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Toxicity and Bioaccumulation of Cadmium in Experimental Cultures of Duckweed, *Lemna polyrrhiza* L.

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Knowledge of the mechanism of Itai-Itai disease (Friberg et al. 1976) aided the research concerning the bioaccumulation of heavy metals in plants and aquatic organisms. Because of their characteristics, lemnaceae can be considered as an interesting experimental materiel (Hillman 1961). Their small size, rapid growth and vegetative reproduction permitted us to obtain experimental cultures and to study the effect of cadmium: toxicity and bioaccumulation. The species Lemna polyrrhiza having a very voluminous root system, was used in this work. The effects of cadmium chloride ($Cd\ Cl_2$-$2.5\ H_2O$) and Cadmium sulfate ($3\ Cd\ SO_4$-$8H_2O$) were compared.

The toxicity effect was approached by the numeration of plants and determination of different data :
The concentration causing 50 % decrease of plant multiplication and growth : multiplication and growth E.C. 50 (effective concentration 50). The concentration for which 50 % of the population is morbid (morbidity concentration 50 = MC 50).

MATERIALS AND METHODS

Lemna polyrrhiza SC 83 (Spirodella polyrrhiza) was grown with Tellier's mineral nutritive solution (1963). All plants were continuously illuminated by a white light (1600 Lux) with a dimred light in an air conditioned room at 26 ± 1°C. The control was carried out by counting samples after eight days of growth. Homogenous samples of one hundred plants were made up by selecting colonies of the same aspect (typical colonies of three and four plants). During the numeration, two kinds of population were discriminated : a population of healthy plants (n), a population of morbid plants (n'). These results were used in the calculation of different daily rates (with n_o, starting number of plants and t time in days).

- Rate of growth : $R_G = \dfrac{n - n_o}{n_o \times t}$ %

- Rate of multiplication : $R_M = \dfrac{(n + n') - n_o}{n_o \times t}$ %

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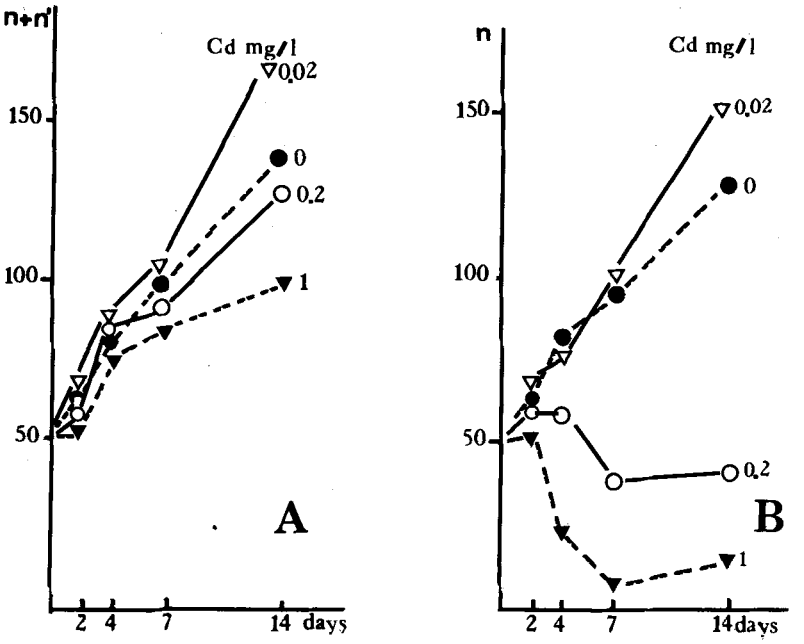

Figure 1. Nutritive solutions supplied with Cd Cl_2. 2.5 H_2O
1A Vegetative multiplication, 1B Growth.

Intervals of normals values were determinated during the control periods :

- R_G = from 12 to 15 %
- R_M = from 14 to 16.5 %

Toxicity was investigated through two series of experiments, one with Cd Cl_2 2.5 H_2O, another with 3 Cd SO_4 8 H_2O with concentrations : 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8, 1 mg/l. Each starting sample was set up with 50 healthy plants. The R_G and R_M were calculated for each level of contamination and used respectively for the determination of EC 50 of growth and EC 50 of multiplication by Log-probit plot.

