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A Comparison of Plant Toxicities of Some Industrial Chemicals in Soil Culture and Soilless Culture

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The toxicities of 10 industrial chemicals to three types of plants were determined according to OECD Guideline 208. Apart from two types of soil, soilless culture was used in the test. The results with terrestrial plants in these tests were also compared with those of the same chemicals tested with aquatic plants (duck weed, algae) in nutrient solution. It was found that in soilless culture the EC_{50} values obtained were much lower than those in soil culture. Soilless culture of terrestrial plants and tests with aquatic plants yielded about the same results. It is concluded that adsorption of the chemicals to soil particles is the main factor governing the results. In an additional experiment with Cd, it was demonstrated that the Cd content of reacting plants was about the same in tests carried out in soil and in nutrient solution, pointing to the obvious fact that the amount of chemical taken up is the basis for the toxic effect. In the interpretation of tests carried out with chemicals added directly to soil, this should be taken into account. It is recommended that soilless culture of terrestrial plants be seriously considered in ecotoxicological testing.

INTRODUCTION

Whereas the testing of chemicals for aquatic toxicity is of long standing (cf. Mayer and Ellersieck, 1986), a more standardized approach to the terrestrial ecotoxicity of industrial chemicals is a rather recent development. Instrumental in this are the requirements of the so-called 6th Amendment level 1 testing of the European Community (*Official Journal CEC* L259 of 15.10.1979) and the development of OECD Guidelines in this matter (OECD, 1984). Until now, few results based on these requirements have been published in the literature. In these regulations, tests with both terrestrial plants and worms are envisaged. The following paper, for reasons of methodology, will be restricted to experiences with plants using the official test and some modifications of it which appeared to be useful.

In terrestrial tests the chemicals to be tested are usually added directly to a soil of a certain prescribed type. In the case of the OECD Guideline 208, as used by us, this is a loam-type soil. The type of soil may influence test results with plants in two ways:

(1) The rate of growth of plants may be different in different soils.

(2) Adsorption of the test chemical to soil particles may be involved.

In order to gain some insight into the practical importance of these factors, the authors arranged for a comparative assay of 10 different industrial chemicals in tests with terrestrial plants. Three different species of test plants (tomato, oats, lettuce) were cultured in two types of soil (loam and humic sand) as well as in soilless culture (aquaculture). In earlier tests (Adema, 1985) six of the chemicals had already been

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tested against different aquatic plants,[1] which offered a further possibility of comparison of test results under different conditions. In particular, the importance of possible adsorption of test chemicals to soil particles might be observed as well as the influence of these different growth conditions.

MATERIALS AND METHODS

The compounds used in the tests were potassium bichromate (Cr), cadmium chloride (Cd), potassium chlorate ($KClO_3$), trichloroacetic acid (TCA), diisopropylamine (DIPA), 2,4-dinitrotoluene (DNT), 2,6-dimethylquinoline (DMQ), 2,4-dichloroaniline (DCA), tetrapropylene benzene sulfonate (TPBS), and 2,4,6-trichlorophenol (TCP). TPBS was used as a 5% solution in water; DNT contained about 40% water, but based on dry weight its purity was 98%. The other chemicals were pure compounds with purities > 98%.

Plants used were *Avena sativa* (oats); *Lycopersicum esculentum*, Bellina RZ (tomato); and *Lactuca sativa*, Ravel RZ (lettuce).

Two types of soil were used: humic sand (organic matter 3.7%, pH 5.1, silt 5.8%, sand 90.5%) and loam (organic matter 1.4%, pH 7.5, silt 17.1%, sand 71.7%). In addition, the plants were tested on a complete aqueous nutrient solution according to Steiner (1968).

Soil tests were carried out according to OECD Guideline 208 (1984), the water content of the soils being 80% of their maximum water holding capacity. Test concentrations were spaced by a factor of 3.2 and expressed as milligrams per kilogram of dry soil.

DNT, DMQ, DCA, and TCP were dissolved in acetone and added as concentrated solutions to fine silver sand. The acetone was evaporated at room temperature as prescribed by OECD Guideline 208 and the sand mixed with the soil. The other chemicals were added as concentrated solutions in water to the soil, such that the desired final concentrations of chemicals and water in the soil were achieved.

