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The role of the quality of soil organic matter in cadmium accumulation in plants

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Abstract. Two acid soils (pH_{CaCl_2} 4—4.5) synthesized by mixing 1 part by weight of Carex or Sphagnum peat with 9 parts by weight of clay, were compared in a pot and an incubation experiment. In the pot experiment the experimental plant was the radish, the cadmium content of whose tops rose in the first harvest by 23 mg/kg and in the second by 16 mg/kg when 5.12 mg of cadmium labelled with ^{115m}Cd had been added to the mixture of Carex peat and clay (3 kg/pot). The corresponding contents in radishes grown in a mixture of Sphagnum peat and clay were 51 and 33 mg/kg. In the roots, the corresponding contents were only 1/3—1/4 of the preceding. Of the cadmium added to the mixture of Carex peat and clay, radish tops and roots took up an overall 3.9 %. In the mixture of Sphagnum peat and clay the corresponding proportion was 9.5 %.

In the incubation experiment, differences in the solubility of the added cadmium accounted satisfactorily for the differences found in the plant experiment. It was concluded from the results of the pot and incubation experiments and from autoradiographic tracings of cadmium uptake in radishes, that radishes were able to take up cadmium dissolved in soil water with little hindrance, and that translocation occurred in the plant in conjunction with the transpiration stream.

A large amount of organic matter limits the availability to plants of cadmium added to the soil. In the studies which enabled this conclusion to be reached (eg. JOHN et al. 1972, JAAKKOLA and SIRÉN 1976), the connection between the amount of organic matter or organic carbon and cadmium availability was somewhat tenuous, although a group of other factors bearing upon the relationship were considered simultaneously. Obviously, part of the variation in cadmium availability is due to random influences, but part undoubtedly derives from factors not taken into account. Soil organic matter has a complex makeup, and is a combination of substances with properties different from one another. One can expect that variations in the composition of the organic matter will be reflected, for instance, in its ability to retain cadmium added to the soil and to release it to plants. The purpose of this study was to determine

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to what extent the type of organic matter affects the retention and availability to plants of added cadmium. For comparison, two types of peat were chosen, whose mixtures with a heavy clay were used as the experimental soils.

Material and methods

The material consisted of a pot experiment for determining the cadmium uptake of radishes, an incubation experiment for determining the solubility of cadmium retained by the soil, and an autoradiograph which was used to trace the translocation of cadmium in the radish plants. The experimental soils, which will be referred to as »Carex clay» and »Sphagnum clay», were prepared by mixing Carex peat or Sphagnum peat with clay in proportions giving an organic matter content of 10 % in the synthetic experimental soils (Table 1). The peats were taken from a virgin soil; the clay was taken from the subsoil of a cultivated soil.

The pot experiment

The »Carex clay» or »Sphagnum clay» was used at the rate of about 3 kg per pot. The treatments, which were made on 16. 5. 1974 five weeks before the radishes were sown, consisted of an addition of cadmium chloride containing 5.12 mg/pot of cadmium labelled with ^{115m}Cd, and of 5.00 mg/pot of unlabelled cadmium to previously mixed »Carex clay» or »Sphagnum clay» or to their components (clay, A or peat, B) in the following manner:

Treatment	Labelled Cd added to	Unlabelled Cd added to
AB	neither component	neither component
(AB)*	mixture clay + peat	»
A*B	clay	»
A B*	peat	»
A*B^1	clay	peat
A^{1}B*	peat	clay

On the 16. 5. 1974, the activity of the labelled cadmium addition intended for each pot was 99.76 μCi. The experiment was made in four replications. The previously mixed soils (treatments AB and (AB)*) and their components (the other treatments) were kept moist by watering them from time to time. Evaporation was minimized by covering the pots with a plastic film.