During the bioaccumulation test, measurements were performed on the plants and nutritive media. For each sample, 2 gr (fresh weight of plants) were washed in distilled water and lyophilised. 0.1 gr (dry weight) was heated until dryness in 5 ml HNO_3 and then, 2 ml H_2O_2 were added and heated at 90°C. The final sample was adjusted to five ml by acidified water. Those digests were quantified using flame atomizer spectrophotometer, instrumentation laboratory aa/ae. The lowest levels of contamination were quantified using a carbon graphite furnace spectrophotometer Varient AA 1275. Results were interpreted by calculating the speed of removal and factors of concentration. During the bioaccumulation study, the plants grew 48 hours in media containing 0, 0.1

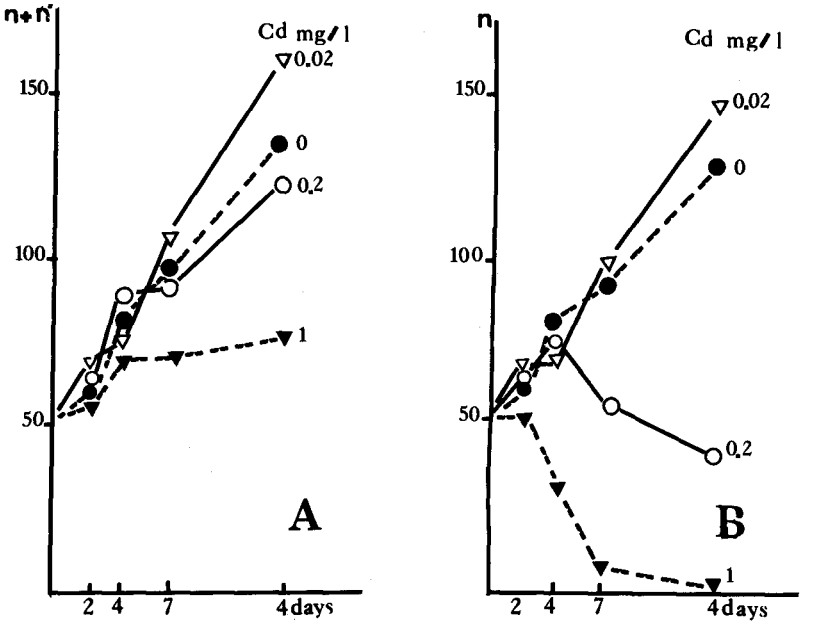

Figure 2. Nutritive solutions supplied with 3 Cd SO_4. 8 H_2O
2A Vegetative multiplication, 2B Growth.

or 1 mg/l Cd. The same plants were afterwards quickly washed with distilled water and placed on contaminated media for the release study.

RESULTS AND DISCUSSION

Typical results for growth and vegetative multiplication are shown by the graphics (see Fig. 1 and 2) at different Cd concentrations. There is no signifiant variation of response between the two compounds (Cd Cl_2 and Cd SO_4).

Stimulation of growth were observed for 0.02 mg/l and multiplication stimulation up to 0.1 mg/l. High concentrations entailed a fast decrease of the healthy population whereas the impact on the total population was not obvious. Multiplication EC 50 were much higher than growth EC 50 (see table 1).

Cadmium principally involved an aging process of the plants but no particular inhibition of their multiplication. EC 50 values are similar to those usually obtained with unicellular algae (Mugel and Ferrard 1978) and Lemnaceae studies (Chouikki 1980).

Neverthless, previous publications (Hutchinson and Coyrka 1975) did not show any stimulation effect at low levels of Cd for Lemnaceae. These results are quite typical for algeae (Lue Kim et al. 1980, Devi Prasad and Devi Prasad 1982) and cell cultures.

In another way, controls showed a linear growth of populations during the 14 days experiment. So the plants did not suffer any

nutritional diseases and observed effects were only caused by toxic compounds added to the nutritive solutions.

Table 1. Statistical data

Exposure in days	EC 50 of growth mg/l		EC 50 of veget. multip. mg/l		MC 50 mg/l	
	$CdCl_2$	$CdSO_4$	$CdCl_2$	$CdSO_4$	$CdCl_2$	$CdSO_4$
4	0.17	0.25			0.62	0.80
7	0.11	0.10			0.20	0.39
14	0.09	0.08	0.90	0.60	0.10	0.15

First experiment showed that the transfer of Cadmium from the nutritive solution to plants was fast. The system then reaches a state of equilibrium. Those two periods are clearly shown during 48 hours of culture (Fig. 3).

The rates of Cd accumulation in plants (Sq) and Cd epuration in solutions (Se) were calculated after 6 h (period of fast transfer). Those data were compared to concentration factors (Fc) obtained at the equilibrium after 48 hours of growth (see table 2).

