Short communication

Influence of diethyldithiocarbamate on cadmium and copper toxicity to freshwater macrophyte *Spirodela polyrhiza*

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Abstract

Toxic effects of two heavy metals, cadmium (Cd) and copper (Cu), and a fungicide, diethyldithiocarbamate, have been evaluated, alone and in association, on *Spirodela polyrhiza* duckweed cultivated in a synthetic mineral medium and in a distilled water medium. The composition of the culture medium influenced the toxicity of the three compounds and the effects of their associations were clearly shown in the distilled water medium. Copper has an antagonistic effect on Cd and reduced its absorption by duckweed. On the other hand, Cd in the culture medium increased Cu absorption. The most significant effect was observed with diethyldithiocarbamate simultaneously associated with Cu and Cd. The diethyldithiocarbamate association largely inhibited the absorption of Cd and Cu by duckweed and so appeared to have a complexant effect that reduced the toxicity of these two metals.

Introduction

The natural environment is a particularly complex medium in which biological organisms can be exposed to various associations of chemical compounds (Babich and Stotzky, 1985; Friberg et al., 1976). Most ecotoxicological laboratory tests evaluate isolated chemicals, although it is known that interactions always occur in the environment when they are associated. On the other hand, one estimates that these interactions are often conducted to an enhancing toxicity (synergism) while not caring about reducting effects (antagonism). The aim of our study was to simulate interactive phenomena linked to the natural medium complexity and to evaluate the interactions between two heavy metals cadmium, (Cd) and copper (Cd), and a fungicide, diethyldithiocarbamate (DDTC). The choice of these three chemicals was based on the fact that Cd and Cu are common and worrying environmental pollutants while diethyldithiocarbamate is a fungicide, but is also a well-known complexing compound used as an antidote for human detoxification treatment (Grafsträn and Greene, 1980).

For this work, we used a strain of duckweed *Spirodela polyrhiza* maintained on a synthetic culture medium. Previous studies have shown the interest of this test species for the evaluation of toxic phenomena involving heavy metals, especially Cd (Charpentier et al., 1987). Duckweed is easy to cultivate in a laboratory. Its short duration vital cycle and the homogeneity of its populations are favorable factors for their use in ecotoxicological evaluations and constitute a species of choice for the study of the toxic impact of chemicals on freshwater ecosystems (Charpentier and Garnier, 1985).

Cd, Cu and DDTC were first studied separately by determining the influence of the composition of duckweed culture medium. Different assays were performed in parallel studies with a synthetic mineral medium and with a distilled water medium.

In a second step, we compared the effects of different possible associations between Cd, Cu and DDTC on the metal biosorption of duckweed *Spirodela polyrhiza* which was cultivated in a distilled water medium.

TABLE 1
Composition of the Homes modified culture medium

NaH_2PO_4, 2 H_2O	0.6 x 10^{-3} mol/ℓ
KNO_3	2.3 x 10^{-3} mol/ℓ
$MgSO_4$, $7H_2O$	0.5 x 10^{-3} mol/ℓ
$Ca(NO_3)_2$, $4H_2O$	0.7 x 10^{-3} mol/ℓ
EDTA	16.0 x 10^{-6} mol/ℓ
H_3BO_3	1.6 x 10^{-6} mol/ℓ
$MnSO_4$	8.0 x 10^{-6} mol/ℓ
$CuSO_4$	1.0 x 10^{-6} mol/ℓ
$(NH_4)_6Mo_7O_{24}$, $4H_2O$	0.94 x 10^{-8} mol/ℓ
pH	5.4 $\pm$ 0.2

Material and methods

Spirodela polyrhiza L., strain S.C. 83, was harvested in 1983 in a freshwater pond in Normandy and then acclimatised and maintained in our laboratory. The strain was maintained in an exponential growth phase in a synthetic culture medium (Thellier, 1963) which is a modified Homes culture medium (Table 1). Culture bottles were placed in a 24$\pm$1°C thermostated room under a 1600 lux continuous white light. Every 8 d, which is the maximum delay for macrophyte population doubling, fronds were transferred to the fresh culture medium.

