EFFECT OF SOIL CONTAMINATION WITH FLUORINE ON THE CONTENTS OF CALCIUM AND MAGNESIUM IN THE BIOMASS OF CROP PLANTS

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ABSTRACT: The aim of this study was to determine the effect of soil contamination with fluorine, analyzed with the addition of neutralizing substances such as lime, charcoal, and loam, on the contents of calcium and magnesium in eight species of crop plants. Pot experiments were carried out in the years 2009–2011 at the vegetation hall of the University of Warmia and Mazury in Olsztyn, Poland. The study used brown soil taken from the topsoil with the granulometric composition of loamy sand. In the experiments, two factors were considered: (i) the increasing contamination of soil with fluorine in a form of potassium fluoride and (ii) substances neutralizing the soil contamination with fluorine. The increasing contamination of soil with fluorine had a significant effect on the contents of calcium and magnesium in all species and organs of the tested plants. In general, it resulted in a reduction in the contents of calcium and magnesium, with the lowest contents of both analyzed macroelements found in the objects to which fluorine had been introduced in the largest quantity. The calcium content in the tested plants ranged on average from 0.7 g Ca/kg dw in the grain of spring triticale to 23.7 g Ca/kg dw in the above-ground biomass of black radish. In turn, the highest magnesium content was found in the roots of phacelia, on average, 7.0 g Mg/kg dw, and the lowest one in the roots of spring triticale, on average, 0.8 g Mg/kg dw. The above-ground parts of the plants concerned contained more magnesium than their roots. The neutralizing substances added to the substrate in order to inactivate the adverse effects of fluorine on the plants had a positive effect on the contents of calcium and magnesium in the plants. Lime had the most positive effect on the contents of both elements, then loam followed by charcoal.

Keywords: Calcium; Charcoal; Crop plants; Lime; Loam; Magnesium; Soil contamination with fluorine.

INTRODUCTION

Fluorine is one of the elements that are widespread in nature and it occupies the thirteenth position in the ranking of abundance in the lithosphere.[1] The presence of fluorine in the natural environment is associated with the processes of weathering of minerals rich in this element, volcanic eruptions, and the release of fluorine from the surfaces of seas and oceans.[2] Human industrial activity is also responsible for the contamination of the natural environment with fluorine compounds, including primarily aluminum metallurgy, manufacturing of phosphorus fertilizers, and the operation of ceramic plants, glass-making plants, and brickyards.[3]

Fluorine contained in the soil is taken up by the roots of plants, and transported to their above-ground parts, where it is accumulated.[4]

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Bioavailability of fluorine to plants depends on, *inter alia*, the type and pH of the soil, and the content of other elements, e.g., calcium, aluminum, and phosphorus, in the substrate. According to Romar et al., in soils rich in calcium, fluorine is bound by the soil into insoluble compounds such as CaF_2 or apatite compounds with a similar composition, thus reducing the bioavailability of fluorine to plants.[6] In turn, Kau et al. demonstrated that fluorine exhibits considerable affinity to aluminum present in loam, which is easily bound by this mineral through the anion exchange; in addition, fluorine is bound by magnesium and calcium and is present in considerable quantities in this material in sparingly soluble compounds.[7-9]

The effects of fluorine on plants through its impact on seed germination,[10,11] the amount of biomass produced,[12] the content of chlorophyll,[13] and the activity of cellular enzymes,[14] are well documented in the scientific literature.

On the other hand, there are few studies concerning the effects of fluorine on the uptake of particular nutrients from the soil, and the development of the contents thereof in plants.

Therefore, a study was undertaken in order to determine the effects of soil contamination with fluorine on the contents of calcium and magnesium in eight species of crop plants under the conditions of the use of various fluorine-inactivating substances, namely lime, charcoal, and loam.

MATERIAL AND METHODS

The study was carried out based on eight pot experiments conducted in the years 2009–2011 at the vegetation hall of the University of Warmia and Mazury in Olsztyn, Poland. The experiments used a topsoil layer of brown soil with a granulometric composition of loamy sand. The pH of the used soil in H_2O was 5.89; in 1 mol KCl/dm^3 it was 4.43, and the hydrolytic acidity was 30.7 mmol/kg.

The characteristics of the basic chemical properties of the neutralizing substances used in the experiments are presented in Table 1.

Table 1. Chemical composition of the substances used for the inactivation of fluorine

Neutralizing substance	Element (g/kg dry weight)					
	Fluorine (total)	Phosphorus	Potassium	Magnesium	Calcium	Sodium
Lime (CaO)	0.50	0.12	0.75	2.55	339.21	0.09
Charcoal	2.00	0.71	9.30	2.62	7.33	0.79
Loam	0.088	0.40	21.0	17.7	23.92	7.99

The content of assimilable ingredients in the soil material used was as follows: phosphorus: 43.2 mg P/kg dw of soil, potassium: 124.5 mg K/kg dw of soil, and magnesium: 30.0 mg Mg/kg dw of soil. The following contents were also determined in the soil: organic carbon: 6.0g/kg dw of soil, total nitrogen: 0.62g/kg dw of soil, and total fluorine: 125mg/kg dw of soil. For the inactivation of fluorine in the soil, lime, charcoal, and loam were used.

