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UPTAKE AND TRANSPORT OF CADMIUM BY PERENNIAL RYEGRASS FROM FLOWING SOLUTION CULTURE WITH A CONSTANT CONCENTRATION OF CADMIUM

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SUMMARY

Perennial ryegrass was sown in flowing solution culture at 7, 6, 5 and 4 weeks before the addition of cadmium to the nutrient solution. The concentration of cadmium in solution was held constant at 0.01 ppm for the following 15 days during which period uptake by the 4 sets of plants of different ages was followed by plant analysis at 3-day intervals. During the 15-day period the total uptake per g (dry weight) root remained nearly constant. The cadmium content of the roots was much greater than that of the corresponding shoots and, although older plants contained more cadmium than younger plants, the proportion of the total content retained by the roots was much the same in the 4 sets of plants, *i.e.* >90 per cent. It is concluded that the roots of ryegrass restrict the transport of cadmium to the shoots. The concentration in the shoots increased only slightly during the 15-day period but to a different extent amongst the 4 sets of plants. These differences reflect differences in growth rate; thus the shoots of the younger sets of plants had lower growth rates and contained correspondingly higher concentrations of cadmium.

INTRODUCTION

Cadmium is receiving increasing attention as an environmental contaminant that may be harmful to human and animal health[17]. There are extensive, low level inputs of cadium to soil and vegetation through the atmosphere, arising mainly as emissions from the smelting and refining of zinc and some other metal ores. The inputs are much higher in areas adjacent to such activities[3 15]. Cadmium is also added extensively, but at low levels, to agricultural soils in phosphatic fertilizers[19 20 21] and less extensively in some sewage sludges in concentrations as high as 1500 ppm[1]. In order to devise methods for minimizing the content of cadmium in crops an understanding is required, not only of its origin and environmental behaviour, but also of factors influencing its movement along the food chain. Under most circumstances the major route of movement is from soil to plant to animal, and uptake by plant roots and transport to their shoots are, therefore, basic links in the food chain. It has been reported that cadmium is readily taken up after addition to either soil[7 12 13] or nutrient solutions[6 11 18] and that there is an accumulation in the above-ground parts[13 18]. The processes controlling uptake and transport in plants are, however, poorly defined and the following studies were designed to examine some aspects of these.

The uptake and transport of cadmium by perennial ryegrass from a flowing nutrient solution containing added cadmium was measured over a 15-day period by sampling plants for analysis at regular intervals. Previous studies have followed uptake and transport after a single 3-day exposure to cadmium in the absence of phosphate in the nutrient solution[9]; in the present studies the concentration of cadmium in the nutrient solution was held constant and phosphate was supplied throughout the 15-day experimental period. A comparison was made of plants that were of different ages at the time cadmium was added to the nutrient solution.

METHODS

Perennial ryegrass (*Lolium perenne* var. S23) was grown in the glasshouse in the system of flowing solution culture that has been described fully elsewhere[4] and used in a recent study of the uptake of cadmium[9]. This system comprises plant culture units constructed of polyethylene, each of which contains 300 l solution circulated through 24 culture vessels of 1.2 l capacity connected in parallel. Each culture vessel has a screw cap holding 6 growth tubes with nylon mesh attached to their lower ends; the mesh is held below the level of the circulating solution and provides support for the experimental plants which are grown from seed in the growth tubes. Six plants were grown in each tube, *i.e.* 36 plants per vessel. The air in the glasshouse was continuously circulated and filtered through charcoal. Day and night temperatures were maintained at 18–25°C and 14–16°C, respectively.

Seeds were sown in 6 culture vessels in each of 2 culture units at 4 one-week intervals to provide 4 sets of plants of different ages. The nutrient solution was that of Hoagland as described by Hewitt[8] but with macronutrients at 1/20 strength and micronutrients at 1/10 strength, and was renewed every 14 days until 7 weeks after the first set of plants was sown (*i.e.* 4 weeks after the fourth set). At this time (day 0) the solution was again renewed but cadmium, as Cd $(NO_3)_2$, was added to both units to give a concentration of 0.01 ppm. During the following 15-day period of growth the cadmium concentration was maintained constant by daily analysis of the nutrient solution, and by the addition of $Cd(NO_3)_2$. The nutrient solution was also analysed daily for potassium and its concentration adjusted to 10 ppm (1/20 strength Hoagland) by the addition of a solution containing an appropriate concentration of potassium. This solution also provided all other nutrients, including phosphate, each supplied in the ratio to potassium that was present in the initial nutrient solution. Iron was supplied throughout as $FeSO_4$, and pH was held at 4.5.