Study of Cd, Cu and DDTC alone

Concentrated toxic solutions were prepared in freshly distilled water with cadmium chloride ($CdCl_2$), copper sulphate ($CuSO_4$,$5H_2O$) and sodium diethyldithiocarbamate (DDTC-Na). These concentrated solutions were diluted in distilled water or in a mineral medium to obtain a concentration range (Cd 0, 0.05, 0.1,

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0.2, 0.3, 0.5 mg/ℓ; Cu 0, 0.1, 0.2, 0.4, 0.8, 1.2 mg/ℓ; DDTC 0, 50, 100, 150, 200, 400 mg/ℓ).

For each concentration, four bottles were prepared with 50 mℓ reference pure culture medium or added toxic culture medium. According to previous studies (Charpentier et al., 1987), we placed in each 50 mℓ medium bottle, five whole frond couples, each composed of an association of a mother frond and a daughter frond. Each test was repeated thrice.

In order to assess mortality and growth inhibition in distilled water and in a mineral medium, test solutions with only distilled water medium and culture medium were conducted simultaneously for each series of toxicant.

Bottles were then closed with gauze and placed in a culture room (24 ± 1°C and 1600 lux continuous white light) for 4 d. The test could not excessed 4 d, that is the no-effect maximum delay without effect for duckweed in a distilled water medium. Therefore, after a 2 d and a 4 d culture, every bottle was visually observed and resulting frond multiplication were noted.

The observation of duckweed cultures has allowed us to differentiate between two typical populations: a population of healthy macrophytes and a population of morbid macrophytes. Every frond presenting a necrotic point or simply faded is considered as being morbid. Thus, the numeration of duckweed allows one to calculate two indices that were assessed against fronds having grown in test solution without toxicant.

The morbidity index represents the number of morbid fronds in proportion to the total population of fronds exposed to toxins. For each toxic concentration, we calculated a morbidity index:

$$\text{morbidity index} = (Nm/Nt) \times 100$$

where:
- Nm: number of morbid fronds
- Nt: total number of fronds.

The multiplication index (or growth inhibition index) takes into account the fluctuation of vegetative multiplication for a toxic concentration in proportion to control:

$$\text{multiplication index} = (Nt - Nm)/(Nt_c - Nm_c) \times 100$$

where:
- Nt: total number of fronds,
- Nm: number of morbid fronds,
- Nt_c: total number of fronds in control,
- Nm_c: number of morbid fronds in control.

The calculation of morbidity and multiplication indices for each toxic dose allows one to respectively determine by interpolation the MC50 which is the toxic concentration inducing 50% morbidity in the population and IC50 which is the toxic concentration inducing 50% inhibition multiplication of the fronds.

Study of interactions between Cd, Cu and DDTC

To evaluate the influence of DDTC on Cd and Cu toxicity to *Spirodela polyrhiza*, it was difficult to realise an experiment with many concentrations for all possible cases of mixtures. Moreover, these kinds of experiments associating several chemicals were often difficult to interpret. Thus, we decided to estimate the effect of DDTC on Cd and Cu biosorption after exposing duckweed to isolated or associated chemical solutions.

In order to be sure that medium composition could not influence the interaction study, assays were conducted with distilled water as a culture medium. On the other hand, to ensure good growth conditions so that we could preserve duckweed from toxic effects, we chose three chemical concentrations (Cd 0.1 mg/ℓ; Cu 0.2 mg/ℓ; DDTC 20 mg/ℓ) being lower than MC50 we had determined in a distilled water medium for 4 d exposure.

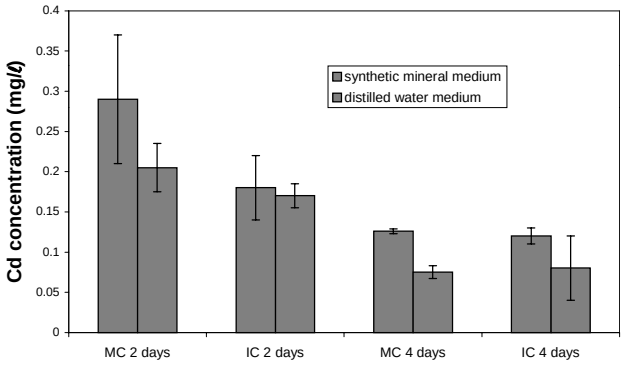

Figure 1

Effects of Cd alone on duckweed after 2 and 4 d exposure in synthetic and distilled water medium (MC50 morbid concentration; IC50 inhibition concentration)