Five pots (disposable plastic, diameter 8 cm, height 6 cm) were filled from each batch of dosed soil; one pot was used for analysis of the chemical at the start of the test and four were sown with 10 seeds each. Since only five plants per pot were required, any additional germinating plants were discarded. Twenty plants were thus exposed to each concentration of each chemical. The pots were covered with glass plates until germination of the plants. The plants were grown in a culture room at 25 ± 2°C, 65% relative humidity, and 6500 lx from fluorescent tubes (color 57).

Evaporation of water was accurately compensated daily by addition of distilled water; in this way pots without holes could be used and cross-contamination or contamination of the surroundings could be avoided.

Two weeks after germination the plants were cut off just above the soil and the wet weight was immediately determined using an automatic balance, connected to a computer for data processing.

The pH and water content of the soil were determined at the start and end of the test.

[1] Lemna minor (duck weed), several unicellular algae, and a flagellate, viz., *Scenedesmus* sp. *Euglena* sp.

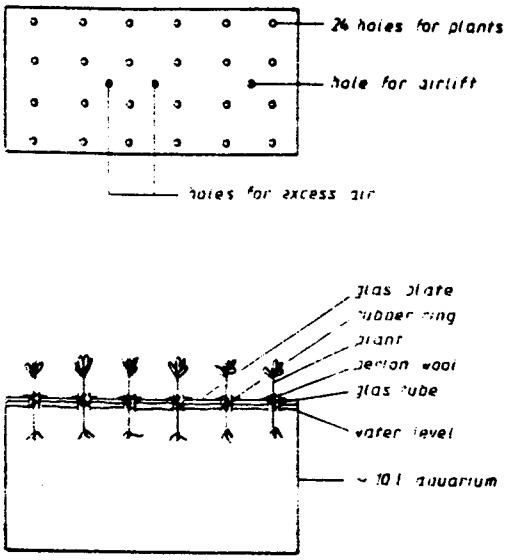

FIG. 1. Test system for plant growth test in nutrient solution (soilless culture).

Tests with soilless culture (aquaculture) were carried out in 10-liter aquaria as indicated in Fig. 1. The water was aerated and recirculated with an airlift. Plants were sown as precultures in perlite soaked with the test solution (nutrient solution with the test chemical concentration) to be tested in the aquaria. Two to three days after germination the young plants were taken from the pots with perlite and placed with their roots in the test solutions in the aquaria. Test concentrations were spaced by a factor of 3.2 and expressed in milligrams per liter of nutrient solution. One aquarium, with 20 plants, was used for each test concentration.

The test conditions and measurements were as far as possible analogous to those in the soil tests as noted above; in addition, the oxygen content of the test solutions was monitored during the test.

As far as possible the concentration of the chemicals was determined by chemical analyses at two or three dose levels in the soils and nutrient solution at the start and end of the test. Chemical analyses were not carried out for $KClO_3$ or TCA, or for DCA in soil. The other test compounds were analyzed after mild extraction (e.g., with acetone, ethanol, or methanol) of the soil and usually by direct analysis of the nutrient solutions. Cleanup procedures were used where necessary. Usual analysis and detection methods were used (AAS for metals, GC with ECD or FID, or spectrophotometric analysis for the organic compounds). Additionally, cadmium was also analyzed in some of the plant samples.

For each plant species, test compound, and medium, a curve of the average wet weight per plant with its standard deviation after 2 weeks of growth versus the concentration of the test compound was constructed (examples in Figs. 2 and 3) and from this relation the EC_{50} (and its confidence limits) was calculated according to Kooijman *et al.* (1983):

$$W_{(c)} = W_0 \Big/ \left[1 + \left(\frac{C}{EC_{50}} \right)^s \right],$$

where $W_{(c)}$ is the weight at concentration c. W_0 is the weight of the control plants, g