The test plants included: maize (*Zea mays* L.); yellow lupin (*Lupinus luteus* L.*)*; winter rape (*Brassica napus* L.); spring triticale (*Triticosecale wittm.*); narrow-leafed lupin (*Lupinus angustifolius* L.); black radish (*Raphanus sativus*); phacelia (*Phacelia juss.)*; and lucerne (*Medicago sativa* L.).

For seven species, the amount of the biomass of both the above-ground parts and the roots was determined, while for lucerne, only the amount of the biomass of the above-ground parts following the 1st mowing was measured.

In the experiments, two factors were considered. The factor of the first order included the increasing contamination of soil with fluorine in a form of potassium fluoride (the commercial form), which was used by means of simulation to contaminate the soil, while the second factor included the comparison of three substances for neutralizing the soil contamination with fluorine.

Depending on the sensitivity of the tested plants, the values for the contamination of soil with fluorine amounted to:

• for sensitive plants, i.e., narrow-leafed lupin: 0, 20, 40, and 60 mg F/kg of soil

• for medium-sensitive plants, i.e., lucerne: 0, 50, 100, and 150 mg F/kg of soil

• for low-sensitive plants, i.e., maize, winter rape, spring triticale, black radish, and phacelia: 0, 100, 200, and 300 mg F/kg of soil.

The selection of doses was guided by the average content of total fluorine in the soils of Poland according to Kabata-Pendias and Pendias.[15]

For two experiments, i.e., those involving narrow-leaved lupin and lucerne, a lower level of soil contamination with fluorine was applied as compared with the other experiments due to the fact that the leguminosae plants are more sensitive to the presence of various xenobiotics in the soil.

As regards the group of plants being sensitive to soil contamination with fluorine, yellow lupin which had been sown as an aftercrop following the harvest of maize was also tested. For this plant, the follow-up effect of the soil contamination with fluorine as adopted under the maize was assessed. The sensitivity of the plants to the contamination with fluorine was estimated based on the preliminary studies carried out prior to the establishment of proper pot experiments.

The following were used in the experiments as the substances neutralizing the soil contamination with fluorine: lime (at a dose equivalent to 1 Hh of the soil, Hh = hydrolytic acidity of soil), charcoal, and loam, with each of the latter two substances being at the level of 3% of the weight of the soil in the pot.

In addition to the neutralizing substances, in order to satisfy the nutritional requirements of the plants, supplementary mineral fertilization with NPK was applied at the same level in all experiments. Nitrogen was applied in a form of urea at an amount of 111 mg N/kg of soil, phosphorus in the form of triple superphosphate 46% at an amount of 48 mg P/kg of soil, and potassium in the form of a 57% potassium salt at an amount equal to 111 mg K/kg of soil.

In total, each experiment included 16 objects, with each being conducted in triplicate. The soil at an amount of 9.0 kg was thoroughly mixed with mineral fertilizers and, in the appropriate objects, with fluorine and the neutralizing substances, and then transferred to appropriately marked pots. Immediately after filling the pots with the soil with the particular components, the tested plants were sown, with 13 plants being ultimately left in a pot in all experiments, with an exception of black radish, for which 8 plants were left in a pot in each experiment.

During the plant vegetation, the moisture content of the soil in the pots was maintained at a level of 60% of the capillary water capacity. The plants were harvested at the stage of technological maturity; at this time, samples of plant material, divided into the above-ground parts and roots, were also taken for laboratory analyses. The biomass obtained from the pots was combined into pooled samples corresponding to the particular combinations. The samples were broken up and dried at a temperature of 60ºC.

The study results obtained were statistically processed using the Statistica1 0.0 program, and by the two-way analysis of variance (ANOVA), while the least significant differences (NIR) were determined at a level of significance $\alpha=0.01$ using the Duncan test.[16] The relationships between the fluorine contamination and the contents of calcium and magnesium in the plants were determined using the polynomial regression equations, and Pearson's simple correlation.

The content of calcium was determined by the method of atomic emission spectroscopy (ESA), while the content of magnesium was determined by the method of atomic absorption spectroscopy (ASA).[17]

For lucerne, the contents of calcium and magnesium were only determined for the above-ground biomass following the 1st mowing.

RESULTS AND DISCUSSION

The contents of calcium and magnesium in the considered organs of the tested plants were dependent on the level of soil contamination with fluorine, on the fluorine-inactivating substance used, and on the species and organs of the tested plants.

The results of our research indicate that the increasing contamination of soil with fluorine had a significant effect on the contents of calcium and magnesium in all species and organs of the tested plants (Tables 2–11). In general, it resulted in a reduction in the content of analyzed macroelements in most of the tested plants. The lowest contents of calcium and magnesium were found in the plants cultivated in the soil to which fluorine had been introduced at the largest amount, i.e., 60 mg F/kg of soil in the experiment involving narrow-leafed lupin, 150 mg F/kg of soil in the experiment involving lucerne, and 300 mg F/kg of soil for other plants. It should be noted that more pronounced changes were observed for the content of calcium as compared with the content of magnesium. Under the influence of 300 mg F/kg of soil, the largest reductions in the average content of calcium were noted for the roots of yellow lupin and winter rape at 63% and 47%, respectively, in relation to the control objects, i.e., those non-contaminated with fluorine.