Immediately before the addition of cadmium (on day 0), each of the 4 sets of plants was sampled by removing the intact plants from a single culture vessel in each unit; this procedure was subsequently repeated at 3-day intervals until day 15. The roots were washed in 3 successive lots of deionised water and the plants then separated into shoots and roots and dried at 100°C.

Samples of the dried material were digested in a 5 : 1 mixture of Aristar nitric and perchloric acids and the resulting solution was diluted to standard volume. Cadmium was determined by atomic absorption spectrophotometry (Varian Techtron AA5) using a Varian Techtron hollow cathode tube (Ne-Cd) as source and an air-acetylene flame. Absorption measurements were made at wavelength 228.8 nm and background, non-atomic absorption was estimated where necessary with an H_2-continuum lamp. Cadmium in the culture solutions was determined directly by atomic absorption spectrophotometry as above.

TABLE 1

Dry weight (g) of 4 sets of ryegrass plants grown in flowing solution for 7, 6, 5 and 4 weeks before the addition of Cd; the plants were then grown for 15 days with 0.01 ppm Cd in solution, and sampled at 3-day intervals. Values are means for plants from 2 culture vessels

Days after adding cadmium	Age of plants at time of Cd addition (weeks)							
	Shoots				Roots			
	7	6	5	4	7	6	5	4
0	9.2	4.5	3.0	1.0	1.6	0.8	0.8	0.3
3	12.6	6.7	3.7	1.6	2.3	1.3	0.9	0.5
6	14.3	7.6	5.6	2.0	2.2	1.2	1.0	0.5
9	16.7	7.7	4.4	2.9	3.4	1.4	1.2	1.1
12	19.9	11.9	8.3	3.1	3.8	2.6	2.1	1.0
15	22.7	15.0	11.2	5.3	4.3	3.2	2.9	1.7
SE (19 df)*	1.23				0.29			

* excludes values for day 0

RESULTS

Table 1 shows the dry weights of the 4 sets of plants immediately before adding cadmium and at 5 intervals during the subsequent 15-day period of growth. Uptake of cadmium continued throughout the whole of this period but was most rapid during the first 3-day interval. At the end of the final 3-day interval, younger plants contained less cadmium ($P < 0.001$) than older plants, and the total contents (shoots plus roots) ranged from 588 μg in the youngest set to 1597 μg in the oldest (Table 2). Although total uptake increased with time, uptake per unit weight of root, as expressed by μg cadmium in the plant per g of root (dry weight), did not increase after the first 3-day interval and was, on average for all plants over the 15-day period, 345 μg/g root (Tables 1 and 2). The roots of the youngest set of plants initially took up more cadmium (400 μg/g root) than those of the other sets (on average, 325 μg/g root), but this difference was not apparent beyond the first 3-day interval. The roots of all plants always contained more cadmium than the corresponding shoots ($P < 0.001$) (Table 2). Furthermore, the proportions of the total content that were retained in the roots were similar in the 4 sets of plants at the end of each 3-day interval (Table 3): the mean percentages over the 5 intervals ranged from 93.5 to 95.3. There was, however, a tendency in all sets of plants for the proportion retained in the roots to decrease with time after the addition of cadmium, *e.g.* the mean

TABLE 2

Cadmium content (μg) of 4 sets of ryegrass plants grown in flowing solution for 7, 6, 5 and 4 weeks before the addition of Cd; the plants were then grown for 15 days with 0.01 ppm Cd in solution, and sampled at 3-day intervals. Values are means for plants from 2 culture vessels

Days after adding cadmium	Age of plants at time of Cd addition (weeks)							
	Shoots				Roots			
	7	6	5	4	7	6	5	4
3	19	7	7	7	717	362	312	213
6	21	13	13	9	627	373	350	173
9	55	21	22	28	1119	421	418	363
12	81	44	46	25	1149	781	663	286
15	104	89	80	55	1493	1071	885	533
SE (19 df)	9.9				102.6			

TABLE 3

Cadmium content of ryegrass roots expressed as a percentage of the total in the plant; values are calculated from Table 2

Days after adding cadmium	Age of plants at time of Cd addition (weeks)			
	7	6	5	4
3	97.3	98.1	97.8	96.8
6	96.7	96.4	96.1	95.1
9	95.3	95.0	94.8	92.8
12	93.4	94.7	93.4	92.0
15	93.5	92.4	91.7	90.7

percentages retained were 97.5 and 92.1 at the end of the first and final 3-day intervals, respectively.