In 1 l bottles, we firstly placed 400 ml of culture medium (control; Cd; Cu; Cd + Cu; Cd + DDTC; Cu + DDTC; Cd + Cu + DDTC) in which we added 2 g of duckweed at the beginning of the experiment. Each series was repeated 5 times so to allow to sample a bottle for analytical determinations in duckweed after 4, 8, 16, 24 and 48 h exposure respectively (Charpentier et al., 1987). Condition culture was the same as previously described. After different exposure delays, all duckweed from each bottle was carefully sampled, rinsed with EDTA 10^{-4} M solution to take into account only the fraction of toxic actually absorbed by fronds and then freeze-dried for 12 h. Finally, 0.1 g dry material was mineralised and analytic dosages were assayed by atomic spectrophotometry absorption with a Varian AA 1275 model equipped with a graphic furnace to evaluate Cd and Cu biosorption.

Results and discussions

Influence of the composition of culture medium on the toxicity of Cd, Cu and DDTC

The synthetic culture medium normally used, is a solution with a high mineral element content. Interferences between elements of culture medium and studied ions can occur and may influence test results. To evaluate this possible error, the toxicity of the three elements Cd, Cu and DDTC were previously compared by exposing *Spirodella polyrhiza* duckweed in parallel in a synthetic culture medium and in a distilled water medium.

Toxicity tests were conducted after 2 d and 4 d exposure. Indeed, preceding tests (Charpentier and Garnier, 1985) have shown that the absence of nutritional elements had no effect on the duckweed after 2 d, but could not exceed 4 d. At the end of 96 h, morbidity and multiplication percentages in reference cultures were 23.5±4% and 64.5±4% respectively when *Spirodella polyrhiza* was cultivated on a distilled water medium compared to macrophytes cultivated on a synthetic medium.

The toxicity of Cd, Cu and DDTC was evaluated by determining 50% morbid concentrations (MC50) and 50% inhibition concentrations (IC50) after 2 and 4 d exposure in synthetic medium and in distilled water medium.

Toxicity of Cd (Fig. 1)

In both the media (synthetic or distilled water) and for both the biological criteria (MC50 or IC50), Cd toxicity increased with the duration of exposure, as expected. On the other hand, morbid concentrations were greater than inhibition concentrations. The inhibition of multiplication fronds criterion was the most sensitive measure to evaluate the intoxication level induced by Cd.

The comparison of toxicity conducted in a synthetic medium and in a distilled water medium showed that, whatever the criterion and the duration of exposure, the toxicity of Cd is always higher in a distilled water medium than in a synthetic medium. Several authors (Whitton and Shehata, 1982; Polar and Küçukcezzar, 1986; Nasu et al., 1988) have already suspected a lesser availability of Cd for the duckweed in a mineral medium. The difference between a distilled water and a mineral medium could be explained by a possible complexation of Cd with different mineral components of the synthetic medium. However, this hypothesis is not necessarily realised and other phenomena like competition in uptake mechanisms could occur. Although the duration of the assays did not exceed 4 d so that the lack of nutrients would not negatively affect the growth of duckweed placed in distilled water (Charpentier and Garnier, 1985), it is possible that plants exposed to a synthetic medium were better able to survive the toxicants simply because of the availability of food.

Toxicity of Cu (Fig. 2)

Contrary to Cd experimental results, inhibition concentrations (IC50) for Cu were greater than morbid concentrations (MC50) after 2 d exposure. Therefore, the toxic impact of Cu on *Spirodela polyrhiza* appears on the morbidity of fronds.

If no difference appears between culture media after 4 d exposure, we can observe that Cu toxicity after 2 d in a distilled water medium is higher than toxicity in a synthetic medium. So complexation phenomena of Cu and Cd by mineral elements from synthetic culture media or competition during uptake might occur and could be the reason of the observed differences.

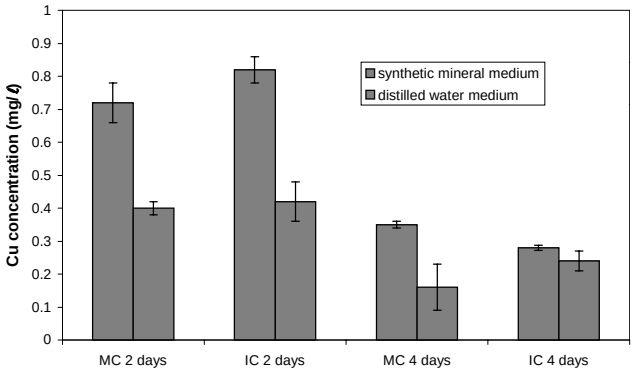

Figure 2

Effects of Cu alone on duckweed after 2 and 4 d exposure in synthetic and distilled water medium (MC50 morbid concentration; IC50 inhibition concentration)