Table 2. Concentration of calcium (g Ca/kg dry weight) in analyzed plants (maize and yellow lupin) with different levels of soil contamination with fluorine (F) (mg/kg) and applied neutralizing substances

Soil F level mg/kg	Concentration of calcium in analyzed plants (g Ca/kg dry weight)									
	Type of neutralizing substance								Mean	
	Without neutralizing substance		Lime according to 1Hh		Charcoal, 3% of soil mass		Loam, 3% of soil mass			
	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots
MAIZE										
0	3.6	2.1	4.6	5.4	5.3	2.1	4.0	3.3	4.4	3.2
100	3.4	2.9	3.9	4.0	4.0	3.9	3.4	2.1	3.7	3.2
200	3.4	3.1	3.2	4.9	3.3	5.9	3.6	2.4	3.4	4.1
300	2.7	2.3	4.0	5.8	5.1	4.9	4.4	2.9	4.1	4.0
Mean	3.3	2.6	3.9	5.0	4.4	4.2	3.9	2.7	-	-
r	–0.87‡	0.22	–0.56	0.35	–0.18	0.82‡	0.40	–0.22	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.123*; b: –0.123*; a-b: –0.246*						
	Roots			a: –0.160*; b: –0.160*; a-b: –0.321*						
YELLOW LUPIN										
0	9.9	8.4	12.9	13.1	11.5	13.4	13.1	11.4	11.9	11.6
100	9.9	6.4	11.4	6.6	11.4	9.6	11.3	5.7	11.0	7.1
200	8.0	6.0	10.4	6.0	8.1	8.4	8.6	3.7	8.8	6.0
300	8.0	4.0	10.1	6.0	7.9	4.4	8.2	2.9	8.5	4.3
Mean	9.0	6.2	11.2	7.9	9.7	8.9	10.3	5.9	-	-
r	–0.88‡	–0.97‡	–0.95‡	–0.82‡	–0.90‡	–0.98‡	–0.96‡	–0.92‡	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.299*; b: –0.299*; a-b: –0.598*						
	Roots			a: –0.242*; b: –0.242*; a-b: –0.485*						

LSD (least significant difference) for: a: F soil contamination; b: neutralizing substance;
a-b: interaction of F soil contamination and neutralizing substance;
*significant for α=0.01; n.s.: no significant difference;
†correlation coefficient (r) significant for α=0.05;
‡correlation coefficient (r) significant for α=0.01.

Table 3. Concentration of calcium (g Ca/kg dry weight) in analyzed plants (winter oilseed rape and narrow-leaf lupin) with different levels of soil contamination with fluorine (F) (mg/kg) and applied neutralizing substances

Soil F level mg/kg	Concentration of calcium in analyzed plants (g Ca/kg dry weight)									
	Type of neutralizing substance								Mean	
	Without neutralizing substance		Lime according to 1Hh		Charcoal, 3% of soil mass		Loam, 3% of soil mass			
	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots
WINTER OILSEED RAPE										
0	12.3	5.7	10.7	9.1	12.3	5.6	11.4	4.3	11.7	6.2
100	13.1	4.3	10.6	5.1	11.5	3.1	12.1	4.3	11.8	4.2
200	12.2	4.3	14.6	3.6	14.0	2.9	13.9	3.4	13.7	3.6
300	11.6	3.4	12.7	3.7	11.1	3.3	12.2	2.6	11.9	3.3
Mean	12.3	4.4	12.2	5.4	12.2	3.7	12.4	3.7	-	-
r	–0.58†	–0.93‡	0.68†	–0.89‡	–0.10	–0.72‡	0.50	–0.94‡	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.422*; b: n.s.; a-b: –0.845*						
	Roots			a: –0.124*; b: –0.124*; a-b: –0.248*						
NARROW-LEAF LUPIN										
0	18.6	13.6	26.8	12.3	20.7	12.9	19.3	6.9	21.4	11.4
20	17.7	8.9	25.4	9.6	14.4	8.6	11.0	6.4	17.1	8.4
40	11.7	6.4	12.7	9.1	13.1	8.3	10.9	5.9	12.1	7.4
60	10.6	7.4	11.2	8.4	12.7	7.7	10.3	5.9	11.2	7.3
Mean	14.6	9.1	19.0	9.8	15.2	9.4	12.9	6.3	-	-
r	–0.95‡	–0.85‡	–0.93‡	–0.92‡	–0.87‡	–0.85‡	–0.81‡	–0.91‡	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.464*; b: –0.464*; a-b: –0.928*						
	Roots			a: –0.273*; b: –0.273*; a-b: –0.547*						

LSD (least significant difference) for: a: F soil contamination; b: neutralizing substance; a-b: interaction of F soil contamination and neutralizing substance;
*significant for α=0.01; n.s.: no significant difference;
†correlation coefficient (r) significant for α=0.05;
‡correlation coefficient (r) significant for α=0.01.