The concentration of cadmium in the shoots and roots immediately before its addition to the nutrient solution (on day 0), was on average 0.15 (±0.032) and 0.60 (±0.160) ppm, respectively; this is presumably due to the presence of cadmium in the seed and uptake from impurities in the salts that were used in the nutrient solution. As the result of adding 0.01 ppm cadmium to the nutrient solution there was, during the first 3-day interval, a 30-fold increase in its concentration in the shoots of the youngest set of plants but only a 10-fold increase in those of the other sets (Table 4). The concentration in the shoots of all sets of plants increased during the next 4 intervals until at the end of the final one it ranged from 4.5 to 10.3 ppm. In each 3-day interval there was a general relationship between

TABLE 4

Cadmium content (ppm) of 4 sets of ryegrass plants grown in flowing solution for 7, 6, 5 and 4 weeks before the addition of Cd; the plants were then grown for 15 days with 0.01 ppm Cd in solution, and sampled at 3-day intervals. Values are means for plants from 2 culture vessels

Days after adding cadmium	Age of plants at time of Cd addition (weeks)							
	Shoots				Roots			
	7	6	5	4	7	6	5	4
3	1.5	1.1	1.9	4.7	308.6	278.5	342.1	390.5
6	1.5	1.7	2.4	4.4	291.4	310.7	342.8	387.7
9	3.3	2.8	5.0	9.3	326.6	298.7	345.7	337.1
12	4.0	4.8	5.5	8.2	296.5	305.1	315.2	296.3
15	4.5	5.9	7.3	10.3	349.5	335.5	305.4	306.4
SE (19 df)	0.80				17.30			

the age of the plants and the concentration of cadmium in the shoots; thus, it was higher in shoots of younger than in those of older plants ($P < 0.001$). In the roots the concentration was always much greater ($P < 0.001$) than in the corresponding shoots. Initially, the roots of the 2 youngest sets of plants had higher concentrations than those of the other 2 sets but, over the 15-day growth period there was, on average, little difference between the concentration in the 4 sets of plants.

DISCUSSION

In previous studies of cadmium uptake by ryegrass over a period of 4 hours we concluded that it was, in the short term, predominantly an exchange adsorption process[9]. With lead there is evidence that in the longer term there may be a component of uptake, both by ryegrass[10] and by barley[2], that is under metabolic control. The present investigation with cadmium further indicates that even over the longer term, *i.e.* 15 days, uptake from a flowing nutrient solution containing 0.01 ppm added cadmium is predominantly an exchange adsorption process. Thus it was rapid, and during the first 3 days after adding cadmium, an average of 339 μg/g root was taken up; this rate was maintained as the roots increased in weight during the next 12 days. In contrast to lead, however, there was no overall increase in uptake per unit weight of root, and at the end of the 15-day period the figure for uptake was still only 352 μg/g root.

The roots of ryegrass have been shown to act as a 'barrier' restricting transport of cadmium to the shoots following a single 3-day exposure after it was added (in the absence of phosphate) to a flowing nutrient solution[9]. Thus, 88 per cent of the total amount taken up by the plants was retained in the roots during this 3-day period. When the nutrient solution was changed to contain no added cadmium, and phosphate as normal, there was no further transport to the shoots. In the present investigation cadmium was added to the nutrient solution which contained phosphate as normal. It is notable that, at the end of the first 3-day interval after adding cadmium, 97.5 per cent (mean) of the total amount taken up by the plants was retained in the roots (Table 3). Although there was a gradual decrease in this proportion during the 15-day period of uptake it did not fall below 90.7 per cent. These results are consistent with our earlier observation[9] that the roots of ryegrass restrict the transport of cadmium to the shoots and the suggestion that this may be related to the solubility of cadmium phosphate.

It is unlikely that the plants in the present investigation suffered toxicity since there were no visible signs and, furthermore, we have previously found that there were no effects on the growth of perennial ryegrass even although the cadmium contents were as high as 1004.3 ppm in the roots and 45.7 ppm in the corresponding shoots[9]. Other studies with ryegrass have shown that contents of 50 to 100 ppm in the shoots are required to produce significant effects on growth[5].

