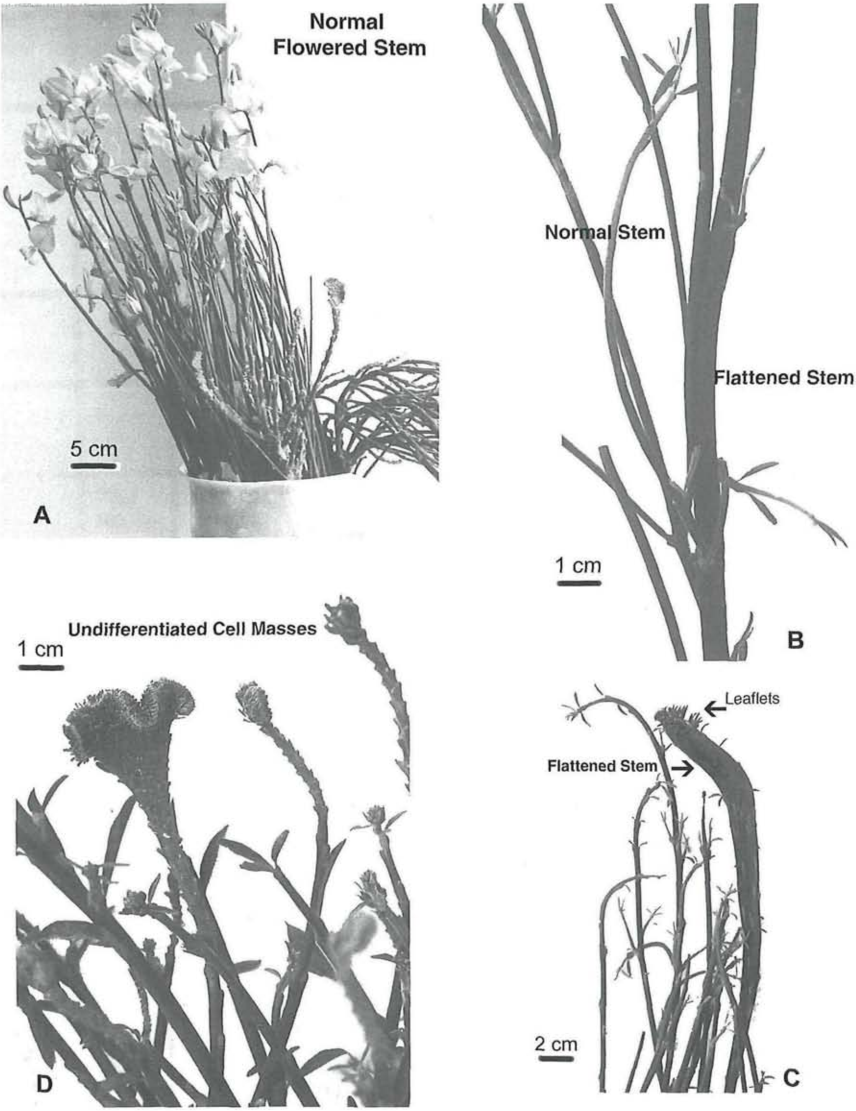


Fig. 1. (A) Normal flowered stems of *S. junceum*. (B) Normal stems associated with flattened stems. (C) Flattened stems with emergent leaflets (arrow). (D) Undifferentiated cell masses forming a cockscomb.

