

## Metal uptake by *Caryophyllaceae* species from metalliferous soils in northern Greece

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**Key words:** *Caryophyllaceae*, *Dianthus*, *Minuartia*, *Scleranthus*, *Silene*. – Metal uptake, metalliferous sites, northern Greece.

**Abstract:** Plant samples collected from 17 populations of 9 taxa of the genera *Dianthus*, *Minuartia*, *Scleranthus*, and *Silene* (*Caryophyllaceae*), growing in 14 metalliferous sites of N Greece, and surface soil samples from their growth area were analysed for Cu, Pb, Zn, Cd, Ni, Cr, Fe, Mn, Ca, Mg. Metal uptake varies considerably between the populations. A resistance mechanism excluding Cu and Pb from the aerial parts of the plants over a wide range of the soil concentrations up to a critical soil level appeared to exist, whereas Zn concentrations in plants were analogously related to those of the soil. *Scleranthus perennis* subsp. *perennis* showed the highest Cu concentration (205 µg/g), whereas *Minuartia* cf. *bulgarica* hyperaccumulated Pb (1175 µg/g). Ca concentrations in plants were in most cases much higher than those in soil, whereas the contrary was true for Mg. As a result the Ca/Mg ratio, which was in almost all cases lower than 1 in the soil, was much increased in the plants.

Soils characterized by toxic concentrations of metals (Ni, Cr, Co, Cu, Pb, Zn, Cd, Mg), such as in serpentine or mine areas, are also, among others, settled by *Caryophyllaceae* species. *Silene otites* CL. WIB., *S. maritima* WITH., *S. cobalticola* DUVIGN. & PLANCKE, *S. dioica* (L.) CLAIRV., *S. cucubalus* (L.) WIB., *Minuartia verna* (L.) HIERN, and *Viscaria alpina* G. DON have been reported as being resistant to metal toxicity (GAMS 1975; BAKER 1978a, b; BROOKS & al. 1980; BAKER & al. 1983; BABALONAS & REEVES 1988; VERKLEIJ & PRAST 1989; SIEGHARDT 1990; RUNE & WESTERBERGH 1992). Furthermore, *Minuartia verna* and *Silene cucubalus*, characterize the vegetation of metalliferous soils in Central Europe, which is classified by ERNST (1974) in the class *Violetea calaminariae* BR.–BL. & TX. 1943.

According to KRAUSE (1958), vascular plants of the families *Caryophyllaceae*, *Polypodiaceae*, *Gramineae*, *Leguminosae* (*Genisteae*), and *Ericaceae* growing in serpentine substrate generally show low values of Ca/Mg ratio, as well as low Ca concentrations, in their tissues. This allows them to grow in the hostile edaphic environment of serpentine soils, which are rich in Ni, Cr, and Mg and poor in Ca. KINZEL & WEBER (1982) support the idea that *Caryophyllaceae* species occupying serpentine substrate tolerate the hostile edaphic conditions by concentrating low

Ca quantities in their tissues and counteracting excess Mg in the form of oxalate salts (Oxalat-Typ).

The purpose of the present work, which is a part of a more extensive investigation in northern Greece (KONSTANTINOU 1992), is to gain further information on the behaviour of populations of the genera *Silene*, *Scleranthus*, *Dianthus* and *Minuartia* (*Caryophyllaceae*) growing on soils with varying mineralization.

### Material and methods

In the aerial part of plant specimens collected from 17 populations of nine taxa of the family *Caryophyllaceae*, growing in 14 metalliferous sites in northern Greece, the concentrations of the metals Cu, Pb, Zn, Cd, Cr, Ni, Fe, Mn, Ca, and Mg were measured and related to the soil total ones. The concentrations in plants were measured in mixed samples comprising 5–6 individuals whereas in soil concentrations were measured in surface samples (0–10 cm) collected from the area where the plant populations grew. The taxa analysed, the sites of collection and the main parent rocks associated with mineralization in each site are given in Table 1. Further details of the geological environment and the mineralization

Table 1. *Caryophyllaceae* taxa analysed and the sites of collection with their geological substrates. Abbreviations of the names and the collection sites are given in parentheses

