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The Ecology of Plant Populations Growing on Serpentine Soils

II. Ca/Mg Ratio and the Cr, Fe, Ni, Co Concentrations as Development Factors of *Buxus sempervirens* L.

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Summary

KARATAGLIS S., BABALONAS D. & KABASAKALIS B. 1982. The ecology of plant populations growing on serpentine soils. II. Ca/Mg ratio and the Cr, Fe, Ni, Co concentrations as development factors of *Buxus sempervirens* L. — Phyton (Austria) 22 (2): 317—327. — English with German summary.

The ecotype of the *Buxus sempervirens* plant growing on ophiolithic soils of the chromium mine in Skoumtsa (NW-Macedonia) has been studied and the following have been established.

Soils of the area studied differ from the adjacent non-ophiolithic ones as far as their content in Ca, Mg, Cr, Fe and Ni is concerned. Vegetation occurring on this site is rather poor, while certain species absenting from the neighbouring non-serpentine regions do take part in its composition. Although chromium is found to exist in great quantities in the soil, those on the leaves are hardly traceable, a fact attributed to the low "availability" very small chromium amounts present in the plant. In comparison with other plant species *Buxus sempervirens* does not engage large amounts of heavy toxic metals (Cr, Ni, Co, Fe) except Mg. The ability the plant has to accumulate very large quantities of Ca from those existing in the soil, may constitute one of the probable physiological mechanisms the plant develops in order to compensate the toxic action of these heavy metals.

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Zusammenfassung

KARATAGLIS S., BABALONAS D. & KABASAKALIS B. 1982. Die Ökologie von Pflanzenpopulationen auf Serpentinböden. II. Das Ca/Mg-Verhältnis und Cr, Fe, Ni und Co als Entwicklungsfaktoren von *Buxus sempervirens* L. — Phyton (Austria) 22 (2): 317—327. — Englisch mit deutscher Zusammenfassung.

Ein Ökotyp von *Buxus sempervirens* auf Serpentinböden der Chrom-Mine Skoumtsa (NW-Mazedonien) wurde untersucht. Die Böden des Untersuchungsgebietes unterscheiden sich von den umliegenden nicht serpentinischen Böden hinsichtlich ihres Ca-, Mg-, Cr-, Fe- und Ni-Gehaltes. Die Vegetation auf diesen ist ziemlich arm, hingegen beteiligen sich Pflanzen, die auf den umliegenden nicht serpentinischen Böden fehlen, an ihrer Zusammensetzung. Obwohl Chrom im Boden in großer Menge vorhanden ist, kann es in den Pflanzen nur schwer nachgewiesen werden, was einer nur geringen Menge für die Pflanze verfügbaren Chroms zugeschrieben wird. Im Gegensatz zu anderen Pflanzen nimmt *Buxus sempervirens* keine großen Mengen von Schwermetallen (Cr, Ni, Co und Fe) auf, dies gilt aber nicht für Mg. Die Fähigkeit der Pflanzen, große Mengen Ca aus dem Boden aufzunehmen könnte einen physiologischen Mechanismus darstellen, den die Pflanze entwickelt hat, die toxische Wirkung solcher Metalle zu kompensieren.

(Editor transl.)

Introduction

The nature, form and composition of the soil in combination with water deficiency are undoubtedly some of the most restrictive factors concerning plant spreading and growth. The fact that it is possible for different types of soil to exist in neighbouring areas, more usually makes up one of the most basic factors of vegetation boundary setting thus rendering the landscape a somewhat peculiar aspect (KRAUSE 1958, KRUCKEBERG 1951, LYON *et al.* 1971).