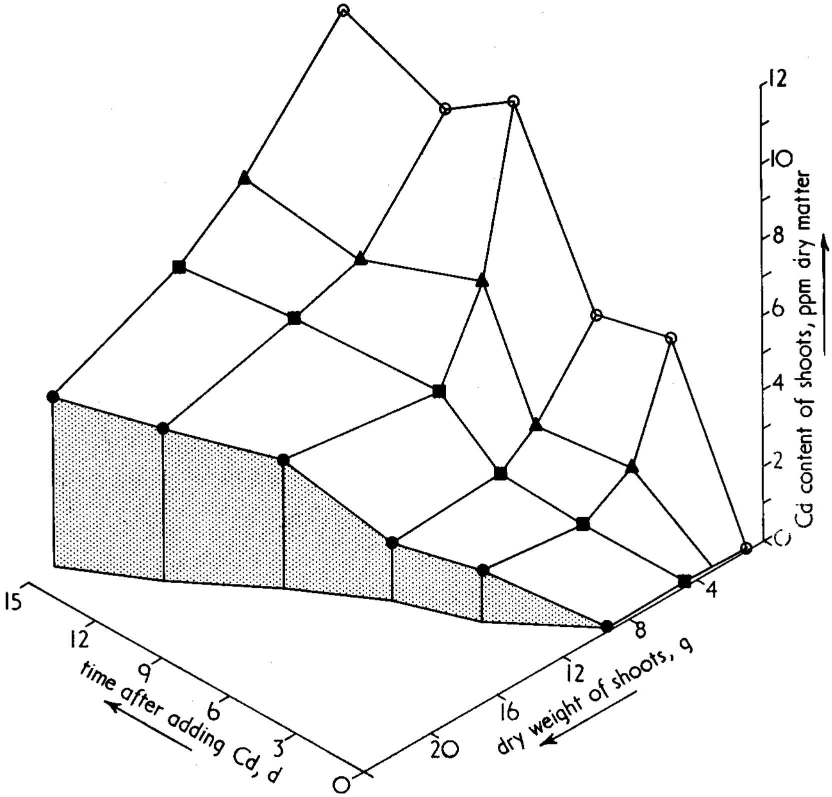

Fig. 1. Relation between dry weight (g) and Cd content (ppm) of the shoots of 4 sets of ryegrass plants grown in flowing solution for 15 days with 0.01 ppm added Cd. The plants were sown at 7 (●), 6 (■), 5 (▲) and 4 (○) weeks before the addition of Cd and then sampled at 3-day intervals.

The relationship between growth and the concentration of cadmium in the shoots is illustrated in Fig. 1 and calls for comment. In all sets of plants there is an increase in cadmium concentration with time which reflects the gradual decrease in the proportion of total uptake that was retained in the roots (Table 3). It is notable that this increase was faster in younger than in older sets of plants. This may be explained by considering the rate at which the plants were growing. The dry weight of the shoots of each set increased linearly over the 15-day period, but the growth rate was lower in younger than in older sets (Table 1); calculated values for the youngest to the oldest are 0.26, 0.51, 0.65 and 0.87 g per day. The cadmium transported to the shoots of the youngest set was therefore diluted to a markedly lesser extent than in the oldest with a corresponding difference in concentration in the dry matter. The other two sets of plants had intermediate growth rates and intermediate cadmium concentrations in their shoots. This effect is further illustrated in Fig. 2, which also shows the increases in cadmium concentration that occurred with time (*i.e.* during 9 days) as a result of decreases in the proportion retained in the roots.

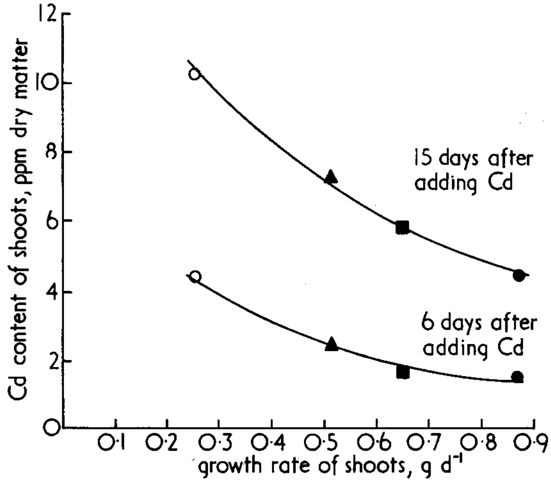

Fig. 2. Relation between growth rate (g d^{-1}) and Cd content (ppm) of the shoots of 4 sets of ryegrass plants grown in flowing solution for 15 days with 0.01 ppm added Cd. The plants were sown at 7 (●), 6 (■), 5 (▲) and 4 (○) weeks before the addition of Cd and then sampled at 3-day intervals. Plotted points are for days 6 and 15.

Other effects of growth on the concentration of heavy metals in plant shoots have been described. For example, substantial increases in lead concentration have been found in the shoots of plants whose growth rate was slow due either to a nutrient deficiency[14] or to season[16]. In situations where additions of heavy metals to soil are likely to increase uptake by plants, the rate at which they are growing therefore becomes an important factor in the movement of heavy metals along the food chain. Plants whose inherent growth rate is slow, or which are growing slowly because of environmental or nutritional constraints, are likely to contain elevated concentrations of heavy metals such as cadmium and lead and will represent a greater hazard to the animal that ingests such plants than those with higher growth rates. Under such circumstances the adoption of management practices which minimise this hazard, *e.g.* encouragement of optimum growth, choice of appropriate crop species or variety, becomes important.

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