Table 4. Concentration of calcium (g Ca/kg dry weight) in analyzed plants (black radish and phacelia) with different levels of soil contamination with fluorine (F) (mg/kg) and applied neutralizing substances

Soil F level mg/kg	Concentration of calcium in analyzed plants (g Ca/kg dry weight)									
	Type of neutralizing substance								Mean	
	Without neutralizing substance		Lime according to 1Hh		Charcoal, 3% of soil mass		Loam, 3% of soil mass			
	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots
					BLACK RADISH					
0	25.0	7.9	31.4	8.0	28.6	7.9	25.7	7.1	27.7	7.7
100	21.4	7.9	28.6	7.9	24.3	7.9	25.1	7.1	24.8	7.7
200	21.4	8.4	22.8	7.9	21.0	8.3	24.3	8.0	22.4	8.2
300	21.4	8.6	20.0	7.7	20.7	9.1	17.1	7.7	19.8	8.3
Mean	22.3	8.2	25.7	7.9	23.6	8.3	23.0	7.5	-	-
r	-0.75‡	0.84‡	-0.99‡	-0.64†	-0.92‡	0.76‡	-0.85‡	0.74‡	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.741*; b: –0.741*; a-b: –1.482*						
	Roots			a: –0.274*; b: –0.274*; a-b: –0.548*						
					PHACELIA					
0	10.3	11.4	14.3	12.9	13.3	12.7	13.9	8.3	13.0	11.3
100	11.3	8.1	12.4	12.4	12.9	12.6	13.9	7.8	12.6	10.2
200	11.2	8.0	10.4	12.0	12.6	12.4	13.6	7.6	11.9	10.0
300	10.2	7.6	10.2	11.4	11.3	12.1	12.1	6.0	11.0	9.3
Mean	10.8	8.8	11.8	12.2	12.5	12.4	13.4	7.4	-	-
r	-0.08	-0.84‡	-0.95‡	-0.95‡	-0.80‡	-0.42	-0.81†	-0.91‡	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.406*; b: –0.406*; a-b: –0.812*						
	Roots			a: –0.374*; b: –0.374*; a-b: –0.748*						

LSD (least significant difference) for: a: F soil contamination; b: neutralizing substance; a-b: interaction of F soil contamination and neutralizing substance;
*significant for α=0.01; n.s.: no significant difference;
†correlation coefficient (r) significant for α=0.05;
‡correlation coefficient (r) significant for α=0.01.

Table 5. Concentration of calcium (g Ca/kg dry weight) in analyzed plant (spring triticale) with different levels of soil contamination with fluorine (F) (mg/kg) and applied neutralizing substances

Soil F level mg/kg	Concentration of calcium in analyzed plants (g Ca/kg dry weight)								
	Type of neutralizing substance								
	Without neutralizing substance			Lime according to 1Hh			Charcoal, 3% of soil mass		
	Seed	Straw	Roots	Seed	Straw	Roots	Seed	Straw	Roots
	SPRING TRITICALE								
0	0.7	3.1	8.1	0.7	2.9	8.6	0.7	3.1	8.6
100	0.7	3.3	5.6	0.7	3.6	6.1	0.7	3.6	5.4
200	0.6	3.6	5.3	0.6	3.7	5.4	0.6	3.7	4.4
300	0.6	3.6	4.7	0.7	3.6	4.6	0.6	3.9	4.6
Mean	0.7	3.4	5.9	0.7	3.5	6.2	0.7	3.6	5.7
r	–0.87‡	0.91‡	–0.90‡	–0.25	0.76‡	–0.95‡	–0.81‡	0.85‡	–0.86‡

Soil F level mg/kg	Concentration of calcium in analyzed plants (g Ca/kg dry weight)					
	Type of neutralizing substance			Mean		
	Loam, 3% of soil mass					
	Seed	Straw	Roots	Seed	Straw	Roots
	SPRING TRITICALE					
0	0.6	2.9	7.1	0.7	3.0	8.1
100	0.6	3.3	5.7	0.6	3.4	5.7
200	0.6	3.5	5.3	0.6	3.6	5.1
300	0.6	3.4	4.7	0.7	3.6	4.6
Mean	0.6	3.3	5.7	-	-	-
r	0.00	0.81‡	–0.95‡			
$LSD_{0.01}$ for:	Seed			a: –0.021*; b: –0.021*; a-b: –0.042*		
	Straw			a: –0.119*; b: –0.119*; a-b: –0.239*		
	Roots			a: –0.168*; b: –0.168*;.; a-b: –0.337*		

LSD (least significant difference) for: a: F soil contamination; b: neutralizing substance;
a-b: interaction of F soil contamination and neutralizing substance;
*significant for α=0.01; n.s.: no significant difference;
†correlation coefficient (r) significant for α=0.05;
‡correlation coefficient (r) significant for α=0.01.