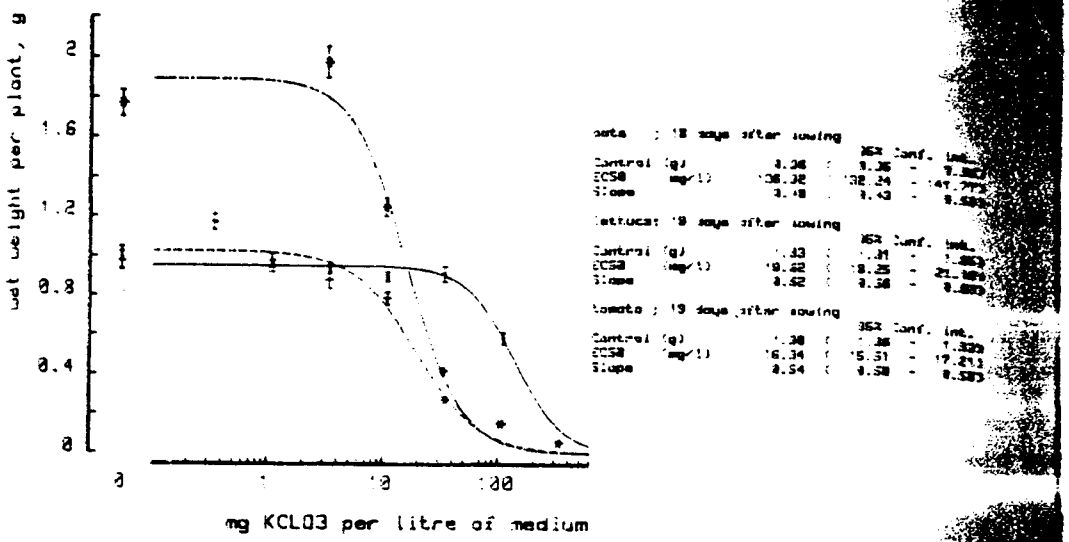

FIG. 2. Growth of oats, lettuce, or tomato at various concentrations of $KClO_3$ in nutrient solution (mg liter^{-1}). (—) Oats, 18 days after sowing; (---) lettuce, 19 days after sowing; (-·-·-) tomato, 19 days after sowing.

is the parameter for the gradient, and EC_{50} is the concentration at which the weight of the plants is half of that of the control plants.

The highest concentration tested that did not cause a plant weight significantly different from the control weight was determined with the Student t test at a 5% level of significance and is indicated as no observed effect concentration (NOEC).

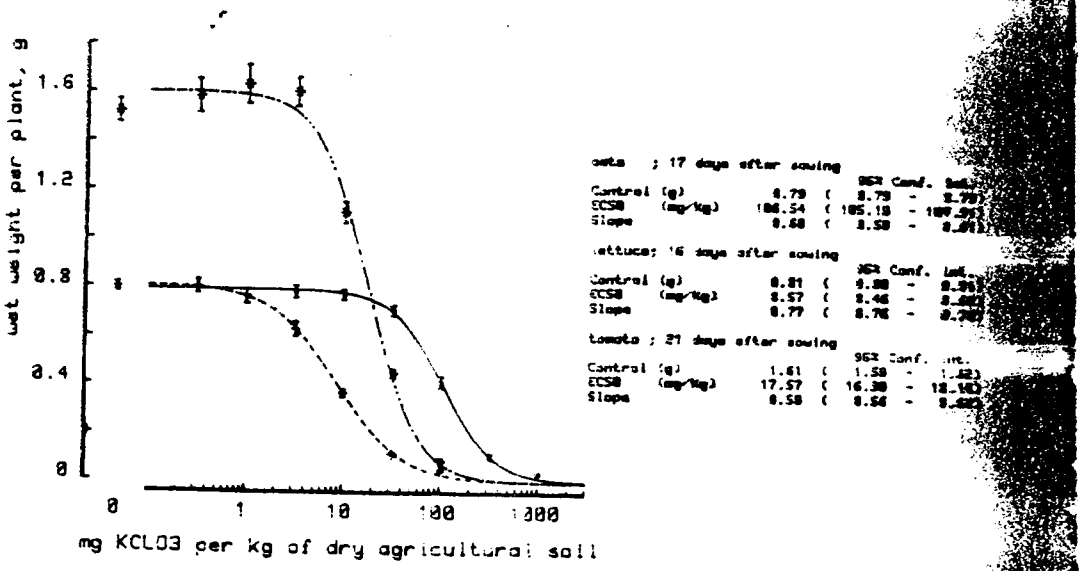

FIG. 3. Growth of oats, lettuce, or tomato at various concentrations of $KClO_3$ in loam (mg kg^{-1} based on dry soil). (—) oats, 17 days after sowing; (---) lettuce, 16 days after sowing; (-·-·-) tomato, 21 days after sowing.