After the incubation period, the peat and clay to be put into the same container were mixed if they had been stored separately (the treatments other than AB and (AB)*). The soil was supplementd with the following amounts (mg) of nutrients per pot, in the form of inorganic salts:

N	P	K	Mg	Mn	Cu	Zn	Mo	B
1 500	400	1 000	200	10	10	10	10	10

In each pot were sown 15 seeds of radish (*Raphanus sativus* cv. Non plus ultra). The 10 individuals allowed to grow after thinning were harvested four weeks after sowing. The roots and tops were harvested separately. The tops were dried (105° C) for dry matter determination. The roots were washed with deionized water before drying.

Table 1. Components of »Carex clay» and »Sphagnum clay».

	Clay	Carex peat	Sphagnum peat
Amount, g per pot			
in »Carex clay»	2 700	350	–
in »Sphagnum clay»	2 700	–	310
pH_{CaCl_2}	5.9	3.6	2.8
CEC, mval/100 g	24.0	110.5	139.5
Clay fraction (< 2 μm), %	76.7	–	–
Ammonium oxalate (pH 3.3) extractable			
Fe, g/kg	2.6	5.4	0.3
Al, g/kg	2.0	3.3	0.3
Org. C, g/kg	6	400	440
Humic acids[1]), g/kg	2	430	50
Fulvic acids[1]), g/kg	12	170	43

[1]) According to Stevenson (1965).

The harvest from a second sowing of radishes was taken from the same pots when the plants were four weeks old.

The incubation experiment

To allow a determination of the solubility of cadmium retained by the soils and their components, and of changes in the solubility, an incubation experiment was run in parallel with the pot experiment. The soils, which were used in 1/10 of the amounts used in the pot experiment, were incubated in 0.5-litre plastic containers maintained under approximately the same moisture regime as in the pot experiment by watering at intervals to a given weight. The temperature varied between 20 and 25° C. On the 16. 5. 1974, the soil in each container received an addition of 0.52 mg of ^{115m}Cd-labelled cadmium as the chloride. The activity was 45.07 μCi per pot. For the determination of the solubility of the native cadmium of the soil, the same soils without cadmium addition were incubated in a series parallel to the main experiment. All five treatments (»Carex clay», »Sphagnum clay», clay, Carex peat and Sphagnum peat) were replicated four times.

The incubated soils were extracted with 1 N HCl, 1 N ammonium acetate (pH 7.0) and 0.01 M $CaCl_2$ on the following days:

Day of extraction	25. 5.	11. 6.	26. 6.	18. 7.	28. 8.
incubation time (d)	9	26	41	63	104

For each extraction, a quantity of soil 1/30 of the amount originally held by the container (1 g of peat, 9 g of clay, 10 g of mixture) was used for the extraction. The amount of extractant used was 50 ml and the shaking time 1 hour.

Autoradiography

The translocation of labelled cadmium to and its distribution among different parts of the radish plant were followed by making on X-ray film autoradiograms of radish individuals at varying stages of growth. For this purpose, radishes were grown in 5-litre containers filled with »Carex clay». Just before sowing, 14 mg of labelled cadmium (1010 μCi ^{115m}Cd) as the chloride were mixed with the synthetic soil. A radish individual was taken from the container for radiographic analysis at 21, 28, 35 and 39 days from sowing.

Methods of analysis

Unlabelled cadmium was determined on extracts of soil and plant ash with a flameless atomic absorption spectrophotometer (model Varian Techtron 1200, Carbon Rod Atomizer 63). Labelled cadmium in plant material was determined by measuring the γ activity directly on a 5 ml sample aliquot dried at 105° C (model Wallac Gamma Sample Counter GTL 500). Labelled cadmium in soil extracts was determined by measuring the β-activity with a geiger counter on the evaporated residue from a 1 ml aliquot of extract.

Results

The pot experiment

The cadmium treatments did not influence the size of the root or top yield in either harvest. Although there were slight differences in yield between the soils, there were no differences between their native cadmium contents (Table 2).