Soils deriving mainly from hyperbasic-ophiolithic rocks actually present a characteristic aspect in their vegetation. Such serpentine and generally hyperbasic soils known from the references (RUNE 1953, WALKER 1954, KRUCKEBERG 1954, WALKER *et al.* 1955, SOANE & SAUNDER 1959, SPENCE & MILLAR 1963, SAROSIEK 1964, PARIBOK & ALEXEYEVA-POPOVA 1966, LYON *et al.* 1968, SHEWRY & PETERSON 1975, 1976) as agriculturally barren or problematic exist in many parts of the world including Greece (MARATOS 1972). These soils have some common characteristics which determine the form of the adjacent vegetation and are the following:

a) They have high Mg content in comparison with Ca.

b) They contain large quantities of heavy metals such as Ni, Cr and Co.

c) They are poor in basic nutrient elements such as nitrogen, phosphoros and potassium.

These odd characteristics contribute to making soils inappropriate for the normal growth of many plant species. As a consequence, the vegetation growing on these soils is mostly scattered, dwarfish, xerophytic and many times species from the neighbouring nonserpentine areas take part in its composition. The sterility of these soils is characterized as serpentine unproductiveness (Jackson 1964).

The main target of this study is to check whether the *Buxus sempervirens* L. individuals growing on ophiolithic soils take up the toxic metals Mg, Ni, Cr, Co etc. in relatively high quantities and to compare these quantities of toxic metals with the existing amount of Ca in the soil, and with that engaged by the plant. We have therefore chosen a characteristic ophiolithic area, its vegetation is sparse, bushy and with different aspect in connexion with the neighbouring areas. This region lies to the west of the village "Chromio" in Western Macedonia. It is the area of the chromium mine of Skoumtsa found at an altitude of about 1000 m. The parent rock of this area is Dunite on which soil material of relatively small thickness (20—30 cm) has developed.

Materials and Methods

The plant material used in our study was collected on a site by the name of Dry Field, near the mine where an extremely superficial occurrence of chromite could be detected. *Buxus sempervirens* was used as experimental material mainly for two reasons: a) It was one of the most dominant species with yellow-brown coloured leaves, and b) the root system of the individuals in which we were more interested was found lying within the surface vein of one meter width and on its both sides as well.

Leaves showing a yellow to dark green colour were selected from the *Buxus sempervirens* shrub and from individuals rooted on the chromite vein. Soil was also collected being in contact with the upper part of the root system (0—15 cm). Relative samplings were performed in turn, on both sides of the vein and vertically. Each sampling place was about 10 m apart from the next one or its preceding adjacent one.

Analytical procedures

All plant material was washed with de-ionized water, dried overnight at 40° C and ashed in a low temperature RF furnace (L. T. A.-302 (2), L. F. E Corporation), (Gleit 1963). This procedure was found very convenient for ashing plant material for its low temperature making obsolete all previous oxidation techniques. The low temperature ashing equipment enables the dry removal of organic materials while retaining the inorganic elements and without the possibility of great quantities reagents contamination. Hence, analyses for trace elements are not only simplified, but we believe that they can be purged to a greater degree of analytical accuracy and stability.

The ash was treated with 6 ml 50% HNO_3 and heated on a sand-bath to about 120° C nearly to dryness, according to the procedure given by Jackson (1962), then 2 ml 6N HCl were added and followed by heating to dryness. Finally, the residue was dissolved in 5 ml 6N HCl and the solution diluted as appropriate.

Chromium, Nickel, Cobalt, Calcium and Magnesium were determined using anatomic absorption spectrophotometer (Perkin Elmer model 503).

Soil samples were air-dried, sub-sampled, ground with pestle and mortar and passed through a 2-mm nylon sieve. Total chemical analysis of soils was carried out by Na_2O_2 fusion in pure nickel crucibles. Nickel constituent of soils was determined in separate soil sample after digestion with boiling conc. HCl (2h) and boiling conc. HNO_3 (30 min.), until only a residue of silica remained. pH measurements were made in soil-water paste.

The macro- and micro-elements present in soil samples were determined using the atomic absorption spectrophotometer as mentioned above after appropriate extraction or dissolution, respectively.