TABLE 1

AVERAGE CONCENTRATIONS OF CHEMICALS IN TEST MEDIA AT THE START (t = 0) AND END (t = 14 d) OF THE TEST IN PERCENTAGE OF NOMINAL VALUES

	Medium					
	Nutrient solution		Loam		Humic sand	
Test compound	t = 0	t = 14 d	t = 0	t = 14 d	t = 0	t = 14 d
Cr	99	113	86	n.d.[a]	86[b]	86[b]
Cd	104	80	115	106[b]	97[b]	85[b]
DIPA	111	84	50	68	48	n.d.
DNT	97	103	63	44	34	65
DMQ	94	72	63	39	74	65
DCA	80	47	n.d.	n.d.	n.d.	n.d.
TPBS	92	57	83	58	78	64
TCP	111	0	75	0.4	92	0.7

[a] Not determined.
[b] Corrected for background levels in the soils.

RESULTS

The results of the chemical analyses (Table 1) revealed that the concentrations of the chemicals for the tests in nutrient solution were within a range of about 20% around the nominal value at the start of the test. DCA and TPBS decreased to about 50 and 60%, respectively, of the nominal value at the end of the test and TCP was no longer detectable. The concentrations of the other test compounds at the end of the test were about the same as those at the start. The concentrations of Cr and Cd in both soils, at the start and end of the test, were—when corrected for the background values for these elements—about equal (within 15%) to the nominal values. The background concentrations were about 20 (loam) or 10 (humic sand) mg Cr per kilogram dry soil and about 1 mg Cd per kilogram dry soil for both soils. The concentrations of the organic compounds in soil were mostly somewhat lower than the nominal values. The concentration of TCP at the start of the test was about equal to the nominal value, but as in the nutrient solution, this compound disappeared almost completely during the exposure period.

The somewhat low figures determined, compared to the nominal values, are assumed to be a consequence of a rapid adsorption to soil and the mild extraction method used in the analytical determinations. Furthermore, since a difference of a factor of 2 in results is not considered of great importance in environmental testing, all results are expressed relative to the nominal concentrations tested.

Figures 2, 3, and 4 present examples of the results of the growth tests. Figures 2 and 3 are straightforward dose–response curves for $KClO_3$ in nutrient solution (Fig. 2) and in one of the soil types (loam, Fig. 3). Figure 4 compares the results in three media (nutrient solution, humic sand, and loam) for one of the three plant species used (oats). In Fig. 4 the concentrations of the chemical in both soil types were calculated in milligram per liter water based on the water content of the soils, e.g., 10 mg per kilogram dry soil equals 33 mg per liter water when the water content of the soil

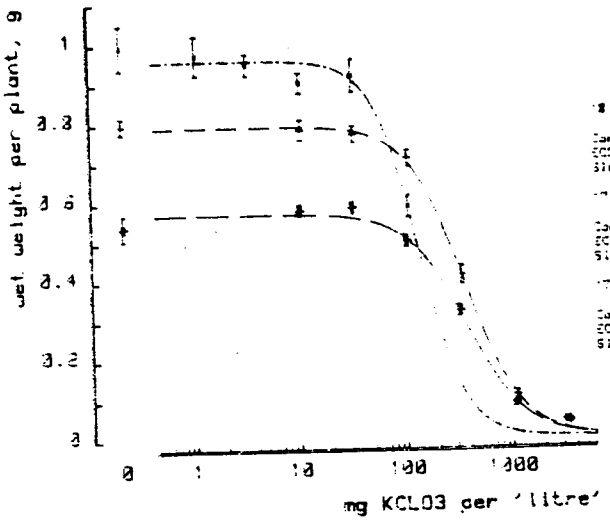

FIG. 4. Growth of oats in three media at various concentrations of $KClO_3$ calculated per liter of aqueous phase (1 liter nutrient solution = 1 liter aqueous phase; 1.3 kg wet loam consists of about 300 ml water and about 1 kg dry loam; 1.3 wet humic sand consists of about 300 ml water and about 1 kg dry humic sand). (—·—) nutrient solution, 18 days after sowing; (--) loam, 17 days after sowing; (—) humic sand, [illegible] days after sowing.