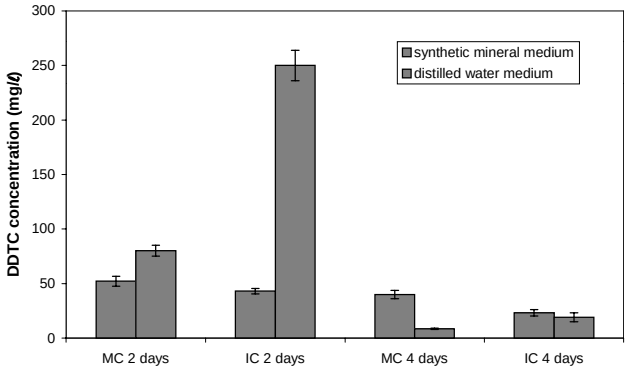

Figure 3

Effects of DDTC alone on duckweed after 2 and 4 d exposure in synthetic and distilled water medium (MC50 morbid concentration; IC50 inhibition concentration)

Toxicity of DDTC (Fig. 3)

Results concerning the study with DDTC are clearly different according to the duration of exposure. After 2 d of exposure to toxicant, a toxic effect of DDTC was observed which was much more pronounced in a synthetic medium that in a distilled water medium for IC50, while differences are a lot less marked between the two media after 4 d exposure. On the other hand, DDTC seemed to exert a toxic effect on frond morbidity after 2 d exposure. In order to clearly observe an effect on frond multiplication, a 4 d DDTC exposure was necessary.

These preliminary studies on isolated Cd, Cu and DDTC showed clearly that two factors influencing the toxicity of these compounds on *Spirodela polyrhiza* were, on the one hand, the exposure duration and, on the other hand, the culture medium composition. To limit interferences linked to the presence of mineral elements in the culture medium and to observe enhanced or reduced toxic effects between these three toxicants, further tests were conducted with a distilled water medium. To limit the effect linked to the absence of nutritional elements, these tests were conducted over a 48 h period.

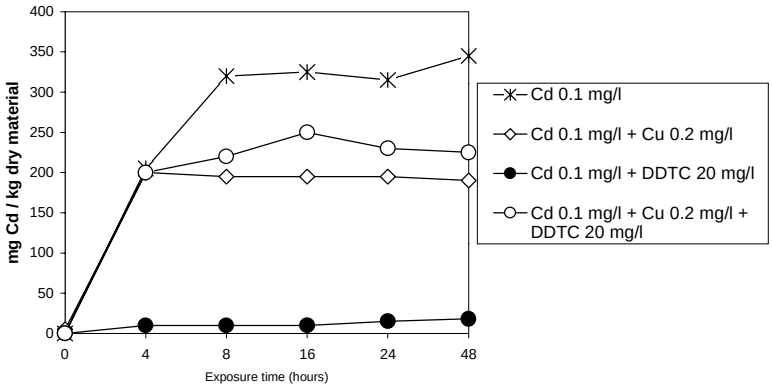

Figure 4

Influence of Cu and DDTC on Cd absorption by Spirodela polyrhiza

Toxicity of Cd, Cu and DDTC in association

Toxicity tests of Cd, Cu and DDTC in different combinations were conducted in a distilled water medium during 48 h exposure of *Sprirodela polyrhiza* to Cd 0.1 mg/ℓ, Cu 0.2 mg/ℓ and DDTC 20 mg/ℓ concentrations as single toxicants or associated toxicants.

Influence of Cu and DDTC on Cd absorption (Fig. 4)

We firstly observed that Cd concentrations found still increased after 4 h in duckweed (expressed in mg of Cd per kg dry material) and were appreciably the same after 8, 16, 24 or 48 h of test. The absorption of Cd by duckweed was optimal during the first hours of the intoxication and did not increase after this initial period.