In both harvests, an addition of cadmium to the »Carex clay» caused a considerably smaller increase in the cadmium content of radish tops and roots than an addition to the »Sphagnum clay» (Table 3). The effect of the cadmium added to »Carex clay» was independent of the method of addition. In the case of the first harvest of tops from the »Sphagnum clay», the cadmium content was higher when the cadmium had been added to either component of the soil mixture than when it had been added to the mixture. In the second harvest, there appeared to be a similar trend. Between cadmium contents of the roots no such differences were found. An addition of unlabelled cadmium to the peat seemingly reduced the effect of labelled cadmium added to the clay in all harvests of tops and roots, although only some of the differences were significant. An addition of cadmium to the clay affected the cadmium in the peat in a similar

Table 2. Radish yields and contents of native cadmium (treatment AB) on D.M. basis.

	1st harvest		2nd harvest	
	tops	roots	tops	roots
»Carex clay»				
yield, g/pot	4.09	3.50	4.38	3.90
Cd, mg/kg	1.7	0.6	1.7	0.6
»Sphagnum clay»				
yield, g/pot	3.93	4.19	4.50	4.19
Cd, mg/kg	1.5	0.6	1.7	0.6

Table 3. Contents of labelled cadmium appearing in radish tops and roots, mg/kg of D.M.

	1st harvest		2nd harvest	
	tops	roots	tops	roots
»Carex clay»				
(AB)*	23.0^{d}	6.6^{a}	15.5^{m}	4.1^{k}
A*B	23.3^{d}	7.1^{a}	15.7^{m}	4.2^{k}
A B*	24.6^{d}	7.4^{a}	16.1^{m}	3.9^{k}
A*B^1	24.4^{d}	6.7^{a}	15.2^{m}	3.9^{k}
A^{1}B*	22.3^{d}	6.9^{a}	15.3^{m}	4.2^{k}
»Sphagnum clay»				
(AB)*	50.8^{f}	17.0^{c}	32.5^{no}	8.4^{l}
A*B	57.2^{g}	15.5^{bc}	34.8^{op}	9.0^{l}
A B*	56.2^{g}	16.2^{c}	35.5^{p}	8.5^{l}
A*B^1	46.8^{e}	12.5^{b}	31.8^{n}	7.0^{l}
A^{1}B*	48.7^{ef}	12.0^{b}	33.2^{nop}	7.8^{l}

Values not followed by a common letter differ significantly (P = 0.05) according to Duncan's test.

Table 4. Uptake of labelled cadmium by radish, per cent of added amount.

	1st harvest	2nd harvest	Total	
	tops and roots	tops and roots	tops and roots	tops
»Carex clay»				
(AB)*	2.3^{a}	1.7^{k}	3.9^{r}	3.1^{a}
A*B	2.2^{a}	1.8^{k}	4.0^{r}	3.2^{a}
A B*	2.5^{a}	1.9^{k}	4.3^{r}	3.5^{a}
A*B^1	2.3^{a}	1.6^{k}	3.9^{r}	3.1^{a}
A^{1}B*	2.2^{a}	1.8^{k}	3.9^{r}	3.2^{a}
»Sphagnum clay»				
(AB)*	5.5^{cd}	4.0^{lm}	9.5^{tu}	7.4^{cd}
A*B	5.8^{d}	4.3^{m}	10.1^{u}	8.0^{d}
A B*	5.7^{d}	4.3^{lm}	10.0^{u}	7.8^{cd}
A*B^1	4.7^{b}	3.6^{l}	8.3^{s}	6.5^{b}
A^{1}B*	4.9^{bc}	4.1^{lm}	8.9^{st}	7.2^{c}

Values not followed by a common letter differ significantly (P = 0.05) according to Duncan's test.

manner. For the first harvest, the content of labelled cadmium in the roots after cadmium addition to single components was lower than when cadmium was added to the mixture.

Of the cadmium added to the »Carex clay», 3.9—4.3 % was taken up by the plant (Table 4). The method of addition did not influence the results. The »Sphagnum clay» released about twice as much of its cadmium, 8.3—10.1 %. The method of addition gave rise to differences similar in direction to those in cadmium contents, which is natural since yield could not be shown to be dependent upon the method of addition. Radish tops contained altogether significantly more labelled cadmium when the labelled cadmium had been added to the peat and unlabelled cadmium to the clay than when labelled cadmium had been added to the clay and unlabelled cadmium to the peat.