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|   |
|---|
| <i>Silene bupleuroides</i> L. (Sb)  |
| (D) Doirani, 700 m south of the village at the “English Monument”. 21/6/1987.         |
| Rhyodacite-Trachytandesite  |
| <i>Silene compacta</i> FISCHER (Sc)   |
| (Kk) Kokkinolakkos, 2 km SW of Stratoni village. 12/7/1987. Amphibolites              |
| <i>Scleranthus perennis</i> L. subsp. <i>perennis</i> (Spp)                           |
| (Sk) Skuries, 3.5 km NE of M. Panagia village. 11/7/1987. Granite porphyry            |
| <i>Scleranthus perennis</i> L. subsp. <i>dichotomus</i> STOJ. & STEF. (Spd)           |
| (K) Kirki, 20 km NW of the town of Alexandroupolis at “King Arthur”. 3/6/1987.        |
| Volcanosedimentary formation  |
| <i>Dianthus pinifolius</i> SIBTH. & SM. subsp. <i>pinifolius</i> (Dpp)                |
| (Kk) Kokkinolakkos, 2 km SW of Stratoni village. 12/7/1987. Amphibolites              |
| <i>Dianthus pinifolius</i> SIBTH. & SM. <i>lilacinus</i> WETTST. (Dpl)                |
| (A) Akritas, 2.5 km SE of the village at the “Black Rock”. 21/6/1987. Rhyolite        |
| <i>Minuartia</i> cf. <i>bulgarica</i> GRAEBNER (Mb)                                   |
| (K) Kirki, 20 km NW of the town of Alexandroupolis at “King Arthur”. 3/6/1987.        |
| Volcanosedimentary formation  |
| <i>Minuartia hirsuta</i> HAND.-MAZZ. subsp. <i>falcata</i> MATTF. (Mhf)               |
| (V) Vathi, S and SE slope of the hill situated in the W of the village. 28/5/1987.    |
| Rhyodacite  |
| (Ak) Akropotamia, 1 km away from the village and 100 m S of the road                  |
| Akropotamia-Laodikino. 31/5/1986. Bimica gneisses                                     |
| (L) Laodikino, 2.5 km SW of the village. Bimica gneisses                              |
| (St) Stagira, at the “Aristotle statue” close to the village. 11/7/1987. Amphibolites |
| (Kk) Kokkinolakkos, 2 km SW of Stratoni village. 12/7/1987. Amphibolites              |
| (Kt) Kteni, 2 km N of the village. 17/6/1987. Dunite, harzburgite                     |
| (T) Triadi, 1 km E of the village. 11/6/1988. Dunite                                  |
| <i>Minuartia verna</i> HIERN subsp. <i>collina</i> HALLIDAY (MVC)                     |
| (G) Grammeni mine, 1.5 km E of Panorama village. 19/6/1987. Marbles                   |
| (Lg) Lagos, 1 km E of Granitis village. 17/6/1987. Marbles                            |
| (Vv) Vavdos, SW of the village at the mine area. 18/6/1988. Dunite, serpentinite      |

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are presented in KONSTANTINOY (1992). Specimens of all populations have been deposited in the Herbarium of the Aristotle University of Thessaloniki (TAU). The field work was carried out in 1986–1988.

The plant samples were washed thoroughly in tap-water and were air-dried. Then they were pulverized and oven-dried at 105 °C for 2 h. The soil samples were air-dried and passed through a 2 mm sieve. Before analysis the soils were ground gently with an agate pestle and mortar, passed through a 0.2 mm sieve and oven-dried at 105 °C for several hours (ALLEN & al. 1974, BROOKS 1983).

Elemental concentrations in 1 g of plant and 1 g of soil samples were measured in an Atomic Absorption Spectrophotometer (Perkin Elmer 2380) after wet digestion with a 4 : 1 mixture of nitric and perchloric acids. The analyses were carried out twice.

## Results and discussion

The analytical results (Table 2) show that overall generalisations about the behaviour of the populations studied are impossible because it varies considerably. However, it is obvious in many cases that the amount of metals in the soil affects their uptake by plants. For example, *Minuartia* populations, which show high or the highest concentrations of Cu (43–75 µg/g), Pb (29–1175 µg/g), Zn (260–1200 µg/g), Cr (20–24 µg/g), and Ni (19–37 µg/g), grew on soils with high or the highest contents of these metals. The same could be stated for the populations of *Silene* and *Scleranthus* for Cu, Pb, Zn, and Ni.

The behaviour of the two subspecies of *Scleranthus perennis* from the sites Skuries and Kirki differs from the one of the two subspecies of *Dianthus pinifolius* from Kokkinolakkos and Akritas. In *Scleranthus perennis*, the concentrations of Cu, Pb, Zn, Fe, and Mn are higher in the subspecies growing in the soil with highest concentration (Fig. 1). In contrast, in the two subspecies of *Dianthus pinifolius* this is obvious only for Cu and Zn, while the reverse is true for Pb, Fe, and Mn (Fig. 2).

**Pb.** Lead concentrations in all populations studied are less than 79 µg/g, when total Pb concentrations in the soil range between 31–19500 µg/g. The population of *Minuartia* cf. *bulgarica* from Kirki site, which shows hyperaccumulation of Pb (1175 µg/g), is the only interesting exception, considering that there are few reports in the literature of plant species hyperaccumulating Pb (REEVES & BROOKS 1983, KONSTANTINOY & BABALONAS 1992). This population needs further taxonomic research and it may well be a new ecotype or a new ecospecies according to the conception of TURESSON (1922 cited in ERNST 1974), adapted to the particular substrate of Kirki, which is rich in Cu, Pb, and Zn. It is well known that metal-rich soils may act as places of plant evolution (BOYD & MARTENS 1992). The Pb concentrations found in the other taxa analysed are lower than 79 µg/g and thus are higher than the normal range of Pb in plants (0.05–3 µg/g) given by ALLEN & al. (1974).