Results

Physiognomy of the vegetation

Vegetation physiognomy was quite typical. On the whole, the vegetation was not dense leaving more than 50% of the uncovered area. The stunted development of some plant species (*Quercus coccifera*, *Potentilla detomasii* *Rumex acetosella* etc.) was macroscopically evident. In addition, the dwarfish of certain species and the yellow to dark green colour on the leaves as well was also obvious when referring to known and widely spread species.

The floral structure of vegetation on the sample site and covering an extent of 40 m^2 is scheduled in Table 1.

Quercus pubescens was scattered all around the area while its individuals bore an average size, which did not account for the senile symptoms the species presented in many cases. *Juniperus oxycedrus* was not observed to have a developed central trunk in all the area. On the contrary, it had the typical form of a shrub with overhanging branches.

Measurements of Ca, Mg on the soil and in plants

Table 2 depicts the average concentration of Ca and Mg in soil samples of the investigated area. For the benefit of comparison, the same table also presents results of analyses of other workers produced from several regions. In both cases the total amount of Mg found on these soils is appreciably high, while the Ca concentrations of all samples were at low levels.

The Ca and Mg values for the Greenhill, (Aberdeenshire), Kraubath (Austria) areas, of the Unst islands (Shetland) and the Maryland (Ireland) regions, when compared to the five different measurements of the Chromio Kozani site (Greece) were found to be generally lower. The Mg and Ca values of our five different measurements appeared to be somewhat uniform. An exception to this is the position D, in which Ca was found to be in double content than in all the other positions.

The Ca and Mg contents of the *Buxus sempervirens* leaves are given in Table 3. It is apparent that the total amounts of these elements and the Ca/Mg ratio vary in several positions. The Ca amounts on the leaves of the plant were up to 10 times higher than those in the soil. It was also seen that despite the very high content of Mg in the soil against Ca, an inversion was observed on the leaves with the result that Ca was found in double quantity than that of Mg.

Table 1

Floristic composition of the studied area (40 m^2)

Quercus pubescens WILLD.	+.1
Juniperus oxycedrus L.	1.2
Buxus sempervirens L.	1.2
Melica ciliata L.	+.1
Koeleria macrantha (LEDEB.) SCHULTES	1.1
Hypericum rumeliacum BOISS.	1.1
Fumana bonabartei MAIRE & PETIMENGIN	1.2
Thymus teucrioides BOISS & SPRUNER	1.2
Convolvulus boissieri STREUDEL subsp. *compactus* (BOISS) STACE.	1.1
Festuca sp.	1.1
Draba lasiocarpa RAHEL	1.1
Alyssum montanum L. subsp. *montanum*	1.1
Sedum sartorianum BOISS.	1.1
Thlaspi praecox WULFEN	1.1
Carex humilis LEYSSER	1.2
Minuartia verna (L.) HIERN.	1.1
Anthemis orientalis (L.) DEGEN	+.1
Bromus racemosus L.	+.1
Linum austriacum L.	+.1
Helianthemum nummularium (L.) MILLER	+.1
Acinos alpinus (L.) MOENCH	+.1
Teucrium montanum L.	+.1
Aethionema saxatile (L.) R. BR.	+.1
Iris sp.	+.1
Sedum caespitosum DC.	+.1
Medicago sp.	+.1
Hieracium sp.	+.1
Poa perconcinna J. R. EDMON.	+.1
Centaurea sp.	+.1
Alyssum sp.	+.1
Chrysopogon gryllus IRIN.	+.1
Veronica austriaca L.	+.1
Rumex acetosella L.	+.1
Stachys scardica (GRISEB.) HAYEK	+.1
Scorzonera sp.	r.1
Armeria canescens (HOST.) BOISS.	r.1

Measurements of Ni, Cr, Co and Fe on the soil and plants

The various Ni values deriving from the soil and leaves analyses of different plants are scheduled in Table 4. The content of Ni in different positions of the Chromio area is at high levels thus making these soils toxic enough for plant vegetation. Despite the fact that the Ni quantities did not present any significant differences in comparison with the soil content of other sites, the *Buxus sempervirens* plants, however, deposited no big amounts on leaves, as was the case with the *Homalium, Hybanthus* and *Pimelea* plants which accumulated much larger quantities than those of their soil (= hyperaccumulators). The Ni amounts observed on the *Buxus* leaves, although relatively smaller, were nevertheless at levels higher than normal.