The incubation experiment

The contents of native cadmium extractable in 1 N HCl in the experimental soils and their components were on average only 2 % of the cadmium added. Other extractants (1 N NH_4OAc and 0.01 M $CaCl_2$) released even less of the native cadmium, whose influence on the retention and release of cadmium can thus be disregarded.

Almost 90 % of the cadmium added to both of the pot experiment soils was still extractable in hydrochloric acid at the end of the experiments 104 d from their establishment (Table 5). There were no differences between the soils. The proportion extractable from »Sphagnum clay» was found to diminish during the course of the experiment.

Table 5. Percentage of added cadmium extractable with 1 N HCl.

	Incubation time, days				
	9	26	41	63	104
»Carex clay»	92^{ab}	99^{g}	93^{k}	86^{rs}	85^{x}
»Sphagnum clay»	99^{b}	98^{g}	92^{k}	88^{rs}	87^{x}
Clay	89^{a}	89^{f}	87^{k}	80^{r}	76^{w}
Carex peat	89^{a}	89^{f}	87^{k}	92^{s}	94^{y}
Sphagnum peat	96^{ab}	97^{g}	95^{k}	94^{s}	92^{xy}

Values in the same column not followed by a common letter differ significantly (P = 0.05) according to Duncan'a test.

$LSD_{0.05}$ between columns: 6.

Table 6. Percentage of added cadmium extractable with 1 N NH_4OAc, pH 7.0.

	Incubation time, days				
	9	26	41	63	104
»Carex clay»	30^{ab}	27^{g}	30^{l}	28^{s}	27^{b}
»Sphagnum clay»	48^{c}	40^{i}	43^{m}	39^{t}	49^{d}
Clay	27^{a}	21^{f}	18^{k}	14^{r}	12^{a}
Carex peat	33^{b}	31^{h}	32^{l}	31^{s}	31^{c}
Sphagnum peat	56^{d}	56^{j}	59^{n}	62^{u}	59^{e}

Values in the same column not followed by a common letter differ significantly (P = 0.05) according to Duncan's test.

$LSD_{0.05}$ between columns: 3.

Table 7. Distribution of added cadmium in extracts made with 0.01 M $CaCl_2$ and in soil.

	Cd added to soil mg/kg (1)	Cd after incubation for 9–104 days		
		in $CaCl_2$ extract µg/l (2)	in soil mg/kg (3)	(2)/(3)
»Carex clay»	1.88	28.9	1.71	16.9
»Sphagnum clay»	1.91	46.4	1.61	28.8
Clay	2.12	10.5	2.05	5.1
Carex peat	17.38	123	10.95	11.2
Sphagnum peat	19.77	261	3.97	65.7

As a rule, significantly less was exrtactable from clay than from the soils used in the pot experiment. The extractable proportion decreased during the course of the experiment and was finally less than 80 %. The proportion extractable from peat alone was generally at least as large as that extractable from the mixtures, but 26 days from the beginning of the experiment the Carex peat released less than the »Carex clay».

Ammonium acetate extracted about 30 % from the »Carex clay» and from the »Sphagnum clay» considerably more, over 40 % (Table 6). The proportion extractable did not alter during the incubation period. After 9 d from addition, clay alone released almost 30 %, after 104 days from addition only a little over 10 %. The Carex peat released slightly more than the corresponding clay mixture. The Sphagnum peat released considerably more than the »Sphagnum clay».

In the »Carex clay», the ratio of cadmium extractable from the soil in 0.01 M $CaCl_2$ and the cadmium content of the soil was noticeably smaller than in the »Sphagnum clay». In Table 7, only averages of different extraction runs are shown, since no statistically significant differences were found between them. In Carex peat alone, the ratio was smaller than in the corresponding clay mixture. In clay alone, the ratio was smaller still. However, in the Sphagnum peat the ratio was much larger than in the other soils, which shows that it retained cadmium very poorly.

