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INVESTIGATION INTO THE 2,4—D ACCUMULATION OF WATER-PLANTS

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Abstract

Water-plants are capable of accumulating the residues of weedicides in direct ratio to 2,4-D concentration. By this herbicide accumulation in the *in vitro* systems, however, there is always drawn away considerably less herbicide from the solution used as an experimental medium than it happens in the natural ecosystem. A decrease in the pesticide pollution can therefore only be expected in living systems as a result of the water-vegetation. At the chemical weed control of the water-plants considered to be harmful — what is today a mostly unsolved problem, as yet — the application of 2,4-D is to be determined with due regard for species. In case of the *Elodea canadensis* investigated by us, the efficient dose is so high from the point of view of protecting water quality that, due to the acute danger, it is not allowed to be used even if in living waters the water-vegetation is able to achieve comparatively rapidly a high degree of accumulation.

Introduction

Among the anthropogenic effects befalling the living waters, the industrial pollutions are still outstanding quantitatively in our days, but the agricultural pollutions, and among them primarily those induced by plant-protecting insecticides, are quite as much exposed, owing to their hidden dangerousness. Every link of the aquatic ecosystem is touched by the pesticide pollution of the surface waters and even the residues of the protective agents got into are exposed to several biological effects.

The different algal species respond differently to the effect of 2,4—D. The 2,4—D concentration is, for instance, of toxic effect on Cylindrospermum above 0,8 ppm (SINGH 1974), on Chlorella pyrenoidosa, Chlamydomonas eugametos, and Scenedesmus quadricauda already above 0,2 ppm (VANCE & SMITH 1969). On the other hand, it is true that in case of a smaller residue of herbicides, in a synchronous culture of due cell density, the herbicide put in the water is decomposed by the latter species (VALENTINE & BINGHAM 1974).

The most characteristic biological indicators of the pollution of living waters by herbicides are the water-plants because these species are able to accumulate a very large amount of the residues of herbicides (FUNDERBURK & LAWRENCE 1963), and in this way they can diminish in certain cases the pesticide level of waters.

The decomposition of 2,4—D in water takes practically place in the same way as in the soil (HURLE & RADEMACHER 1970). There is an adaptation of bacterial flora, here too, but similarly under aerobian conditions alone. And this is important in this medium because the molecules of the agents present in the water can settle down in the mud, as well. Under normal conditions there are aerobian relations here and 2,4—D is breaking down well (ALY & FAUST 1964). But the anaerobity of mud or of a layer of mud may ensure the herbicidal pollution of water because under anaerobic conditions there is no decomposition possible. In a polluted water, the herbicidal accumulation in fish is also very considerable. From the different tissues, the least undecomposed 2,4—D was found in the muscle, on the other hand, there was very much 2,4—D metabolite there (SCHULTZ 1973).

Materials and Methods

Elodea canadensis, grown under artificial conditions, was applied as an experimental plant. It was desirable that, before the treatment carried out under laboratory conditions, the experimental object should be influenced by no herbicidal effect because the determination needing a high analytical precision could be considerably disturbed by the possible presence of chemical agents or other extraneous substances. The time of treatment was determined in eight and fifteen days. At the doses, a 2,4-D concentration of 0,6 3,0 6,0 166,0 333,0 ppm was applied.

The 2,4-D content of the vegetable dry-matter samples was determined by a gas-chromatographic methodics. Extraction was performed with the procedure of *Faust & Suffet* (1972), the qualitative demonstration and quantitative measuring were carried out on the basis of the experimental method of ZWEIG & SHERMA (1972).

Results and evaluation

The results, the majority of which were achieved in a direct way from a gas-chromatographic analysis, were given indirectly by our earlier peroxidase-activity and indole-hydroxylation investigations concerning the action-mechanism of 2,4—D (KERESZTES 1976). While namely the effect of treatment on the amount of herbicides, getting into the water-plant and accumulating there, could only be concluded — by reason of the biochemical indicators — from the results of the two former methods, by the gas-chromatographic procedure it was possible to evaluate this phenomenon immediately. The formation of the quantities of herbicide-residues was measured at the date of the treatment and the result is shown in Table 1.

It is apparent that the accumulated 2,4—D values are growing directly proportional to the increase in concentrations, what does correspond to the facts established so far. (We have used different quantitites of the vegetable matter prepared for being analysed because it was advisable owing to the peculiarities of the method, the possible limits of traceability, etc.) A much more interesting picture was, however, obtained after studying the changes in the accumulation of herbicide-residues between 8 and 15 days, as depended on the treatment periods. The results achieved are shown in Tables 2 and 3.