**Zn.** Our populations analysed from soils with normal Zn content (i.e., <300 µg/g, ALLEN & al. 1974) show concentrations lower than 100 µg/g, while others growing on soils with much higher Zn quantities (830–4400 µg/g) contain more than 100 µg/g up to 1200 µg/g. Correlation analysis for plant-soil relationships for each metal, carried out on the data (results not shown), gave only for Zn a highly significant correlation ( $r = 0,8936$ ,  $p < 0,001$ ).

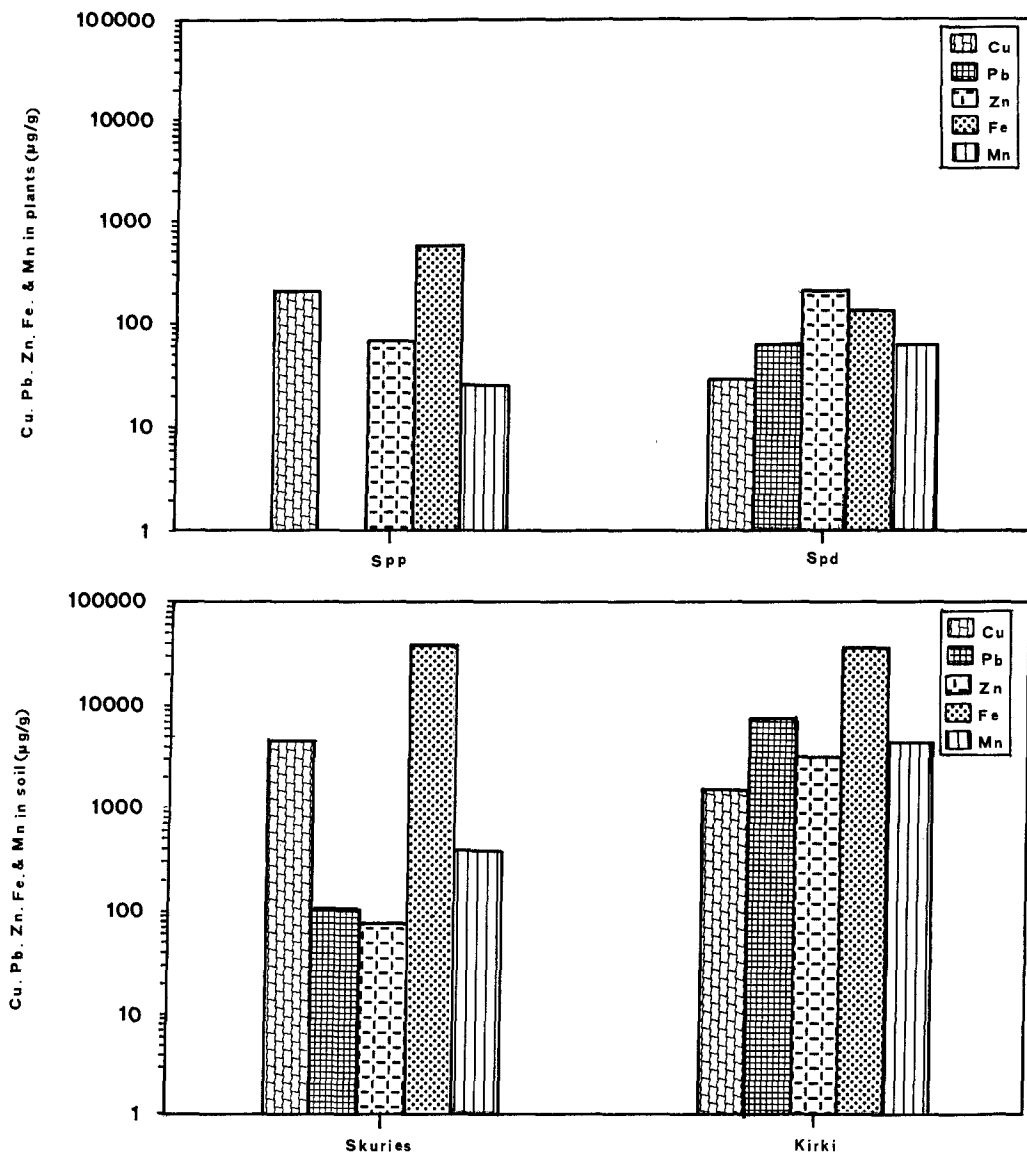


Fig. 1. Copper, lead, zinc, iron and manganese concentrations ( $\mu\text{g/g}$  d.w.) in the aerial parts of *Scleranthus perennis* subspecies and in the corresponding soils. For taxon abbreviations see Table 1