Autoradiography

Cadmium is fairly evenly distributed within the cotyledons (Fig. 1 a), but in the first true leaves the bulk of the cadmium accumulated in small spots. Apparently most of the cadmium is present in the leaf veins. In subsequent autoradiograms (Fig. 1 b–c) the point-like accumulations in the true leaves showed up very clearly. The older leaves appeared to contain more cadmium than the young leaves and in the former some of the cadmium was distributed between the points of accumulation. The roots contained considerable amounts of cadmium whereas the swollen stem base which acts as a nutrient store contained very little.

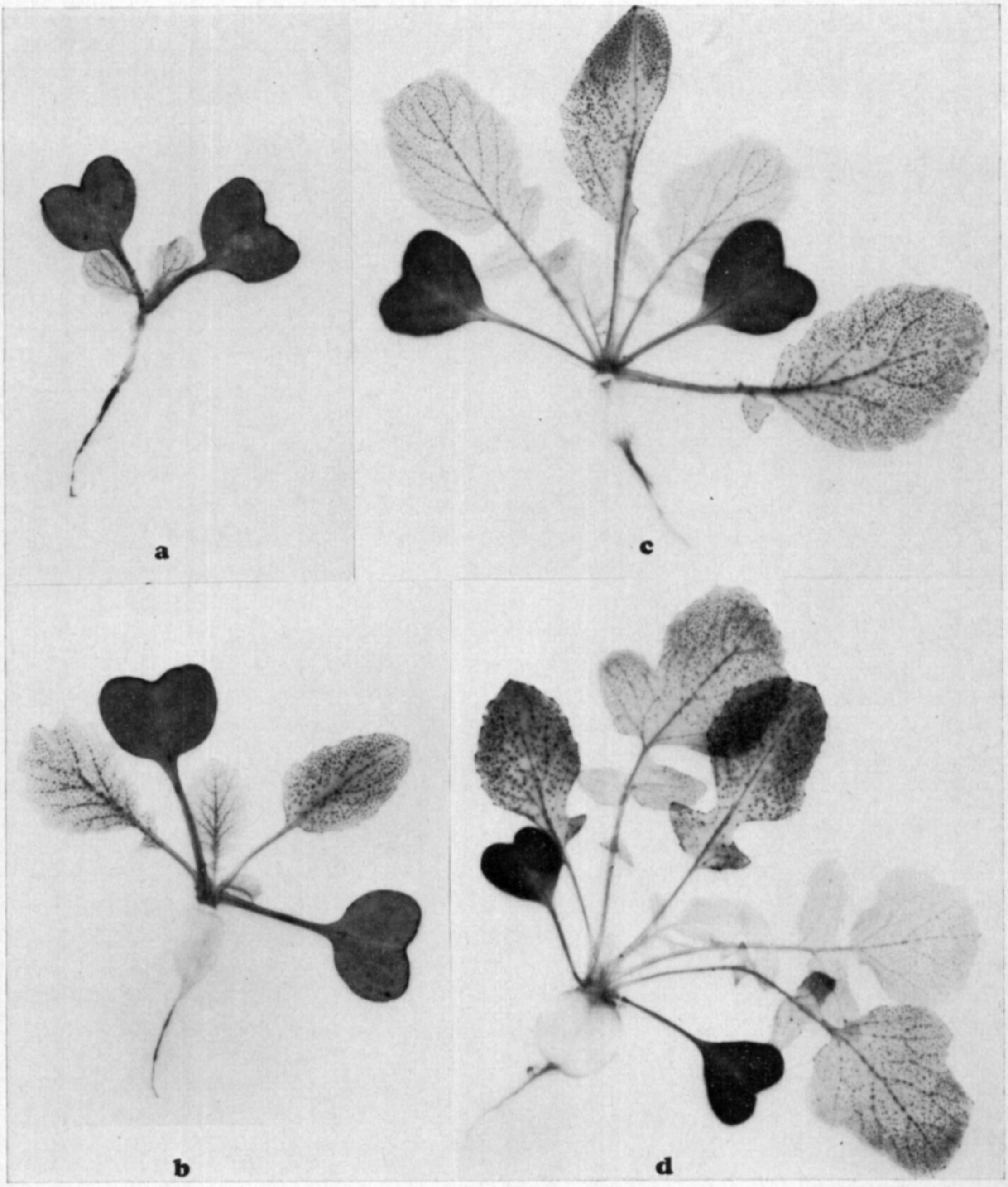

Fig. 1. Autoradiograms of 21 (a), 28 (b), 35 (c) and 39 (d) day old radish seedlings.