Table 6. Concentration of calcium (g Ca/kg dry weight) in analyzed plant (alfalfa) with different levels of soil contamination with fluorine (F) (mg/kg) and applied neutralizing substances

Soil F level mg/kg	Concentration of calcium in analyzed plants (g Ca/kg dry weight)									
	Type of neutralizing substance								Mean	
	Without neutralizing substance		Lime according to 1Hh		Charcoal, 3% of soil mass		Loam, 3% of soil mass			
	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots
					ALFALFA					
0	12.1	-	10.0	-	11.1	-	12.3	-	11.4	-
50	10.7	-	11.6	-	10.7	-	9.5	-	10.6	-
100	10.7	-	11.0	-	10.4	-	9.4	-	10.4	-
150	10.0	-	10.0	-	10.3	-	9.1	-	9.8	-
Mean	10.8	-	10.6	-	10.6	-	10.1	-	-	-
r	–0.90‡	-	–0.10	-	–0.60†	-	–0.83‡	-	-	-

$LSD_{0.01}$ for:	Above-ground parts	a: –0.344*; b: –0.344*; a-b: –0.688*
	Roots	-

LSD (least significant difference) for: a: F soil contamination; b: neutralizing substance; a-b: interaction of F soil contamination and neutralizing substance;
*significant for α=0.01; n.s.: no significant difference;
†correlation coefficient (r) significant for α=0.05;
‡correlation coefficient (r) significant for α=0.01.

Table 7. Concentration of magnesium (g Mg/kg dry weight) in analyzed plants (maize and yellow lupin) with different levels of soil contamination with fluorine (F) (mg/kg) and applied neutralizing substances

Soil F level mg/kg	Concentration of magnesium in analyzed plants (g Mg/kg dry weight)									
	Type of neutralizing substance								Mean	
	Without neutralizing substance		Lime according to 1Hh		Charcoal, 3% of soil mass		Loam, 3% of soil mass			
	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots
					MAIZE					
0	1.5	1.4	1.8	1.7	1.7	2.4	1.8	2.3	1.7	1.9
100	1.3	1.4	1.4	1.7	1.3	2.1	1.7	2.2	1.4	1.8
200	1.1	1.4	1.2	1.3	1.2	1.8	1.6	1.6	1.3	1.5
300	1.0	1.4	1.2	1.2	1.1	1.4	1.5	1.6	1.2	1.4
Mean	1.2	1.4	1.4	1.5	1.3	1.9	1.6	1.9	-	-
r	–0.98‡	0.00	–0.91‡	–0.93‡	–0.91‡	–0.98‡	–0.96‡	–0.92‡	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.042*; b: –0.042*; a-b: –0.085*						
	Roots			a: –0.057*; b: –0.057*; a-b: –0.115*						
					YELLOW LUPIN					
0	3.8	1.8	3.7	1.8	3.4	2.0	4.3	2.1	3.8	1.9
100	4.0	1.6	3.7	1.7	3.7	1.6	4.4	1.8	4.0	1.7
200	4.1	1.7	3.8	1.6	3.7	1.6	4.3	1.6	4.0	1.6
300	4.1	1.7	3.7	1.4	3.6	1.4	4.3	1.6	3.9	1.5
Mean	4.0	1.7	3.7	1.6	3.6	1.6	4.3	1.8	-	-
r	0.78‡	–0.29	0.16	–0.97‡	0.36	–0.89‡	–0.12	–0.92‡	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.127*; b: –0.127*; a-b: n.s.						
	Roots			a: –0.054*; b: –0.054*; a-b: –0.108*						

LSD (least significant difference) for: a: F soil contamination; b: neutralizing substance;
a-b: interaction of F soil contamination and neutralizing substance;
*significant for α=0.01; n.s.: no significant difference;
†correlation coefficient (r) significant for α=0.05;
‡correlation coefficient (r) significant for α=0.01.

Table 8. Concentration of magnesium (g Mg/kg dry weight) in analyzed plants (winter oilseed rape and narrow-leaf lupin) with different levels of soil contamination with fluorine (F) (mg/kg) and applied neutralizing substances

Soil F level mg/kg	Concentration of magnesium in analyzed plants (g Mg/kg dry weight)									
	Type of neutralizing substance								Mean	
	Without neutralizing substance		Lime according to 1Hh		Charcoal, 3% of soil mass		Loam, 3% of soil mass			
	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots
	WINTER OILSEED RAPE									
0	2.4	4.9	2.8	4.9	3.1	3.7	2.8	4.2	2.8	4.4
100	2.2	3.6	2.7	3.6	3.0	3.5	2.7	3.7	2.7	3.6
200	2.1	3.3	2.7	3.4	2.8	3.3	2.7	3.4	2.6	3.3
300	2.0	3.2	2.5	3.2	2.7	3.0	2.5	3.2	2.4	3.1
Mean	2.2	3.7	2.7	3.8	2.9	3.4	2.7	3.6	-	-
r	–0.95‡	–0.88‡	–0.87‡	–0.89‡	–0.82‡	–0.90‡	–0.84‡	–0.96‡	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.088*; b: –0.088*; a-b: n.s.						
	Roots			a: –0.114*; b: –0.114*; a-b: –0.227*						
	NARROW-LEAF LUPIN									
0	3.7	2.1	3.3	2.1	3.3	2.2	4.0	2.1	3.6	2.1
20	3.7	1.9	3.5	1.9	3.0	2.4	4.0	2.4	3.5	2.1
40	3.4	1.9	3.1	1.9	2.8	2.0	4.0	2.4	3.3	2.0
60	3.4	1.9	3.0	1.9	2.8	2.0	3.9	2.4	3.3	2.1
Mean	3.5	1.9	3.2	1.9	3.0	2.2	4.0	2.3	-	-
r	–0.82‡	–0.72‡	–0.74‡	–0.74‡	–0.82‡	–0.61†	–0.40	0.74‡	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.104*; b: –0.104*; a-b: –0.208*						
	Roots			a: –0.067*; b: –0.067*; a-b: –0.135*						

LSD (least significant difference) for: a: F soil contamination; b: neutralizing substance; a-b: interaction of F soil contamination and neutralizing substance;
*significant for α=0.01; n.s.: no significant difference;
†correlation coefficient (r) significant for α=0.05;
‡correlation coefficient (r) significant for α=0.01.