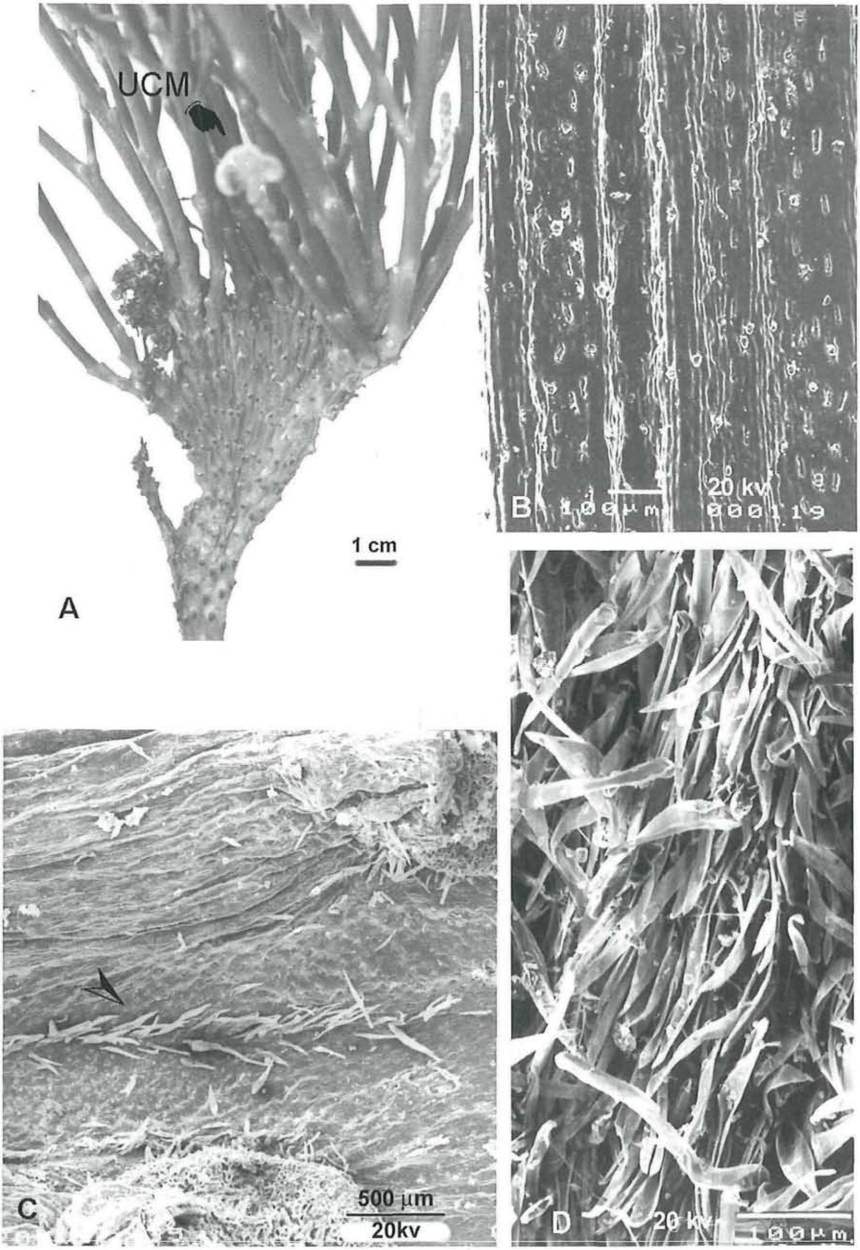


Fig. 2. (A) Cockscomb differentiation with emergent axis and new undifferentiated cell masses (UCM) forming the "witches brooms". (B) In normal stems the numerous rows of aligned stomata. (C) Flattened stems with a few trichomes between two meristematic nodules (arrow). (D) Cell masses (UCM) showing the continuous coat of trichomes.

The average of all mean values of chlorophyll a of the normal stems was 1.3 times higher than the average observed in undifferentiated cell masses (Table 1). A pronounced decrease in Ca, Mg and Mn levels was observed in abnormal plants, especially for undifferentiated cell masses (Table 2). Ca concentrations were 3.9 times lower, Mg 2.5 times lower, and Mn 2.2 times lower than in normal stems. Fe concentrations, on the other hand, were similar in abnormal and normal plants (Table 2). Although weakly, Fe concentrations were also inversely correlated with chlorophyll a concentrations (Fig. 3). The highest Mn levels were always observed in the normal stems (Table 2), where they were not correlated with Chl a. In abnormal plants, Mn levels were weakly correlated to Chl a ($r = 0.36$; Fig. 3).