Discussion

Radishes took up considerably more of the cadmium added to the mixture of heavy clay and Sphagnum peat (»Sphagnum clay») than to the mixture of the same clay and Carex peat (»Carex clay»). Since the radishes grew equally well in either experimental soil, one can assume that there was no difference in cadmium uptake ability of radishes between the mixtures, rather the differences are due to the direct influence of the soil on the added cadmium.

In the incubation experiment, it was found that noticeably less cadmium was extracted by 1 N ammonium acetate from »Carex clay» treated with cadmium chloride than from »Sphagnum clay» after the same treatment. Likewise, extraction with 0.01 M $CaCl_2$ released more cadmium from the former than from the latter soil. Apparently the »Carex clay» retained added cadmium more strongly than the »Sphagnum clay». If one assumes on the basis of the results published by JOHN (1972) that the retention of cadmium observes the Langmuir adsorption therm and that in this case the amount retained is only a fraction of the adsorption maximum, one can obtain an estimate of the cadmium content of the soil water. From measurements made on calcium chloride extracts, the water held in the »Carex clay» (treatment (AB)*) was estimated to contain 29 μg/l Cd and in the »Sphagnum clay» 49 μg/l Cd. In the experiment made by LAGERWERFF and BIERSDORF (1972), the cadmium content of radish leaves increased by about 0.8 mg/kg for a 1 μg/l increase in the cadmium content of the nutrient solution when zinc was available to the plants to roughly the same extent as in the pot experiment in this study. If the radishes had taken up all the cadmium via the soil water, then on the basis of the results of LAGERWERFF and BIERSDORF (1972) the cadmium content of the water during the development of the first harvest would be approximately 30 μg/l (»Carex clay») and 60 μg/l (»Sphagnum clay») and during the development of the second harvest 20 and 40 μg/l, respectively. Apparently cadmium dissolved in the water of these soils provided an important source of cadmium to radishes, and the cadmium content of a 0.01 M calcium chloride extract gave a satisfactory estimate of cadmium availability.

In the incubation experiment, added cadmium was retained by heavy clay much more strongly than by either of the peats. A 0.01 M calcium chloride extract of the heavy clay contained only about 10 μg/l Cd. A similar content would have occurred in extracts made on the clay-peat mixtures had not the binding ability of the clay been modified by the peat. This was not the case. The increase found in the cadmium content was due at least in part to increased acidity. In the clay extracts the pH was on average 4.7, in the extracts made on the mixtures 3.9 (»Carex clay») and 3.6 (»Sphagnum clay»). In the pot experiment the pH values after the last harvest were 4.5 and 4.1, respectively. According to ANDERSSON and NILSSON (1974), clay retains considerably less cadmium at pH 4 than at pH 4.7. The same authors claim that iron oxides and hydroxides, which occurred abundantly in the heavy clay and Carex peat used in this study, begin to retain cadmium only when the pH rises above pH 4.5.

ANDERSSON and NILSSON (1974) have found that the cadmium retaining capacity of organic soils, too, increases when the pH rises over a range within which the »Carex clay» and »Sphagnum clay» fall. On the basis of the results published by these authors, the difference found in cadmium retaining capacity and availability between these soils could not be attributed solely to differences in acidity. The Carex peat with its better retaining capacity was considerably richer in fulvic and humic acids than the Sphagnum peat. The significance of these acids in retaining cadmium may be considerable.

Even though the clay was found to retain cadmium very strongly, apparently part of the cadmium retained was released when the acid peat was mixed with the clay. This is indicated by the fact that the availability of cadmium to the plants did not depend upon whether the cadmium had been added to the mixture of Carex peat and clay or to the clay alone 5 weeks before the peat was added. Correspondingly, the addition of clay to Carex peat treated with cadmium appered to increase the retention of cadmium.