**Cu.** Among the *Caryophyllaceae* taxa analysed, *Scleranthus perennis* subsp. *perennis*, collected from the copper-rich substrate of Skuries, shows the highest Cu concentration ( $205 \mu\text{g/g}$ ). Similar Cu concentrations, reported for other species were considered as being abnormally high (MALAISSE & al. 1979, BROOKS & al. 1982, TIAGI & AERY 1986). Copper concentrations in all other plants analysed are lower than  $75 \mu\text{g/g}$  and lie either within the normal range of Cu ( $2.5\text{--}25 \mu\text{g/g}$ ) given by ALLEN & al. (1974) or are slightly higher, characterized as anomalous.

As Cu is an essential element for plant nutrition, the Cu content of most plants tends to be internally rather than externally regulated, so that concentrations in

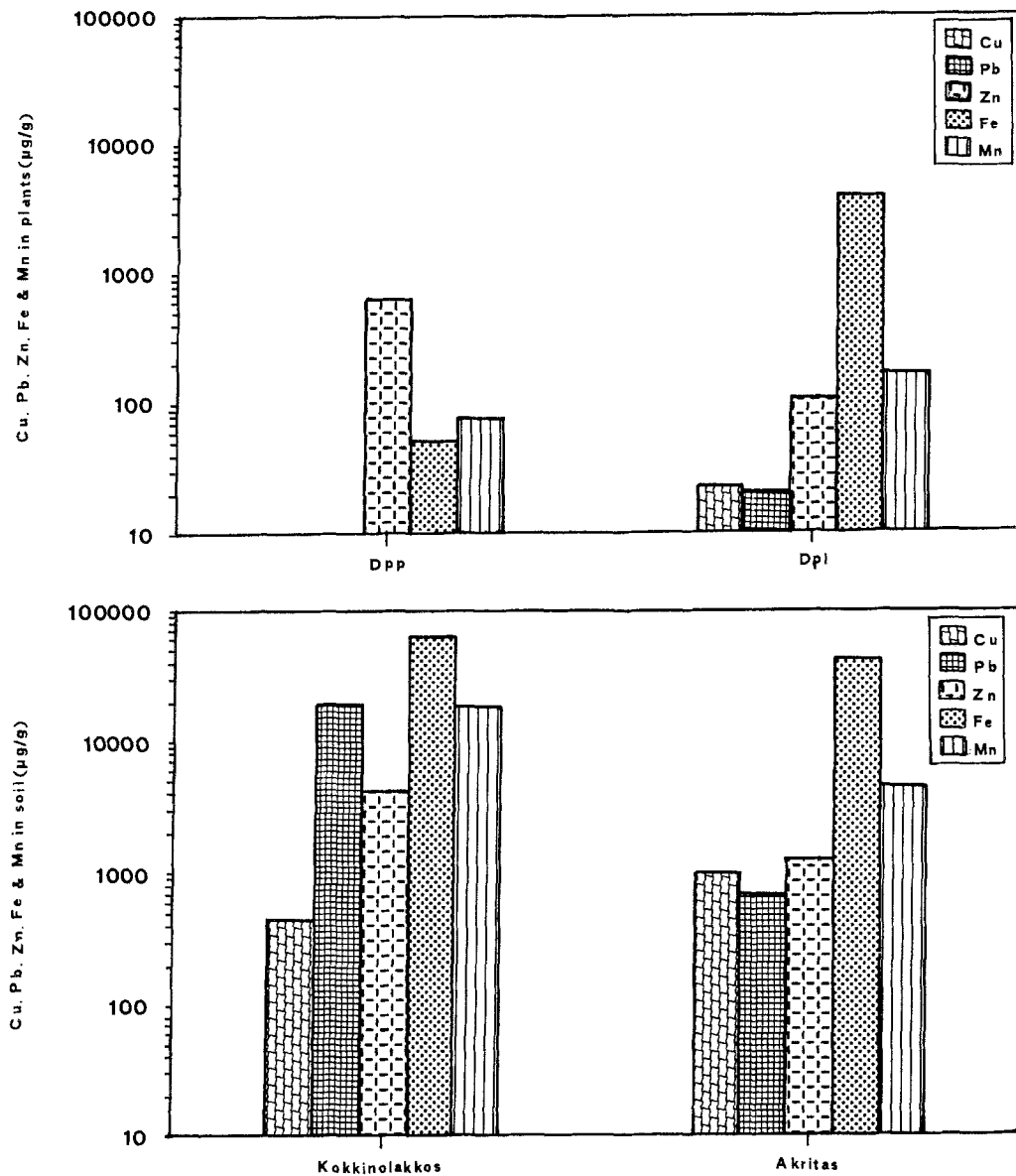


Fig. 2. Copper, lead, zinc, iron and manganese concentrations ( $\mu\text{g/g}$  d. w.) in the aerial parts of *Dianthus pinifolius* subspecies and in the corresponding soils. For taxon abbreviations see Table 1