The analysis of the top soil (0-2cm) revealed that the highest mean Ca concentrations were observed in Stations 1 and 5. This is probably related to the 18% and 13% carbonate content, respectively. Soil Mg had a distribution pattern similar to soil Ca. A particular enrichment of Mn and Fe was noted in the top soil layer of Station 4 (Table 3). Soil Pb increased from the Station 1 to Station 5 reaching an average value of 276 $\mu g\ g^{-1}$. This pattern was not found for the other elements (Table 3). The average Pb concentrations observed in Stations 4 and 5 were significantly different ($P < 0.05$) from those at Stations 1, 2 and 3.

Table 1. Chlorophylls and total carotenoids in *S. junceum* stems.

| | Stations | NS | FS | UCM | NFS |
|---|---|---|---|---|---|
| Chl. a | 1 | 302 ± 21a | 158 ± 34b | | |
| | 2 | 253 ± 20b | | 236 ± 20b | |
| | 3 | 295 ± 24a | | 226 ± 21b | 245 ± 13b |
| | 4 | 266 ± 34a | | 208 ± 28b | 259 ± 25ab |
| | 5 | 332 ± 16a | | 209 ± 42b | 277 ± 23a |
| Chl. b | 1 | 108 ± 9.9a | 54 ± 7.8b | | |
| | 2 | 68 ± 12a | | 92 ± 14a | |
| | 3 | 84 ± 7.9a | | 78 ± 13a | 85 ± 11a |
| | 4 | 87 ± 15a | | 83 ± 11a | 74 ± 6.2a |
| | 5 | 72 ± 6.4a | | 71 ± 14a | 68 ± 8.9a |
| Ctnds. | 1 | 71 ± 4.2ab | 84 ± 7.1a | | |
| | 2 | 62 ± 7.0b | | 67 ± 4.2b | |
| | 3 | 66 ± 6.0b | | 53 ± 7.4c | 59 ± 5.6b |
| | 4 | 60 ± 9.3b | | 62 ± 11b | 50 ± 6.5a |
| | 5 | 67 ± 7.4b | | 50 ± 7.0c | 57 ± 6.8a |

Comparable means (as $\mu g\ g^{-1}$ of fresh weight) not followed by a common letter are significantly different ($P \leq 0.05$) ± standard deviation; n=3. Ctnds = total carotenoids.

Although soils from Stations 4 and 5 generally presented the highest concentrations of the studied elements (Table 3), no direct relationship to