is 30% based on dry weight. This conversion was made to estimate qualitatively the effect of absorption of the chemical on the soil: a large difference between the EC_{50} in milligrams per liter determined in the nutrient solution and that *calculated* in milligrams per liter for the soil type (Table 3) implies significantly decreased availability of the compound to the plants. When the presence in the soil is established as shown by chemical analysis, it is assumed that substantial binding to the soil has occurred.

For each of the 10 chemicals tested, six figures were established, the results of which are summarized in Tables 2 and 3. The EC_{50} and NOEC values in these tables are based on the nominal concentrations. The oxygen concentrations in the nutrient solutions were always >6 mg liter^{-1} and would not have influenced the test results. The pH values of the test solutions and soils were—with a few exceptions—the same as those of the controls; only high (≥300 mg per kilogram or liter) concentrations of DIPA or TCA altered the pH of the media to some extent. In the case of DIPA this may have caused (part of) the effects found. The effects of TCA were found at lower concentrations than those influencing the pH. The water content of the loam and humic sand during testing was about the same in all tests, viz., 29.5 and 30.4%, respectively, based on dry soil.

DISCUSSION

The EC_{50} values in humic sand were in general somewhat higher (2–5 times) than those in loam (Table 2), indicating less adsorption of most chemicals to loam, the soil type prescribed in OECD 208. Only cadmium was probably adsorbed more to loam, as revealed by comparison of the EC_{50} values. Since the adsorption of cadmium to

TABLE 2

EC_{50} AND NOEC VALUES[a]

Test compound	Medium	EC_{50} (mg kg^{-1})			NOEC (mg kg^{-1})		
		Lettuce	Tomato	Oats	Lettuce	Tomato	Oats
Cr	Loam[b]	1.8	6.8	7.4	0.35	3.2	3.5
	Humic sand	>11	21	31	>11	10	11
	Nutrient solution	0.16	0.29	1.4	0.04	0.11	0.12
Cd	Loam	33	171	159	3.2	32	10
	Humic sand	136	16	97	32	<3.2	10
	Nutrient solution	0.84	3.0	6.0	<0.35	1.1	<3.5
$KClO_3$	Loam	8.6	13	107	1.0	3.2	10
	Humic sand	44	20	117	3.2	10	10
	Nutrient solution	20	16	137	3.4	3.3	3.4
TCA	Loam	37	154	4.6	3.2	32	1.0
	Humic sand	560	124	8.1	100	32	3.2
	Nutrient solution	48	59	19	11	3.2	1.1
DIPA	Loam	560	624	725	100	100	320
	Humic sand	953	1118	1880	320	32	100
	Nutrient solution	283	180	594	34	108	108
DNT	Loam	5.8	4.9	46	3.2	3.2	11
	Humic sand	13	10	35	3.2	3.2	10
	Nutrient solution	2.1	2.1	5.3	1.1	0.33	1.1
DMQ	Loam	14	16	26	1.0	10	10
	Humic sand	55	57	78	3.2	10	32
	Nutrient solution	2.3	2.0	3.9	0.34	0.34	0.34
DCA	Loam	18	18	28	10	10	10
	Humic sand	136	68	107	32	32	32
	Nutrient solution	3.5	4.3	6.8	0.35	1.1	1.1
TPBS	Loam	70	111	208	<1.0	32	100
	Humic sand	216	217	567	10	10	100
	Nutrient solution	6.7	12	21	0.34	3.4	3.4
TCP	Loam	180	323	560	32	100	100
	Humic sand	144	398	514	32	100	100
	Nutrient solution	2.2	1.0	6.8	0.11	0.11	<0.35

[a] Based on mg test compound per kg dry soil or mg per liter (= kg) nutrient solution for 10 chemicals.
[b] Loam according to specifications in OECD 208.

clay particles present in silt is known to be high (CEC, 1981), this is to be expected. For all other chemicals the adsorption to humic sand was higher than that to loam, in agreement with the higher content of organic matter of humic sand and the nature of the chemicals (neutral organics or negatively charged ions).