Table 9. Concentration of magnesium (g Mg/kg dry weight) in analyzed plants (black radish and phacelia) with different levels of soil contamination with fluorine (F) (mg/kg) and applied neutralizing substances

Soil F level mg/kg	Concentration of magnesium in analyzed plants (g Mg/kg dry weight)									
	Type of neutralizing substance								Mean	
	Without neutralizing substance		Lime according to 1Hh		Charcoal, 3% of soil mass		Loam, 3% of soil mass			
	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots
BLACK RADISH										
0	3.4	1.4	2.9	1.4	2.6	1.4	3.7	1.4	3.2	1.4
100	2.7	1.7	2.4	1.6	2.5	1.5	3.1	1.6	2.7	1.6
200	2.7	1.9	2.1	1.7	2.4	1.8	2.8	2.0	2.5	1.9
300	2.7	2.1	2.1	1.6	2.2	1.7	2.6	2.1	2.4	1.9
Mean	2.9	1.8	2.4	1.6	2.4	1.6	3.1	1.8	-	-
r	–0.76‡	0.98‡	–0.92‡	0.70†	–0.84‡	0.78‡	–0.96‡	0.97‡	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.085*; b: –0.085*; a-b: –0.171*						
	Roots			a: –0.058*; b: –0.058*; a-b: –0.116*						
PHACELIA										
0	2.5	7.2	2.4	9.9	2.5	6.6	3.0	6.9	2.6	7.6
20	2.1	6.3	2.1	8.4	2.4	6.3	2.7	6.9	2.3	7.0
40	2.0	6.1	1.9	8.2	2.3	6.2	2.5	6.9	2.2	6.8
60	2.0	6.0	1.9	8.0	1.9	6.1	2.5	6.8	2.1	6.7
Mean	2.1	6.4	2.1	8.6	2.3	6.3	2.7	6.9	-	-
r	–0.85‡	–0.87‡	–0.92‡	–0.87‡	–0.87‡	–0.59†	–0.90‡	–0.25	-	-
$LSD_{0.01}$ for:	Above-ground parts			a: –0.075*; b: –0.075*; a-b: –0.150*						
	Roots			a: –0.217*; b: –0.217*; a-b: –0.434*						

LSD (least significant difference) for: a: F soil contamination; b: neutralizing substance; a-b: interaction of F soil contamination and neutralizing substance;
*significant for α=0.01; n.s.: no significant difference;
†correlation coefficient (r) significant for α=0.05;
‡correlation coefficient (r) significant for α=0.01.

Table 10. Concentration of magnesium (g Mg/kg dry weight) in analyzed plant (spring triticale) with different levels of soil contamination with fluorine (F) (mg/kg) and applied neutralizing substances

Soil F level mg/kg	Concentration of magnesium in analyzed plants (g Mg/kg dry weight)								
	Type of neutralizing substance								
	Without neutralizing substance			Lime according to 1Hh			Charcoal, 3% of soil mass		
	Seed	Straw	Roots	Seed	Straw	Roots	Seed	Straw	Roots
	SPRING TRITICALE								
0	1.5	1.0	1.1	1.4	1.0	1.2	1.4	1.0	1.2
100	1.4	1.0	0.7	1.4	0.9	0.7	1.4	1.0	0.8
200	1.3	1.0	0.7	1.4	1.0	0.7	1.4	0.9	0.7
300	1.2	0.8	0.6	1.3	0.8	0.7	1.4	1.0	0.7
Mean	1.4	1.0	0.8	1.4	0.9	0.8	1.4	1.0	0.8
r	–0.98‡	–0.76‡	–0.87‡	–0.70†	–0.67†	–0.77‡	0.00	–0.20	–0.86‡

Soil F level mg/kg	Concentration of magnesium in analyzed plants (g Mg/kg dry weight)					
	Type of neutralizing substance			Mean		
	Loam, 3% of soil mass					
	Seed	Straw	Roots	Seed	Straw	Roots
	SPRING TRITICALE					
0	1.5	1.0	1.3	1.5	1.0	1.2
100	1.4	1.0	0.9	1.4	1.0	0.8
200	1.3	1.1	0.7	1.4	1.0	0.7
300	1.2	1.2	0.7	1.3	1.0	0.7
Mean	1.4	1.1	0.9	-	-	-
r	–0.97‡	0.92‡	–0.91‡			
$LSD_{0.01}$ for:	Seed			a: –0.046*; b: –0.046*; a-b: –0.092*		
	Straw			a: –0.031*; b: –0.031*; a-b: –0.062*		
	Roots			a: –0.025*; b: –0.025*;.; a-b: –0.051*		

LSD (least significant difference) for: a: F soil contamination; b: neutralizing substance;
a-b: interaction of F soil contamination and neutralizing substance;
*significant for α=0.01; n.s.: no significant difference;
†correlation coefficient (r) significant for α=0.05;
‡correlation coefficient (r) significant for α=0.01.