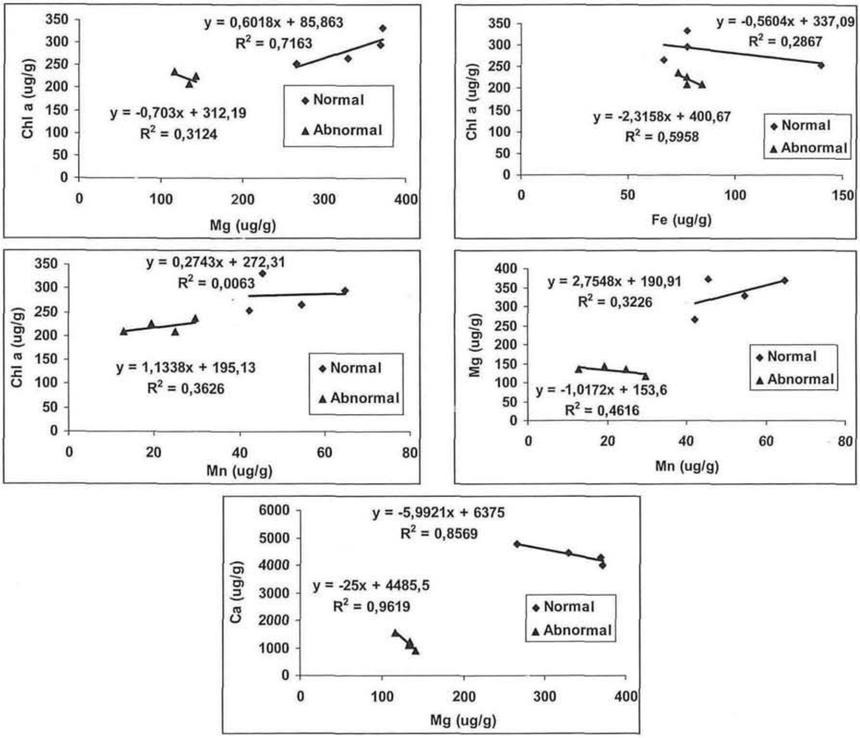


Fig. 3. Chl. a vs Mg, Chl. a vs Fe, Chl. a vs Mn, Mn vs Mg and Ca vs Mg levels in Normal and Abnormal stems (undifferentiated cell masses) in Stations 2 to 5.

their concentrations in both normal and abnormal plants was detected. The values may be related to the geochemistry of the soils, the prevailing air circulation conditions, and the vicinity of pollution sources in addition to the freeway (electrothermal power plant and a metallurgical unit).

In fasciated *S. junceum* plants we detected a pronounced decrease in Ca and to a lesser extent a decrease of Mg and Mn. Broadley & al. 2004 observed strong positive correlations between shoot Ca and Mg concentrations when studying the shoot mineral content of more than one hundred angiosperm species cultivated in hydroponic cultures and approximately 80 angiosperm species collected in the field. We also detected strong inverse correlations between Ca and Mg ($P \leq 0.05$) in both normal and abnormal plants (Fig. 3).

In Norway spruce needles, Mandre & Tuulmets 1997 observed that a decrease in the chlorophyll caused by cement dust resulted in a weak correlation ($r = 0.446$) between the Mn and chlorophyll concentrations. In that study, the concentrations of Mn in the needles ranged between 5–6 mg $kg^{-1}$, approximately 10 times lower than the adequate concentration of 50 mg $kg^{-1}$ (RAVEN & al. 1999). Mn concentrations in the undifferentiated cell masses of *S. junceum* in our study ranged between 12.9–29.7 mg $kg^{-1}$, also indicating deficiency of this element. Since Mn is required for integrity of chloroplast membrane and for $O_2$ release in photosynthesis, one could hypothesise that a deficiency will affect chlorophylls and Mg. However, such a relationship was not assessed by statistical analysis in our study (Fig. 3). The majority of the stems of *S. junceum* were also covered by large amounts of dust which may have implications in photosynthesis. Whether the decrease of photosynthetic pigments is due to the dust or if dust favours the pathogen attack remains unclear.

Table 2. Ca, Fe, Mg, Mn and Pb in the stems of *Spartium junceum*.

| | Stations | NS | FS | UCM | NFS |
|---|---|---|---|---|---|
| | 1 | 5714 ± 874ab | 1907 ± 132cd | | |
| | 2 | 4758 ± 537b | | 1562 ± 128cd | |
| Ca | 3 | 4290 ± 273b | | 899 ± 37.5d | 6891 ± 611a |
| | 4 | 4463 ± 405b | | 1211 ± 144cd | 4282 ± 482b |
| | 5 | 3998 ± 278b | | 1115 ± 127cd | 2630 ± 486c |
| | 1 | 74 ± 15b | 63 ± 14b | | |
| | 2 | 140 ± 16a | | 73 ± 6.6b | |
| Fe | 3 | 77 ± 13b | | 78 ± 8.5b | 62 ± 8.5b |
| | 4 | 67 ± 6.7b | | 78 ± 14b | 76 ± 15b |
| | 5 | 77 ± 6.5b | | 84 ± 4.5b | 65 ± 13b |
| | 1 | 305 ± 23.0a | 216 ± 41.0b | | |
| | 2 | 266 ± 23.6b | | 116 ± 9.55c | |
| Mg | 3 | 368 ± 41.2a | | 142 ± 6.36bc | 374 ± 63.0a |
| | 4 | 329 ± 38.6a | | 134 ± 34.6c | 294 ± 14.9a |
| | 5 | 371 ± 37.7a | | 134 ± 11.8c | 300 ± 35.9a |
| | 1 | 32 ± 7.5b | 18 ± 1.4c | | |
| | 2 | 42 ± 13b | | 30 ± 7.6b | |
| Mn | 3 | 65 ± 7.0a | | 19 ± 4.2c | 27 ± 3.0b |
| | 4 | 55 ± 13a | | 25 ± 2.5b | 16 ± 4.2c |
| | 5 | 45 ± 7.4ab | | 13 ± 0.3c | 16 ± 4.4c |
| | 1 | ND | 1.2 ± 0.2cd | | |
| | 2 | ND | | 5.0 ± 0.4b | |
| Pb | 3 | | | 2.2 ± 0.3c | 1.1 ± 0.4cd |
| | 4 | | | 2.2 ± 0.6c | 1.0 ± 0.1d |
| | 5 | | | 6.3 ± 0.6a | 1.3 ± 0.1cd |