Lettuce and tomato were about equal in sensitivity to the chemicals, with lettuce being slightly more sensitive. Oats were—on average—three times less sensitive than the dicotyledons, except to TCA, a known monocotyledon toxicant.

Table 3 clearly shows lower EC_{50} values (based on dosed amounts) for the chemicals when tested in nutrient solution than when tested in the soils. This indicates that part or most of the chemicals in the soils was not available to the plants, most probably due to adsorption to the soils.

By determining the ratios between the EC_{50} values in soil (based on its water content) and nutrient solution (EC_{50} soil/EC_{50} nutrient solution), the lowest figures (3–

TABLE 3

EC_{50} VALUES[a] BASED ON THE ASSUMPTION THAT THE TEST COMPOUND CONCENTRATION PRESENT IN SOIL IS TOTALLY PRESENT IN THE WATER PHASE, COMPARED TO EC_{50} VALUES IN NUTRIENT SOLUTION

Test compound	Medium	EC_{50} (mg per liter water)		
		Lettuce	Tomato	Oats
Cr	Nutrient solution	0.16	0.29	1.4
	Loam	5.9	22	24
	Humic sand	<38	68	104
Cd	Nutrient solution	0.84	3.0	6.0
	Loam	114	561	560
	Humic sand	430	53	305
$KClO_3$	Nutrient solution	20	16	132
	Loam	29	58	364
	Humic sand	143	65	376
TCA	Nutrient solution	48	59	[illegible]
	Loam	123	510	[illegible]
	Humic sand	1300	407	[illegible]
DIPA	Nutrient solution	283	180	[illegible]
	Loam	1300	2100	[illegible]
	Humic sand	3062	3595	6044
DNT	Nutrient solution	2.1	2.1	[illegible]
	Loam	19	16	46
	Humic sand	42	33	112
DMQ	Nutrient solution	2.3	2.0	[illegible]
	Loam	49	55	87
	Humic sand	171	176	[illegible]
DCA	Nutrient solution	3.5	4.3	[illegible]
	Loam	56	63	[illegible]
	Humic sand	451	225	356
TPBS	Nutrient solution	6.7	12	[illegible]
	Loam	239	382	713
	Humic sand	743	748	1951
TCP[b]	Nutrient solution	2.2	1.0	[illegible]
	Loam	560	1118	[illegible]
	Humic sand	470	1301	1637

[a] In mg per liter water.
[b] Probably rapidly degraded in all media and even faster in soil than in water.

5) in both soils were found for $KClO_3$ and TCA, very water-soluble dissociated compounds for which a relatively low adsorption to soil is to be expected. (The negatively charged ions will not adsorb strongly to clay particles present in silt—which are also charged negatively—or to organic matter.) For DMQ, DCA, and TPBS the ratios were about the same: 20–30 for loam and 70–90 for humic sand. DIPA and DNT also had about the same ratio: 7–8 for loam and 10–20 for humic sand. The ratios were high but irregular for Cr and Cd and exceedingly high for TCP. The latter are neglected because of the probable rapid disappearance of TCP.