plants remain low and relatively constant irrespective of soil content up to a critical soil level. When Cu concentrations in the soil exceed this critical level, the exclusion mechanism breaks down and unrestricted uptake of the metal takes place (SEVERNE & BROOKS 1972, MORRISON & al. 1979, BAKER & BROOKS 1989). Indeed, this is clearly shown in our *Caryophyllaceae* populations studied for Cu, although further research needs to be carried out for confirmation (Fig. 3). Copper concentrations in the soil must exceed ca.  $1000 \mu\text{g/g}$  in order that the plants contain Cu concentrations considerably higher than  $8 \mu\text{g/g}$ . A similar situation seems

Table 2. Elemental concentrations ( $\mu\text{g/g}$  d.w., bold print for soils, normal for plants) in the aerial parts of *Caryophyllaceae* taxa in relation to the concentrations in the soil. Numbers in parentheses refer to the number of soil samples analysed. (The atomic weights of the elements Ca and Mg were taken into account for the calculation of Ca/Mg ratio). For abbreviations see Table 1

| Site          | Species | Cu          | Pb           | Zn          | Cd        | Cr          | Ni          | Fe           | Mn           | Ca           | Mg           | Ca/Mg       |
|---------------|---------|-------------|--------------|-------------|-----------|-------------|-------------|--------------|--------------|--------------|--------------|-------------|
| <b>D (1)</b>  |         | <b>1219</b> | <b>34</b>    | <b>32</b>   | <b>-c</b> | <b>16</b>   | <b>11</b>   | <b>29200</b> | <b>122</b>   | <b>1072</b>  | <b>2325</b>  | <b>0,28</b> |
|               | sb      | 33          | -b           | 25          | -         | -d          | -e          | 389          | 49           | 10050        | 1957         | 3,12        |
| <b>Kk (1)</b> |         | <b>428</b>  | <b>7550</b>  | <b>2750</b> | <b>8</b>  | <b>306</b>  | <b>400</b>  | <b>53100</b> | <b>7550</b>  | <b>2670</b>  | <b>18750</b> | <b>0,09</b> |
|               | Sc      | 50          | 50           | 300         | -         | 8           | 14          | 322          | 210          | 6652         | 1620         | 2,49        |
| <b>Sk (4)</b> |         | <b>4549</b> | <b>106</b>   | <b>75</b>   | -         | <b>351</b>  | <b>265</b>  | <b>38462</b> | <b>387</b>   | <b>1366</b>  | <b>17250</b> | <b>0,05</b> |
|               | Spp     | 205         | -            | 66          | -         | -           | -           | 560          | 25           | 4927         | 1147         | 2,61        |
| <b>K (1)</b>  |         | <b>1500</b> | <b>7445</b>  | <b>3100</b> | <b>7</b>  | <b>32</b>   | <b>29</b>   | <b>35200</b> | <b>4350</b>  | <b>1627</b>  | <b>12450</b> | <b>0,08</b> |
|               | Spd     | 28          | 60           | 198         | -         | -           | -           | 130          | 60           | 5017         | 2550         | 1,19        |
| <b>Kk (1)</b> |         | <b>451</b>  | <b>19500</b> | <b>4250</b> | <b>26</b> | <b>85</b>   | <b>78</b>   | <b>63900</b> | <b>18400</b> | <b>3135</b>  | <b>7575</b>  | <b>0,25</b> |
|               | Dpp     | -           | -            | 640         | -         | -           | -           | 50           | 76           | 6075         | 682          | 5,40        |
| <b>A (1)</b>  |         | <b>1000</b> | <b>692</b>   | <b>1250</b> | <b>6</b>  | <b>35</b>   | <b>24</b>   | <b>42200</b> | <b>4500</b>  | <b>1687</b>  | <b>20250</b> | <b>0,05</b> |
|               | Dpl     | 23          | 20           | 104         | -         | -           | -           | 3840         | 166          | 12600        | 1102         | 6,94        |
| <b>K (1)</b>  |         | <b>1300</b> | <b>9750</b>  | <b>830</b>  | <b>4</b>  | <b>59</b>   | <b>40</b>   | <b>35500</b> | <b>2350</b>  | <b>1942</b>  | <b>9300</b>  | <b>0,13</b> |
|               | Mb      | 43          | 1175         | 260         | -         | 8           | -           | 930          | 119          | 12675        | 1800         | 4,27        |