Comparable means not followed by a common letter are significantly different ($P \leq 0.05$). Values expressed as µg $g^{-1}$ dry weight ± standard deviation; n=3.
Abreviations: NS (Normal Stem); FS (Flattened Stem); NFS (Normal Flowered Stem); UCM (Undifferentiated Cell Masses); ND (Not Detected).

Table 3. Mean values of Ca, Fe, Mg, Mn and Pb in the top layer of the soil (0-2 cm).

| | Station 1 | Station 2 | Station 3 | Station 4 | Station 5 |
|---|---|---|---|---|---|
| Ca | 2.91 ± 0.50a | 0.54 ± 0.02c | 0.47 ± 0.04c | 1.34 ± 0.06b | 3.28 ± 0.23a |
| Fe | 3775 ± 387c | 5892 ± 902bc | 5108 ± 581bc | 9458 ± 918a | 6167 ± 651b |
| Mg | 2799 ± 211a | 1558 ± 170b | 1834 ± 55.9b | 2038 ± 115b | 2698 ± 27.6a |
| Mn | 30.6 ± 3.0b | 18.6 ± 1.84c | 40 ± 9.37b | 141 ± 35.3a | 40.9 ± 15.7b |
| Pb | 16 ± 1.3c | 53.9 ± 10.0b | 87.5 ± 0.7b | 276 ± 13.4a | 250 ± 34.6a |

Comparable means not followed by a common letter are significantly different ($P \leq 0.05$); Average values expressed as $\mu g\ g^{-1}$ dry weight except for $Ca^{2+}$ (dry weight %) ± SD; n=3.

Another possible explanation for the low chlorophyll levels in flattened stems is related to the response to light. The exposition of an organ to sun or moderate shade may influence the chlorophyll content, especially for *S. junceum* leaflets, but not the stems (VALLADARES & al. 2003). In our case, the flattened stems were hidden in the centre of the shrub, in contrast to the undifferentiated cell masses, which were located at the top.

Lead was only detected in bidistilled water extract of undifferentiated cell masses sampled at Stations 2 (0.48 $\mu g$ Pb $g^{-1}$ dry weight) and 5 (1.4 $\mu g$ Pb $g^{-1}$). The bidistilled water extract gives the surface deposited Pb only (not the total uptake). These averages are 9.6% and 22.8% of the total detected there, which is much less than the 85% and 70% detected by TONG & FARRELL 1991 in pine needles and maple leaves, respectively.

A careful surveillance about the occurrence of similar cases in the same area or in others highways under the BRISA authority would be needed.

## Acknowledgements

The authors are grateful to BRISA and to Arq[a] A. C. ALMEIDA, Eng[a] M. J. SILVA (IICT), Eng[o] P. CAETANO and Profs. Dr. M. T. ANTUNES, J. PAIS and J. F. SANTOS OLIVEIRA (F.C.T), for unconditional support and Dr. K. CREGO (Arizona University) for manuscript review.

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Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 2007

Band/Volume: 47_1_2

Autor(en)/Author(s): Reboredo Fernando, Silvares Catarina

Artikel/Article: Fasciation Phenomena and Mineral Balance in Spartium junceum L. 123-132