Cadmium added to the components of the »Sphagnum clay» caused a larger response in the cadmium content of radish tops than did cadmium added to the already prepared mixture. The cadmium content did not depend upon which component the added cadmium had been present in before mixing. It is conceivable that when it moves from one component to another after mixing, cadmium becomes readily available to plants.

On the basis of the results of the pot experiment, it can be concluded that a doubling from about 5 to 10 mg per pot of the cadmium added to the »Sphagnum clay» does not double the cadmium content or cadmium uptake of radishes. Within this range (cadmium content of tops about 30—100 mg/kg), it seems likely that the plant can to some extent restrict its uptake of cadmium. At high levels of cadmium application, there were indications that cadmium added to Sphagnum peat on the one hand and to clay on the other before they were mixed did not move freely from one component to the other, since the proportion added to the clay was found to be less available than that added to the peat.

The autoradiograms confirm the idea gained from the pot and incubation experiments, that cadmium is translocated to the radish plant and moves upwards inside it with the transpiration stream with little hindrance. In contrast to this, translocation towards the root hardly occurs.

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SELOSTUS

Maan orgaanisen aineksen laadun vaikutus kadmiumin kertymiseen kasviin

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Astiakokeessa verrattiin kahden erilaista orgaanista ainesta sisältävän happaman (pH_{CaCl_2} 4—4.5) maan kykyä luovuttaa lisättyä kadmiumia kasville. Maat valmistettiin keinotekoisesti sekoittamalla 1 paino-osa toisaalta sara-, toisaalta rahkaturvetta aitosaveen. Tutkimuksessa käytettiin radioaktiivisella isotoopilla ^{115m}Cd merkittyä kadmiumia.

Saraturpeen ja saven seokseen lisätty kadmium (5.12 mg/3 kg maata) kohotti ensimmäisen retiisisadon maanpäällisten osien kadmiumpitoisuutta 23 mg/kg ja toisen 16 mg/kg. Kadmiumpitoisuuden lisääntyminen oli riippumaton siitä, oliko lisäys suoritettu valmiiseen seokseen vai jompaan kumpaan komponenttiin ennen sekoitusta. Maanalaisissa osissa pitoisuudet olivat vain 1/3—1/4 em. pitoisuuksista ja käsittelyjen väliset erot olivat vastaavasti pienemmät.

Rahkaturpeen ja saven seos luovutti lisättyä kadmiumia selvästi helpommin. Ensimmäisen retiisisadon maanpäällisissä osissa pitoisuus oli 51 mg/kg ja toisen 33 mg/kg, mikäli kadmium oli lisätty valmiiseen seokseen. Ensimmäisen lehtisadon kadmiumpitoisuus kohosi n. 10 %, jos lisäys oli suoritettu vain toiseen komponenttiin, mutta ei riippunut siitä kumpi tämä komponentti oli.

Kaksinkertainen kadmiumannos saraturpeen ja saven seokseen kaksinkertaisti retiisin kadmiumpitoisuuden lisäyksen. Sensijaan rahkaturpeen ja saven seoksessa viljellyn retiisin kadmiumpitoisuus nousi suhteellisesti vähemmän.

Astiakokeen rinnalla suoritetun muhituskokeen perusteella voitiin osa havaituista eroista selittää kadmiumin liukoisuuden eroista johtuviksi. Liukoisuuserojen aiheuttajia ei voitu sitovasti osoittaa. Saraturpeen fulvo- ja humiinihappoihin, joita rahkaturpeessa oli hyvin vähän, saattoi pidättyä kadmiumia merkitsevästi.

Astia- ja muhituskokeen sekä autoradiografian, jossa seurattiin kadmiumin kulkeutumista retiisin eri osiin, perusteella pääteltiin veteen liuenneen kadmiumin pääsevän retiisiin melko esteettömästi ja kulkevan ilmeisesti haihtumisvirtauksen mukana.

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