Table 11. Concentration of magnesium (g Mg/kg dry weight) in analyzed plant (alfalfa) with different levels of soil contamination with fluorine (F) (mg/kg) and applied neutralizing substances

Soil F level mg/kg	Concentration of magnesium in analyzed plants (g Mg/kg dry weight)									
	Type of neutralizing substance								Mean	
	Without neutralizing substance		Lime according to 1Hh		Charcoal, 3% of soil mass		Loam, 3% of soil mass			
	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots	Above-ground parts	Roots
ALFALFA										
0	2.1	-	2.1	-	2.5	-	2.7	-	2.4	-
50	2.2	-	2.1	-	2.4	-	2.7	-	2.3	-
100	1.8	-	1.9	-	2.2	-	2.5	-	2.1	-
150	1.7	-	1.7	-	1.8	-	2.2	-	1.9	-
Mean	2.0	-	1.9	-	2.2	-	2.5	-	-	-
r	–0.86‡	-	–0.93‡	-	–0.92‡	-	–0.90‡	-	-	-
$LSD_{0.01}$ for:	Above-ground parts		a: –0.071*; b: –0.071*; a-b: –0.142*							
	Roots		-							

LSD (least significant difference) for: a: F soil contamination; b: neutralizing substance; a-b: interaction of F soil contamination and neutralizing substance;
*significant for α=0.01; n.s.: no significant difference;
†correlation coefficient (r) significant for α=0.05;
‡correlation coefficient (r) significant for α=0.01.

In the series without the addition of the neutralizing substance, the increasing contamination of soil with fluorine had, in most cases, a highly significant negative effect on the content of tested macroelements in the plants. The demonstrated trend is confirmed by the results obtained by Reddy and Kaur who showed, at the highest level of fluorine contamination (150 mM NaF), a reduction by 53% of the calcium content in *Salicornia brachiata*.[18] In addition, Elloumi et al., in an experiment involving *Amygdalis communis*,[19] Abdallah et al. for *Vitis vinifera*[20], and Li and Ni for *Camellia sinensis*, also noted a reduction in the calcium content as compared with the controls with the increasing doses of fluorine.[21]

A statistical analysis of the results we obtained also showed a highly significant positive relationship between the contamination with fluorine and the contents of the analyzed elements in the plants.

The increase in the content of calcium was noted for the roots of black radish ($r=0.84$, $\alpha=0.01$), and the hay of spring triticale ($r=0.91$, $\alpha=0.01$), and a reduction in the content of magnesium was noted for the above-ground biomass of yellow lupin ($r=0.78$, $\alpha=0.01$) and in the roots of black radish ($r=0.98$, $\alpha=0.01$). A study by Ruan et al. informs of the positive relationships between the soil contamination with fluorine and the calcium content in *Camellia sinensis*.[22]

The noted reduction in the content of calcium in the plants with the increase in the level of soil contamination with fluorine may be explained by the binding of calcium and fluorine into sparingly soluble compounds, e.g., CaF_2, and thus causing the reduction in the bioavailability of calcium to the plants. Similar conclusions were drawn by Arnesen who demonstrated in his studies that the soil contamination with fluorine resulted in a decrease in the uptake of calcium by plants, probably due to calcium forming stable compounds with fluorine.[23]

Results of our own research demonstrated that the calcium content in the tested plants ranged on average from 0.7 g Ca/kg dw in the grain of spring triticale to 23.7 g Ca/kg dw in the above-ground biomass of black radish, with the above-ground biomass of the tested plants being characterised, in most cases, by a higher content of this element as compared with the roots. Khandere and Rao demonstrated in their study that the plants of *Amaranthus gangeticus* (3.97 g/kg dw) contained most calcium, while the plants of potato contained the least of this element (0.2 g/kg dw).[5] Nowak noted a two-times higher content of calcium in the leaves of trees growing on a heap of phosphogypsum as compared with the areas surrounding the heap.[24] It results from the study by Pyś and Pucek that most of the fodder crops cultivated in the area immediately adjacent to the Phosphorus Fertilizer Production Plant in Machów (Poland) were characterised by a very low content of calcium.[25] In turn, Zbierska in her study did not demonstrate an effect of the distance of the Phosphorus Fertilizer Plant in Luboń (Poland) on the content of calcium in plants.[26]

What seems to be particularly important is the effect of fluorine on the content of magnesium which plays a very significant role in the plants, primarily in the photosynthesis process.[27]

The highest magnesium content was found in the roots of phacelia, on average, 7.0 g Mg/kg dw, and the lowest one in the roots of spring triticale, on average, 0.8 g Mg/kg dw. In general, similarly to the case of calcium, the above-ground parts of the tested plants were characterised by a higher content of magnesium than their roots. Nowak reports that the content of magnesium in the leguminosae and grass plants ranged from 1.5 to 2.7 g Mg/kg dw.[24] The results presented by Pyś and Pucek show that the content of the macrocomponent concerned in the green fodder originating from the areas surrounding the Phosphorus Fertilizer Production Plant in Machów did not exceed 1.4 g Mg/kg dw. An exception was the above-ground biomass of maize, 4.1 g Mg/kg dw, and the leaves of fodder beet, 13.7 g Mg/kg dw.[25] On the other hand, Fazlul Hoque et al. demonstrated in their study that the content of magnesium in *Vitex negundo* ranged from 3.2 to 7.4 g Mg/kg dw.[28]