Figure 5 shows the results for determinations of Cd in tomato exposed to contaminated nutrient solution (at four levels) or humic sand (at two levels). On the basis of the dosed amounts of Cd in nutrient solution, the EC_{50} for tomato is 3 mg liter

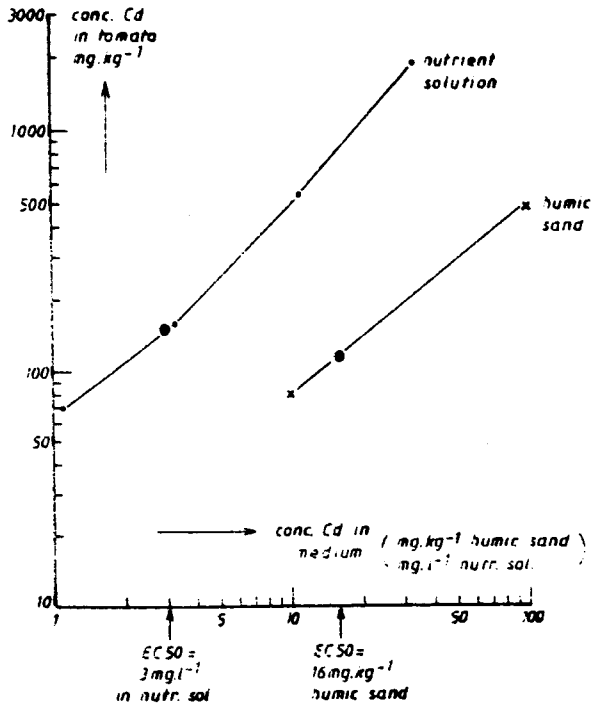

FIG. 5. Relation between internal concentration of Cd in tomato plants and the EC_{50} values obtained in nutrient solution (EC_{50} = 3 mg l^{-1}) or humic sand (EC_{50} = 16 mg kg^{-1}).

(Table 2); from the graph it is estimated that at that concentration the plants contained about 150 mg of Cd per kilogram dry tomato plant leaves. On the basis of the dosed amounts of Cd in humic sand, the EC_{50} for tomato is 16 mg kg^{-1} (Table 2); from the graph it is estimated that at that concentration the plants contained about 120 mg of Cd per kilogram dry tomato plant leaves. Apparently the EC_{50} for Cd is reached at about the same concentration of Cd in the plants. This may taken as an indication that the difference in EC_{50} may be explained mainly on the basis of physical retention (adsorption) by the soil.

Table 4 compares the toxicities of six of the chemicals to the higher plants determined in the aqueous medium with those to duck weed and unicellular green algae (cf. Adema 1985), all based on EC_{50} values for growth. The EC_{50} values for these various organisms are surprisingly close, indicating common sensitivity of these different plants to the chemicals used, which might be due to the involvement of a plant system common to the species. Photosynthesis suggests itself as such.

CONCLUSION

It is suggested that the test in nutrient solution can form a basis for toxicity determinations of chemicals for higher plants. The test is easily conducted; chemical analysis in water is less complicated than that in soils, test concentrations can be maintained when desired (as in aquatic toxicology by continuous flow or renewal systems), and plant growth is standardized (in various soils this is dependent on the soil "quality").

TABLE 4

COMPARISON OF TOXICITY AMONG AQUATIC PLANTS[a]

Chemical	$EC_{50} < 1$					$1 \leq EC_{50} < 10$					$10 \leq EC_{50} < 100$					$100 \leq EC_{50} < 1000$				
	A	D	O	T	L	A	D	O	T	L	A	D	O	T	L	A	D	O	T	L
Cr				0.3	0.2	1.1	1.3	1.4												
DIPA																275	200	590	180	280
DNT						3	2	5	2	2										
DMQ								4	2	2	20	16								
DCA								7	4	4	11	10								
TPBS										7	60	21	21	12						

Note. The data on duckweed and unicellular algae are quoted from Adema (1985). The other data are from the present publication. Toxicity determined in aqueous systems and expressed as EC_{50} values (*E*, growth).

[a] Unicellular algae (A), duckweed (D), and terrestrial plants (oats (O), tomato (T), and lettuce (L)).

Combined with the determination of the adsorption (and degradation if relevant) of chemicals to various soils, the toxicity (EC_{50} value) in those soils may be calculated, rather than determined by soil tests with plants; if necessary the result so obtained can be confirmed by a limited number of actual soil tests. Taking into account the considerable influence of adsorption of test chemicals to soil particles, and the indication by the experiment with cadmium that the internal concentration of the agent is the decisive factor in toxicity, it is suggested that soilless culture of terrestrial plants should also be seriously considered in ecotoxicological testing.

ACKNOWLEDGMENT

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