Table 2

Ca and Mg in serpentine soils (ppm on dry weight basis)

Places	Mg	Ca	Ca/Mg	pH
1) Greenhill, Aberdeenshire	127.000	1090	0.008	
2) Keen of Hamar, Unst	168.000	680	0.004	
3) Dalepark, Unst	137.500	1040	0.008	
4) Kraubath, Austria	159.000	1285	0.008	
5) Dublin, Maryland	77.760	1400	0.018	
6) *New Caledonia (5—15-in layer)*	360	350	0.972	
A Chromio Kozani, Greece	193.015	1071	0.006	6,3
B Chromio Kozani, Greece	172.297	1151	0.006	5,9
C Chromio Kozani, Greece	187.500	1103	0.006	6,0
D Chromio Kozani, Greece	176.056	3080	0.024	5,9
E Chromio Kozani, Greece	126.812	1408	0.008	6,1

1), 6): after Brooks *et al.* 1974; 2), 3): after Shewry & Peterson 1975; 4): after Ritter-Studnička &Dursun — Grom 1973; 5): after Robinson *et al.* (1935)

Table 3

Ca and Mg in *Buxus sempervirens* leaves (ppm on dry weight basis)

Position	Ca	Mg	Ca/Mg
A	8.340	2.601	3,21
B	10.234	4.296	2,40
C	10.607	6.699	1,58
D	9.055	4.478	2,02
E	14.612	7.255	2,01

As for Cr, its content in the soil is quite high (Tab. 4). If we compare the Cr levels found in the Chromio area with those in other regions, we may see that they are much higher. Yet, the amount taken up by the plant is relatively small and even smaller than 10 ppm in all occasions we have checked. On the contrary, in materials used by other researchers and given for comparison we may detect higher levels in the leaves, although the levels in the soils were relatively lower than those obtained by our measurements.

The soil contents in Co did not excede the 200 ppm in neither of our analyses, whereas those produced by the other workers concerning serpentine soils were two or three times higher. The amounts taken up by the *Buxus sempervirens* plant and deposited on its leaves were relatively low (see Table 4).

Another characteristic of the soil of the studied site is its high content in Fe (from 6.607 ppm to 8.272 ppm). However, the quantities found on the plant ranged within normal levels (Table 4). According to Ritter-Studnička & Dursun-Grom (1973) the contents of Fe in plants should excede 1000 ppm if they are to be considered as non-physiological, something shown by comparative measurements in serpentine and non-serpentine soils. Consequently, with *Buxus sempervirens* as our base, we could not characterize the Fe content of the soils of the region as one of the toxically acting factors which shape the physiognomy of vegetation.

Discussion

It is generally accepted nowadays that serpentine soils contain high concentrations of Mg and, equally probable, relatively smaller amounts of other toxic metals such as Ni, Cr or Co to be present while the Ca concentrations are much deficient (Walker 1954, Walker *et al.* 1955). Subsequently these concentrations of the toxic metals and the Ca deficiency in the soil somehow influence increase, development and spread of plant in serpentine soils. Thus, there are some plant species having the ability not only to thrive on such unfavourable soil conditions, but also to develop certain mechanisms so that they accumulate several metals in quantities which greatly exceed the corresponding soil ones (see Table 4), (Rune 1953, Proctor 1971, Brooks *et al.* 1974).