| <b>V (1)</b>  |         | <b>4685</b> | <b>200</b>   | <b>43</b>   | -         | <b>9</b>    | <b>21</b>   | <b>24000</b> | <b>360</b>   | <b>1282</b>  | <b>1275</b>  | <b>0,61</b> |
|               | Mhf     | 74          | -            | 49          | -         | 11          | -           | 334          | 166          | 10725        | 1890         | 3,44        |
| <b>Ak (1)</b> |         | <b>6000</b> | <b>35</b>    | <b>55</b>   | -         | <b>28</b>   | <b>25</b>   | <b>35400</b> | <b>127</b>   | <b>3000</b>  | <b>3150</b>  | <b>0,58</b> |
|               | Mhf     | 75          | -            | 38          | -         | -           | -           | 426          | 109          | 12375        | 1597         | 4,70        |
| <b>L (1)</b>  |         | <b>29</b>   | <b>52</b>    | <b>91</b>   | -         | <b>25</b>   | <b>25</b>   | <b>18950</b> | <b>685</b>   | <b>4080</b>  | <b>2475</b>  | <b>1,00</b> |
|               | Mhf     | -           | -            | 50          | -         | 10          | -           | 377          | 160          | 18975        | 1417         | 8,13        |
| <b>St (1)</b> |         | <b>1855</b> | <b>14300</b> | <b>4400</b> | <b>6</b>  | <b>164</b>  | <b>79</b>   | <b>88500</b> | <b>5600</b>  | <b>2730</b>  | <b>11850</b> | <b>0,14</b> |
|               | Mhf     | -           | 29           | 1200        | -         | -           | -           | 49           | 23           | 7395         | 697          | 6,44        |
| <b>Kk (1)</b> |         | <b>428</b>  | <b>7550</b>  | <b>2750</b> | <b>8</b>  | <b>306</b>  | <b>400</b>  | <b>53100</b> | <b>7550</b>  | <b>2670</b>  | <b>18750</b> | <b>0,09</b> |
|               | Mhf     | -           | 79           | 990         | -         | 20          | 19          | 380          | 220          | 7800         | 1485         | 3,19        |
| <b>Kt (3)</b> |         | <b>30</b>   | <b>38</b>    | <b>63</b>   | -         | <b>1572</b> | <b>1923</b> | <b>76500</b> | <b>1145</b>  | <b>11275</b> | <b>81000</b> | <b>0,08</b> |
|               | Mhf     | -           | -            | 30          | -         | 24          | 37          | 1395         | 62           | 13500        | 2700         | 3,03        |
| <b>T (4)</b>  |         | <b>19</b>   | <b>61</b>    | <b>48</b>   | -         | <b>353</b>  | <b>1662</b> | <b>51200</b> | <b>850</b>   | <b>2552</b>  | <b>73537</b> | <b>0,02</b> |
|               | Mhf     | -           | -            | 19          | -         | -           | 23          | 385          | 44           | 17250        | 2175         | 4,81        |
| <b>G (1)</b>  |         | <b>44</b>   | <b>215</b>   | <b>285</b>  | <b>7</b>  | <b>93</b>   | <b>90</b>   | <b>33100</b> | <b>2300</b>  | <b>15300</b> | <b>8550</b>  | <b>1,08</b> |
|               | Mvc     | -           | -            | 15          | -         | -           | -           | 119          | 109          | 18225        | 877          | 12,61       |
| <b>Lg (1)</b> |         | <b>41</b>   | <b>758</b>   | <b>1550</b> | <b>16</b> | <b>79</b>   | <b>43</b>   | <b>43100</b> | <b>56500</b> | <b>74250</b> | <b>20250</b> | <b>4,22</b> |
|               | Mvc     | -           | -            | 35          | -         | -           | -           | 155          | 265          | 17250        | 2100         | 4,98        |
| <b>Vv (1)</b> |         | <b>8</b>    | <b>31</b>    | <b>21</b>   | -         | <b>233</b>  | <b>895</b>  | <b>29850</b> | <b>420</b>   | <b>33225</b> | <b>93000</b> | <b>0,22</b> |
|               | Mvc     | -           | -            | 16          | -         | -           | 25          | 622          | 24           | 13350        | 7650         | 1,06        |

-a : <8 $\mu\text{g/g}$  Cu, -b : <19 $\mu\text{g/g}$  Pb, -c : <3 $\mu\text{g/g}$  Cd, -d : <8 $\mu\text{g/g}$  Cr, -e : <14 $\mu\text{g/g}$  Ni