Results of our own research indicate that the fluorine applied to the substrate had a negative effect on the content of magnesium in the plants, which is also confirmed by the scientific literature. The reduction in the content of magnesium in the plants is indicated by a study conducted by Li and Ni with the use of *Camellia sinensis*, who demonstrated, at the highest level of fluorine contamination amounting to 0.84 nM, a 2% reduction in the content of magnesium in the leaves of this plant, as compared with the object non-contaminated with fluorine.[21] A study involving *Camellia sinensis* was also conducted by Wang et al., who confirmed the adverse effect of fluorine on the content of magnesium in the tested plant.[29] The toxic effect of fluorine on the development of the content of this macrocomponent was also noted by Elloumi et al. in an experiment involving *Amygdalis communis*,[19] Abdallah et al. in an experiment involving *Vitis vinifera*,[20] and Reddy and Kaur, in 2008, in an experiment involving *Salicornia brachiata.*[18]

The substances added in order to neutralize the soil contamination with fluorine significantly modified the contents of calcium and magnesium in the tested plants (Tables 2–11).

The introduction of calcium into the substrate contributed to an increase in the content of calcium in all the tested plants. In general, a change to the pH of the soil through the use of calcium contributes to an increase in the content of calcium in the roots of plants, which is also confirmed by our own research. The greatest changes to the content of this element were noted for the roots of maize and the roots of phacelia, where, following the application of this additive, the content of calcium increased by 92% and 37%, respectively, as compared to the control series. The introduction of calcium into the substrate also contributed to an increase in the accumulation of magnesium in phacelia, in the roots by 34%, in winter rape, in the above-ground biomass by 23% and in the roots by 3%, and in maize, in the above-ground biomass by 17% and in the roots by 7%. A positive effect of lime application on the increase in the content of both macroelements concerned in the plants should be explained by the natural high content of calcium, and an elevated content of magnesium in this neutralizing substance. Changes to the content of the tested macroelements in the plants, resulting from lime application, are confirmed by the scientific literature, *inter alia* in a study by Ruan et al., where lime application at a dose ranging from 1.05 to 2.7 g/kg of soil contributed to an increase in the contents of calcium and magnesium in the leaves of *Camelia sinensis*, with the content of these macroelements being affected to the highest degree by the dose of 1.65 g/kg of soil.[22]

Charcoal added to the substrate also had a positive effect on the contents of calcium and magnesium in most of the tested plants. In the series with the addition of this neutralizing substance, a trend was noted for an increase in the content of magnesium in the above-ground biomass and for its reduction in the roots of the tested plants as compared with the series non-contaminated with fluorine. The greatest increase in the content of magnesium as compared with the control series was noted for the roots of maize, which amounted to 36%, and in the above-ground biomass of winter rape, which amounted to 32%. As regards calcium, a significant increase in the content thereof was noted for maize, by 33% in the above-ground biomass and by 61% in the roots, followed by the roots of yellow lupin, by 44%, and in the roots of phacelia, by 41%.

The application of loam to the soil had a positive effect on the content of calcium in the above-ground biomass of most of the tested plants, and a negative effect in the roots of the tested plants. The maximum increase in the content of this macroelement was noted for the above-ground biomass of phacelia, and amounted to 24% as compared with the control series. In this study, loam also contributed to an increase in the average content of magnesium in the analyzed plants. The largest increase in the magnesium content under the influence of this substance was noted for maize. For the above-ground biomass the increase was 33% and for the roots it was 36% in relation to the average content of the macroelement concerned in the control series. The higher contents of calcium and magnesium in the plants cultivated in the soil with the addition of loam as compared to the control series may be explained by the natural high content of these elements, introduced to the soil with the application of this inactivating material.

To sum up, it should be emphasised that lime, followed by loam and, to a smaller degree, charcoal, had the most positive effect on the contents of calcium and magnesium in the plants.

CONCLUSIONS

The contents of magnesium and calcium in the considered organs of the tested plants were dependent on the species and organ of the plant, the level of soil contamination with fluorine, and the fluorine-inactivating substance used.

Under the influence of the increasing contamination of soil with fluorine, a reduction in the contents of calcium and magnesium in the tested plants occurred. The content of both elements was the most adversely affected by the soil contamination with fluorine. The highest level of fluorine contamination resulted in the largest reduction in the average content of calcium in the roots of yellow lupin and winter rape, by 63 and 42%, respectively, and of magnesium in the roots of spring triticale, by 42% as compared with the control objects, i.e., those non-contaminated with fluorine.

The application of the neutralizing substances had a significant effect on the contents of the tested macroelements in the plants. The addition of lime, charcoal, and loam to the soil contributed to an increase in the level of both macroelements considered in the plants. In addition, it should be noted that lime, followed by loam and charcoal, had the most positive effect on the contents of calcium and magnesium in the plants.

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