We could establish from Table 2 that during the 15-day treatment time the largest increase in concentration, as compared to the 8-day treatment, was induced by the 6 ppm herbicide treatment. The extent of the increase, taken as a function of concentration, is sine-curve-like. That — together with some other physiological indices — leads us to conclude the optimum of the 6 ppm concentration.

This supposition was strongly confirmed by the results of Table 3 in which we had expressly investigated into the efficiency factors of the accumulation of herbicide-residues. On this occasion, however, we were only taking into consideration the

Table 1. *Development of the quantities of herbicide-residues in Elodea canadensis as a result of the herbicide-treatment*

Treatment period (days)	2,4—D cc. (ppm)	Wet matter g	Dry matter g	Wetness per cent	2,4—D content mg/kg wet matter	2,4—D content mg/kg dry matter
	0,6	17,88	1,10	94,2	0,011	0,18
	3	15,95	0,94	94,4	0,028	0,48
8	6	13,93	0,80	94,6	0,039	0,68
	166	10,79	0,65	94,3	0,920	15,30
	333	10,38	0,56	94,9	2,080	38,50
	0,6	24,55	1,24	04,9	0,025	0,49
	3	22,51	0,91	95,0	0,082	1,63
15	6	16,58	0,83	96,0	0,143	3,54
	166	12,59	0,70	94,4	2,060	37,20
	333	7,19	0,35	95,1	2,170	44,70

Table 2. *Change in the extent of the accumulation of herbicide-residues in Elodea canadensis as a result of the 8- and 15-day herbicide treatment*

Treatment cc. (ppm)	2,4—D content mg/kg wet matter		Extent of growth	2,4—D content mg/kg wet matter		Extent of growth
	8	15 days		8	15 days	
0,6	0,011	0,025	2,27	0,18	0,49	2,72
3	0,028	0,082	2,92	0,48	1,63	3,39
6	0,039	0,143	3,66	0,68	3,54	5,20
166	0,920	2,060	2,23	15,30	37,20	2,43
333	2,080	2,170	1,04	38,50	44,70	1,16

Table 3. *Change in the efficiency of the accumulation of herbicide-residues in Elodea canadensis as a result of the 8- and 15-day herbicide treatment*

Treat-ment cc. (ppm)	Effective increase in cc.	Value of the measured		percentage change in cc.		Differential quotient of the changes in cc. $\left(\frac{\Delta y}{\Delta x}\right)$	
		8	15	8	15	8	15 days
0,6	0	0	0	0	0	0	0
3	5	2,54	3,28	50,8	65,6	0,0007	0,0023
6	2	1,39	1.74	69,5	87,0	0,0036	0,0203
166	27,7	23,58	14,40	85,12	52,5	0,0055	0,0119
333	2	2,26	1,05	113,0	52,17	0,0069	0,0006

results achieved from the vegetable wet matter because here were only decisive the processes taking place in the solution—and not those already developed and fixed. At this evaluation, a great emphasis was laid on the fact that the effective increase in the concentration of treatments, as compared with one another, did not agree, even in a single case, with the relative value of the concentrations observed in the water-plants. The latter value was mostly considerably lower.

The deviation of the two values, expressed in percentage, between the single concentrations on the eighth day was uniformly rising — between the concentrations of 166 and 333 ppm there was only found a single positive deviation, higher than 100 per cent — on the fifteenth day, however, the maximum was represented by the value between the concentrations of 3 and 6 ppm, followed later by a gradual decrease. It is to be noticed that, in the relation of both treatment dates, as well — taking into consideration the similar factors — the largest percentile increase was observed here.

The same results were practically achieved, by calculating the differential quotients of the changes in concentration. The results obtained are represented in Figs. 1 and 2.

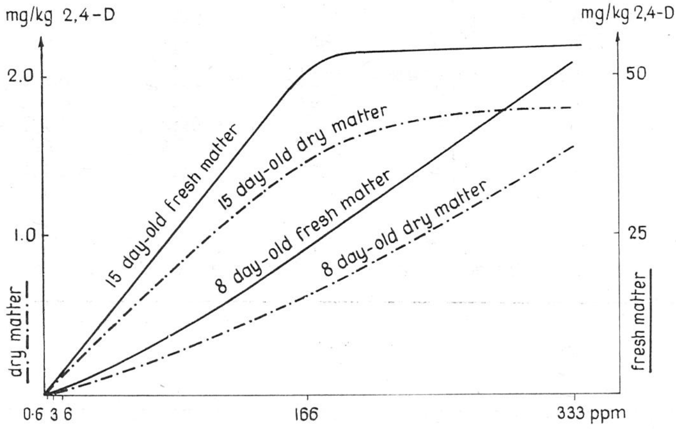

Fig. 1. Accumulation of the agent 2,4-D of different concentration in *Elodea canadensis*.