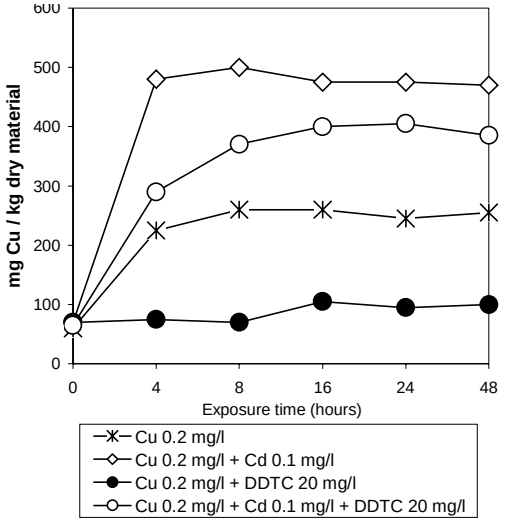

Figure 5
Influence of Cd and DDTC on Cu absorption by Spirodela polyrhiza

The highest concentrations of Cd found in duckweed were those measured in tests with Cd alone at 0.1 mg/ℓ in culture medium. Eight hours after the beginning of exposure, Cd concentrations measured in duckweed were about 320 mg/kg of dry material. When Cu was associated with Cd (Cd 0.1 mg/ℓ and Cu 0.2 mg/ℓ), the absorption of Cd decreased in comparison with tests conducted with Cd alone. The same observation could be made for triple associations with Cd, Cu and DDTC (Cd 0.1 mg/ℓ with Cu 0.2 mg/ℓ and DDTC 20 mg/ℓ).

The most evident effect could be observed when Cd was associated with DDTC (Cd 0.1 mg/ℓ with DDTC 20 mg/ℓ). For these non-toxic concentrations, the biosorption of Cd by the duckweed was nearly non-existent. After 48 h exposure, Cd concentration in duckweed was about 18 mg/kg while it was about 345 mg/kg for culture with Cd alone. It appeared that Cu slightly inhibited the absorption of Cd by the duckweed, but it was especially DDTC that provoked a very marked inhibition of Cd biosorption. The simultaneous presence of DDTC in a culture medium decreased the toxicity of Cd.

Influence of Cd and DDTC on Cu biosorption (Fig. 5)

The initial concentration of Cu in duckweed was about 65± 5 mg/kg. As previously with Cd alone, the absorption of Cu by the duckweed was maximal during the first 4 h of the test.

In Fig. 5, one notes that the addition of Cd (Cu 0.2 mg/ℓ with Cd 0.1 mg/ℓ) and Cd associated with DDTC (Cu 0.2 mg/ℓ with Cd 0.1 mg/ℓ and DDTC 20 mg/ℓ) produced a great increase of the absorption of Cu by *Spirodela polyrhiza*. Contrary to Cd effects, Cu biosorption was favoured by the simultaneous presence of Cd in the culture medium. After 48 h, the concentrations of Cu in duckweed exposed to media with Cd were about 150 to 185 % up on assays with Cu alone.

On the other hand, the presence of DDTC associated with Cu in the culture medium (Cu 0.2 mg/ℓ with DDTC 20 mg/ℓ) produced a quasi total inhibition of the absorption of Cu by *Spirodela polyrhiza*. After 48 h, the Cu concentration in association with DDTC was about 2.5 less than with Cu alone. The presence of DDTC decreased the penetration and therefore the toxicity of Cu for duckweed.

Conclusion

In this study, it appears that the effects of Cd and Cu on *Spirodela polyrhiza* duckweed were greatly influenced by the simultaneous presence of other elements in the culture medium. These interferences can be limited by exposing duckweed to a distilled water medium. However, in this case, the absence of nutritional elements does not allow assays for an exposure duration of more than 48 h.

In a distilled water medium, different effects were observed according to associations with Cd, Cu and DDTC. Copper produces an antagonistic effect for Cd by decreasing its absorption by the duckweed. On the other hand, the presence of Cd in the culture medium favours the absorption of Cu by the duckweed.

From these different tests, the most prominent result concerns the effect of DDTC. When associated with Cd or with Cu, DDTC produces a very significant inhibition of the absorption of these two ions by the macrophyte. DDTC seems to have a complexation effect on Cd and Cu. These results are in agreement with the observations of some authors who recognise the use of DDTC as an antidote for Cu toxicity (Grafström and Greene, 1980; Kojima et al., 1987).

This study clearly shows the chemical reaction complexity that can occur when two or several chemical compounds are associated. Synergism and antagonism phenomena can appear and thus greatly contradict the results obtained when evaluations are conducted with single toxicants only (Kojima et al., 1987; Pommery et al., 1987; Robert, 1984). These results confirm the difficulty to evaluate the true toxicity of a compound in the natural medium which is highly complex by definition.

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