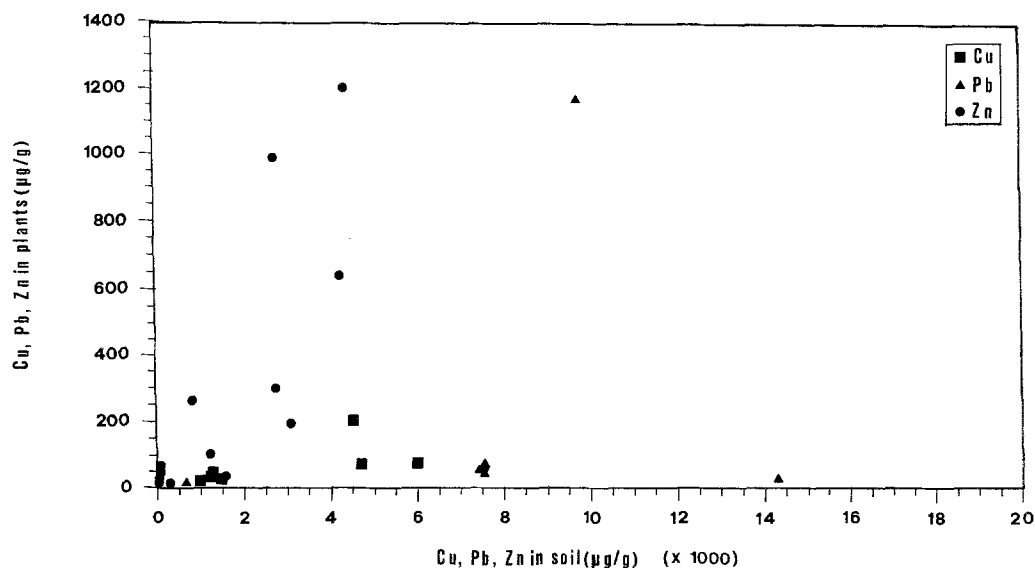


Fig. 3. Copper, lead and zinc concentrations ( $\mu\text{g/g}$  d.w.) in *Caryophyllaceae* expressed as a function of the corresponding concentrations in the soil (see also Table 2). Concentrations of Cu  $< 8 \mu\text{g/g}$  and Pb  $< 19 \mu\text{g/g}$  are not shown

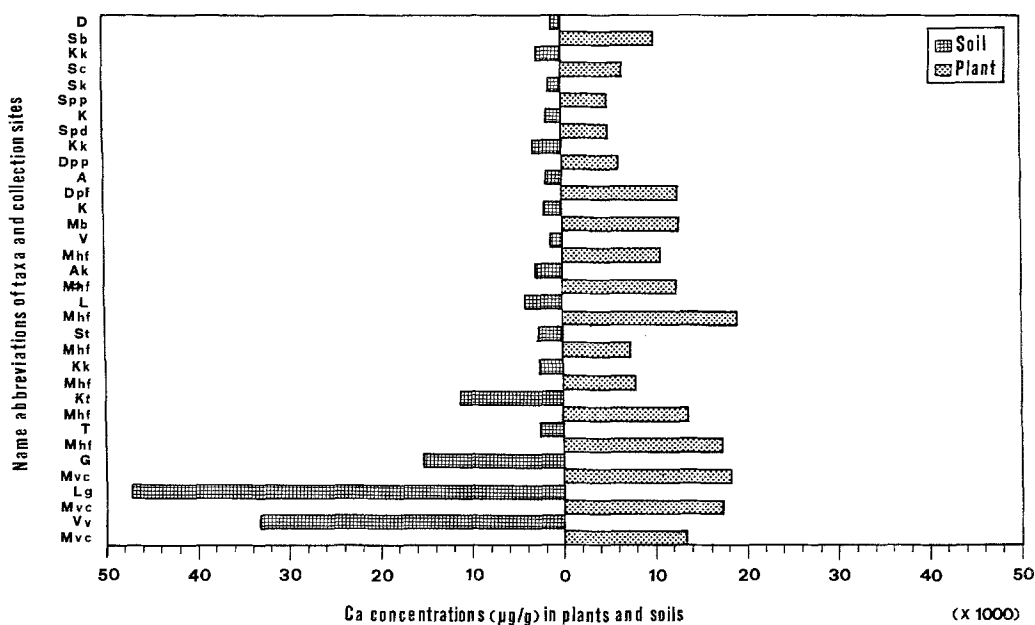


Fig. 4. Calcium concentrations ( $\mu\text{g/g}$  d.w.) in *Caryophyllaceae* and in the corresponding soils. For abbreviations used see Table 1

to exist for Pb. The critical soil level appears to be around  $700 \mu\text{g/g}$  for the plants to contain more than  $19 \mu\text{g/g}$  Pb. This is not true for Zn, which in all cases is readily taken up by the plants from the soil and is translocated to the aerial parts in varying degrees reflecting the soil concentrations (Fig. 3).