Removal rate did not increased with increasing Cd concentrations in nutritive solutions. Higher concentration factors were obtained with controls. This contradiction did not permit us to decide about any relationship between Cadmium concentration in nutritive solutions and bioaccumulation.

Table 2. Removal speeds and concentration factors in bioaccumulation test.

Cd in media		Sa mg/l	Sc mg/l	$Sa_{/Sc}$	FC
Control		-0.04	+0.58		2762
0.1 mg/l	$Cd\ Cl_2$	+4.54	-4.83	939	645
	$CdSO_4$	+3.87	-7.5	516	630
1mg/l	$CdCl_2$	+51.37	-83.3	616	643
	$CdSO_4$	+45.95	-77.6	592	500

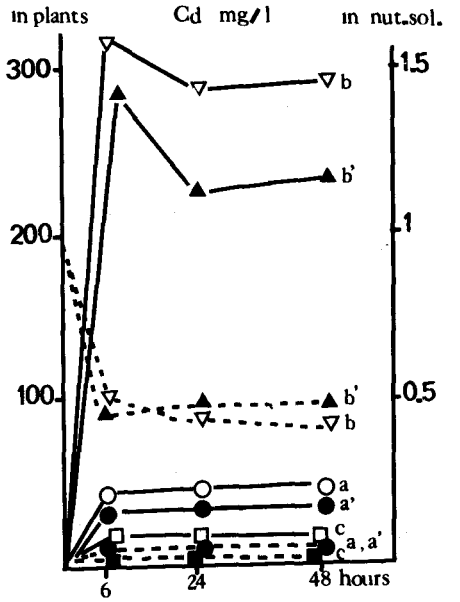

Figure 3. : Cd concentration in plants and solution during a bioaccumulation test. mg of Cd in plants : ——. mg of Cd in nut. sol. : - - -. Starting concentrations : controls c, 0,1 mg/l ($CdCl_2$) a, 0.1 mg/l ($CdSO_4$) a', 1 mg/l ($CdCl_2$) b, 1 mg/l ($CdSO_4$) b'.

Table 3. Cd concentrations in parts of plants

Media	Parts of plants	mg/l of Cd
Control	roots	21
	fronds	10
0.5 mg/l	roots	347
	fronds	111

Variations of the ratio Sa/Sc testify to recovery of Cd in solution by the inner surface of the flasks. This fact explains why the evaporation of the medium during the experiment must not be underestimated in such a static model.

The results in Fig. 4 show outflows of Cadmium from plants to nutritive solution. These slow outflows attest the localization of heavy metal with in the cell partition.

In "Review of Lemnaceae", Hillman (1961) suggested that the roots of these plants were serving chiefly as an anchor to keep the fronds right side up and to protect colonies in dispersal by water motion. Nevertheless the great expansion of root system in Lemna polyrrhiza L could be involved in ionic absorption. In

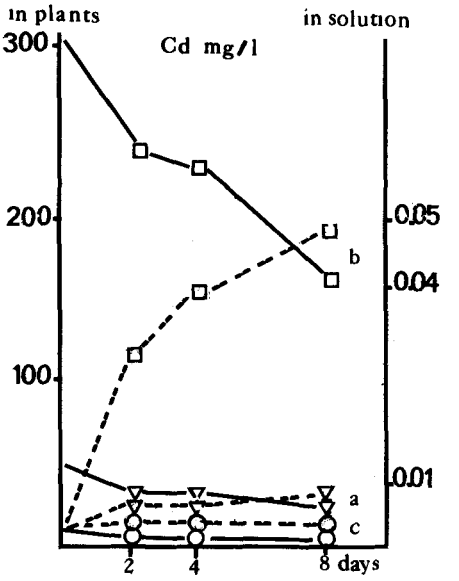

Figure 4. Cd concentrations in plants and solution during the 8 days experiment. —— Cd in plants, - - - Cd in solutions, c - controls, a - plants previously grown in a solution contaminated with 0.1 mg/l Cd, b - plants previously grown in a solution contaminated with 1 mg/l Cd.

order to demonstrate the role of the roots, experiments were made on plants growing in a controlled and contamined media (table 3).

These results demonstrate that in Lemna polyrrhiza L, the roots are the principal Cd absorption organelle and accumulation site of the plants.

This duckweed behaves towards toxic compounds like unicellular algae used in aquatic tests and can bring numerous information in such a test. Nevertheless Lemnaceae are higher plants and these results are more typical of common flora. So this experimental material should be useful in a battery of shorts tests concerning the behaviour of toxic compounds at different stages of aquatic ecosystems.

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