It is visible that the values of the herbicide-residue are showing a tendency of uniform upsweep on the eighth day — in close connection with the above percentile values — and on the fifteenth day they have already the character of a saturation curve. The same is shown by the graph of the differential quotients and it is very expressive that the most efficient change in concentration is indicated by a salient vertex.

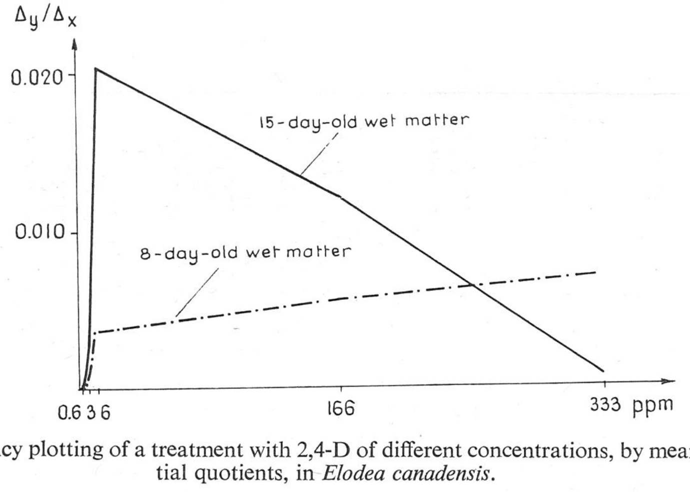

Fig. 2. Efficiency plotting of a treatment with 2,4-D of different concentrations, by means of differential quotients, in *Elodea canadensis*.

But the investigation of the 2,4—D solution itself, containing different concentrations, parallel to examining the water-plant, would have been enabled by the gas-chromatographic methodics applied only with a large material input. We have therefore applied theoretical calculations for establishing the decrease in the 2,4—D concentration of the solution that developed in *Elodea canadensis* as a result of the accumulation of the herbicide-residue. The results achieved in this way have been elaborated in Table 4.

Table 4. *The calculated values of the decrease in concentration of a solution containing 2,4—D in different concentrations, in the course of 8 and 15 days. (The concentration has developed in Elodea canadensis as a result of the accumulation of some herbicide-residues*

Solution cc. (ppm)	Volume *l*	Vegetable wet matter (g)		Vegetable 2,4—D content (mg/kg)		Decrease in 2,4—D concentration				The value of change in cc. between days 8 & 15
						mg/l		p. c.		
		8	15	8	15	8	15	8	15 days	
0,6	1,001	17,88	24,55	0,011	0,025	0,000197	0,000614	0,033	0,102	3,11
3	1,004	15,95	22,51	0,028	0,082	0,000447	0,001846	0,015	0,061	4,12
6	1,007	13,93	16,58	0,039	0,143	0,000540	0,002371	0,009	0,039	4,39
166	0,597	10,79	12,59	0,920	2,060	0,016600	0,025900	0,010	0,015	1,56
333	0,694	10,38	7,19	2,080	2,170	0,031100	0,015600	0,011	0,006	0,50

From the values calculated the surprising conclusions could be drawn hat *in vitro,* in a quite "sterile" milieu, only a minimum change in concentration was induced by the accumulation of herbicides in the water-plant. It is to be supposed that a considerable decrease in the pesticide content of water — owing to the large accumulation of the herbicide-residue — is to be expected only in living systems. In case of the Elodea canadensis, however, the efficiency of the 6 ppm concentration can be measured even here.

The gas-chromatographic investigation has also thrown light upon why the possibility of the inversed sikimi acid — IAA reaction way, brought up by us already earlier (KERESZTES 1976), is only holding good till the eighth day. It is obvious from Fig. 1 that the accumulation curve of the herbicide residue recorded on the eighth day is still of quite open character, as opposed to the saturation curve of the 15th day. It is explained by the picture obtained that the 2,4—D treatment — and, parallel to it, the accumulated 2,4—D concentration — are only effective in physical but mainly in biochemical sense as long as they have not reached the saturation limit. The optimum efficiency of 2,4—D on water-plants depends, therefore, upon three factors: the dose applied, the time of treatment, and the species investigated. According to the statement of our experimental series, the most efficient concentration used by us proved to be 6 ppm, the optimum time of action was found being eight days. By the biochemical changes, developing as a result of the concentrations applied in a wide intervall, even the species particularity was displayed well.

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