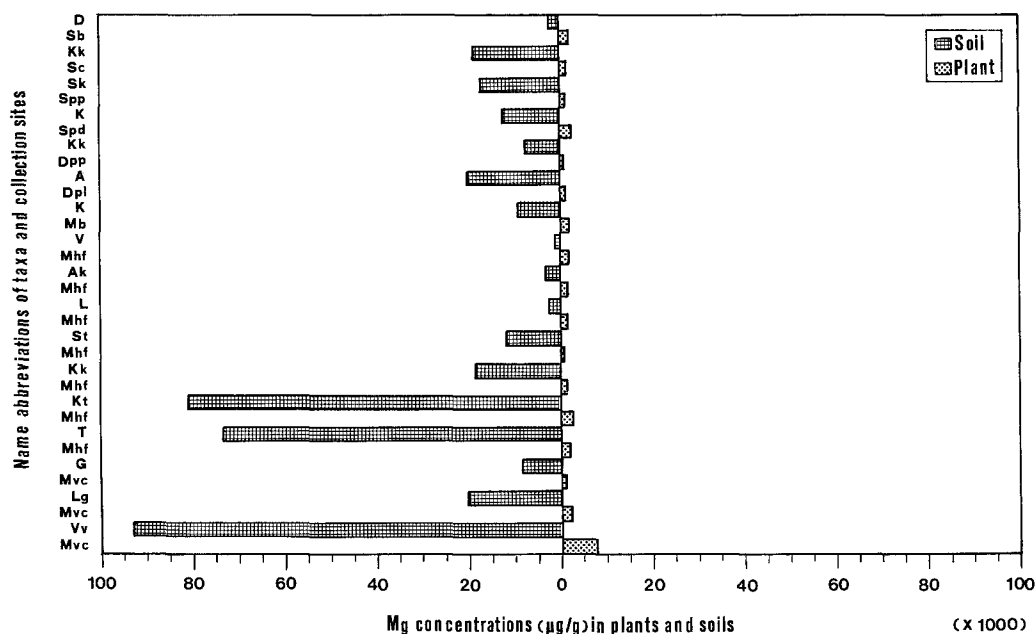


Fig. 5. Magnesium concentrations ( $\mu\text{g/g}$  d.w.) in *Caryophyllaceae* and in the corresponding soils. For abbreviations used see Table 1

Our results agree with the suggestion of BAKER & al. (1983) that resistance mechanisms (BAKER & al. 1983 are using the term tolerance mechanism) involving exclusion of heavy metals demonstrated in metal resistant races of various *Silene* species are also important in the resistance of other members of the *Caryophyllaceae*.

**Cd, Cr, Ni, Mn.** Cadmium concentrations in the plants are below the sensitivity limits of A.A.S. ( $3 \mu\text{g/g}$ ) and those of Cr, Ni, and Mn are lower than  $24 \mu\text{g/g}$ ,  $37 \mu\text{g/g}$ , and  $265 \mu\text{g/g}$ , respectively (Table 2).

**Fe.** Concentrations of Fe ( $49$ – $560 \mu\text{g/g}$ ) in the plants are within the normal range ( $40$ – $500 \mu\text{g/g}$ ) given by ALLEN & al. (1974) except for *Dianthus pinifolius* subsp. *lilacinus* ( $3840 \mu\text{g/g}$ ), *Minuartia hirsuta* subsp. *falcata* ( $1395 \mu\text{g/g}$ ) from Kteni, *Minuartia* cf. *bulgarica* ( $930 \mu\text{g/g}$ ) and *Minuartia verna* subsp. *collina* ( $622 \mu\text{g/g}$ ) from Vavdos. Iron concentrations of the first two taxa are outside the normal range ( $>1000 \mu\text{g/g}$ ) given by RITTER-STUDNICKA & DURSUN-GROM (1973).

**Ca, Mg.** Most noteworthy is the behaviour of the *Caryophyllaceae* populations studied in the Ca and Mg uptake. According to KRAUSE (1958) and KINZEL & WEBER (1982), *Caryophyllaceae* species growing on serpentine soils show low Ca concentrations and low values of Ca/Mg ratio. In the present analysis where the samples come from various metalliferous soils, Ca concentrations in the plants are much higher than those of the soil, except for *Minuartia verna* subsp. *collina* from Lagos and Vavdos. The contrary is true for Mg (Figs. 4, 5). As a result the Ca/Mg ratio, which is in most cases lower than 1 in the soil, is much higher in the plants and varies from 1.08 to 12.61 (Table 2). Similar observations were made for various *Silene* species from serpentine soils by BABALONAS & REEVES (1988), as well as



for other species from metalliferous (non serpentine) soils (BABALONAS & al. 1987). The presence of increased levels of Ca in plants may well be related to the involvement of Ca in resistance against heavy metal toxicity and also against Mg toxicity at high Mg concentrations (e.g., Kteni, Triadi, Lagos, Vavdos). It is known that excess Mg is a factor limiting plant growth (KINZEL & WEBER 1982).

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