The Ca and Mg levels found in the soil where *Buxus sempervirens* grows are almost similar to the corresponding soil levels from other areas (Robinson *et al.* 1935, Birrell & Wright 1945, Shewry and Peterson 1975). Now while the Ca amounts in the soil are small, the ones taken up by the plant are much larger as it was confirmed by Walker (1954), Walker *et al.* (1955), Brooks *et al.* (1974). It seems likely that *Buxus sempervirens* has probably developed some mechanism which permits an excessive Ca amount absenting from the soil to be engaged by the plant. If we combine these

Table 4

Ni, Co, Cr and Fe in several serpentine soils and in the leaves of different plants growing on them
(Results in ppm on dry weight basis)

	Ni		Co		Cr		Fe				
	Soil	Leaves	Soil	Leaves	Soil	Leaves	Soil	Leaves	pH	Plant species	Place of origin
1.	4.900	78.800	540	420	29.640	120				*Homalium kanaliense*	New Caledonia
2.	8.000	225.000	620	270	21.280	360				*Hybanthus austro-caledonicus*	New Caledonia
3.	3.500	5.900	400	95	1.600	150				*Pimelea suteri*	New Zealand
4.	2.000	25	—	—	4.000	10				*Agrostis canina*	Green Hill, Aberdeenshire
5.	— *)	10	—	—	— *)	10				*Agrostis stolonifera*	South of Rönnbäckshön, Sweden
6.	100	5	—	—	150	10				*Agrostis stolonifera*	Oxford (calcareous soil)
A.	3.660	44	179	16	23.814	6.0	6.607	210.5	6.3	*Buxus sempervirens*	Chromio Kozani, Greece
B.	3.540	48	165	39	35.294	5.8	7.155	125.5	5.9	*Buxus sempervirens*	Chromio Kozani, Greece
C.	4.210	52	191	15	88.470	7.4	8.272	137.7	6.0	*Buxus sempervirens*	Chromio Kozani, Greece
D.	3.080	28	174	59	12.790	7.9	7.246	147.6	5.9	*Buxus sempervirens*	Chromio Kozani, Greece
E.	3.345	41	173	29	13.864	6.1	7.747	143.1	6.1	*Buxus sempervirens*	Chromio Kozani, Greece

1—4 after Brooks *et al.* 1974; 5, 6 after Proctor 1971.

*) Serpentine rocks near the area of collection are reported to have a high heavy metal content by Rune (1953)

findings with the fact that Ca is one of the elements contributing to the inhibition of the heavy metals toxic action (JOWETT 1958, 1964, MCNEILLY & BRADSHAW 1968, PROCTOR 1971, KARATAGLIS (in press)), we may then conclude the following. It is possible that the plant engages an excessive amount of Ca in order to compensate the toxic action of various toxic metals and mainly of Mg since that is the metal being taken up in great quantities.

Some researchers have referred to the total content of Ni and Cr on serpentine soils in various parts of the world. A selection of some results is set out in Table 4. This Table also shows that large Ni, and Cr amounts and small ones of Co were found on the soils of the area studied. The Ni and Cr quantities are slightly higher than those described by PROCTOR & WOODELL (1971), BROOKS *et al.* (1974) and SHEWRY & PETERSON (1976).

The total amount of Cr on the soil of the Chromio Kozani areas was, on the average, twice as much as that of Ni. Nevertheless, the amounts of Ni taken up by the plant were about 12 times larger than those of the corresponding Cr ones, while the Cr amounts were hardly traceable. This can be probably attributed to the fact that only a small Cr amount is found to be available for the plant, while its greater amount bears the form of undissolved forms which are not engaged by it. On the contrary, although Ni is found in lower but always toxic for the plant concentrations, it is taken up in larger quantities than Cr. However, it is undoubtful that the Ni engaged amounts are very low in connexion with other plants (see Table 4). This may well be correlated with the pH value of the soil since it is known that values over 5,6 do not favour Ni uptake (MISHRA and KAR 1974).

As for Co, the amounts found in the soil and those taken up by the plant are at low levels and consequently their toxic effects are almost imperceptible.

Ultimately, the amount of Fe uptaken by the plant is on physiological levels, although high amounts of Fe were located in the soil.

From Tables 3 and 4 we may conclude that several plant ecotypes of serpentine soils characteristically take up different amounts of toxic metals. Based on such an observation we may state that different survival mechanisms have been developed by different plant species.

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