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Adsorption of Cd, Cu, Ni, and Pb on Petri Dishes and Filter Materials Used in Bioassay Procedures

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Short-term phytotoxicity tests using higher plants, such as seed germination or root elongation tests, could be practical to assess the bioavailability of Cd, Cu, Ni and Pb in soil solutions and furthermore to link toxicological effects directly to the bioavailable trace element status of soils. Short-term tests have been used to test the phytotoxicity of the trace metals Cd (Ratsch, 1983; Miller et al., 1985; Wang, 1987; Hsu and Chou, 1992), Cu (Miller et al., 1985; Wang, 1987; Hsu and Chou, 1992), Ni (Wang, 1987; Carlson et al., 1991) and Pb (Hsu and Chou, 1992; Xiong, 1998) in synthetic solutions. It is essential that the materials used for the trace metal toxicity test procedures should promote plant growth without a detectable contamination or adsorption of trace metals from the test solution. The surface adsorption of trace metals to container or seed holder materials, usually filter paper, could cause significantly lower metal concentrations in test solutions. Although attempts have been made to characterize the adsorption characteristics of different container surfaces and filter materials, the recommendations of the authors cannot be adapted to the problems faced in toxicity testing. Several authors have favored solution modifications such as acidification (Pellenbarg and Church, 1978; Massee et al., 1981) or addition of strong complexing agents (Hoyle and Atkinson, 1979) in order to decrease sorptive losses. These procedures can not be applied to phytotoxicity tests because they would alter the chemical speciation and thereby the concentrations of the bioavailable toxic ions in the test solution.

Realistic ranges for trace metals in soil solutions, collected from soils in the field, are 0.01-5.7 µg/L Cd, 0.71-78 µg/L Cu, 0.89-15 µg/L Ni and 4-65 µg/L Pb (Bergkvist et al., 1989; Wenzel and Wieshammer, 1995). In order to assess solutions with trace metals at environmentally relevant concentrations, the phytotoxicity tests used must be sensitive and reproducible in these ranges. Furthermore, the adsorption of trace metals to the test materials should not be detectable. The present work was initiated to test different petri dishes and seed holder materials, here filter paper, that have been used or could be used in short-term phytotoxicity tests for their adsorption behavior of Cd, Cu, Ni and Pb in concentrations appropriate to soil solutions.

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MATERIALS AND METHODS

Commonly used 90x15 Pyrex ® glass petri dishes, here referred as glass dishes, and 80x10 Fisherbrand ® polystyrene petri dishes, here referred as plastic dishes were chosen as test dishes. Environmental Express* borosilicate glass fiber filter (area of 64 cm^2, > 0.7 µm particle retention), here referred as BS/GF, Whatman ® glass microfiber filter GF/C (area of 43 cm^2, > 1.2 µm particle retention), here referred as GF/C, and Fisherbrand ® quantitative Q5 filter paper (area of 95 cm^2, > 2 µm particle retention), here referred as Q5 were chosen as seed holder materials. Container and filter materials have been supplied by Fisher Scientific Ltd. except for BS/GF. The holder material BS/GF was supplied by Environmental Express.

All dishes were cleaned with Fisherbrand ® Sparkleen ™, rinsed with tap water, rinsed twice with deionized water, soaked for 20 minutes in 0.01 M Na_2EDTA, rinsed three times with deionized water, three times with double deionized water and then air dried. The test solution was prepared in 1000 mL Pyrex ® glass volumetric using double deionized water and Cd, Cu, Ni and Pb Atomic Absorption Standards in 2-5 % HNO_3 supplied by Seigniory Chemical Products Ltd.

Concentrations of 4 µg/L Cd, 50 µg/L Cu, 20 µg/L Ni and 50 µg/L Pb were prepared for the test solution. The pH of the solution, measured with a Radiometer pH Meter with a combined electrode, was 4.2. Double deionized water that had been acidified with HNO_3 to pH 4.2 was used as the blank control solution. After the test period the solutions were transferred to 20 mL Nalgene ® HDPE bottles to which 0.1 mL of 10 % HNO_3 had been added. The solutions were analyzed for metals using a graphite furnace atomic absorption spectrophotometer Varian SpectrAA 300. The results were statistically analyzed using SAS ® Release 8.0 (SAS Institute Inc., 2000).

In an incomplete factorial design simulating a short-term toxicity test the plastic petri dishes were tested without seed holder materials whereas the glass dishes were tested without and with the holder materials. Different amounts of test solution were added to maintain approximately the same available amount of test solution in the dishes after the holder materials became saturated. The test was performed by adding 10 mL of the test solution to the glass and plastic dishes with BS/GF and Q5 and 8 mL of test solution to the petri dishes without a holder material and with GF/C. All five treatments were replicated three times.

The dishes were layered randomly in a plastic bag inside a cardboard box. A moist paper towel was placed between the layers without any contact to the test solution to control evaporation and avoid dryout of the dishes. The cardboard box was sealed with tape and placed in a controlled environmental chamber for 6 days at 24.5 °C ± 0.5 °C in the dark.

RESULTS AND DISCUSSION

The initial and the final concentrations of the metals after the test are shown for all the different combinations in Figure 1. Despite the fact that the materials were tested with different effective surface areas, the graphs illustrate quite clearly that there are differences in the metal adsorption behavior among the tested materials. It should be noted again that the holder materials were tested in the glass dishes. The final concentrations for the holder materials reflect therefor the adsorption characteristics of the glass dish and the respective holder materials.

To determine if the differences in the adsorption properties of the tested materials were statistically significant, the variability in concentration of each trace element was tested using a one-way ANOVA (see Table 1). Since the calculated *F* values exceed the tabular *F* very significantly, we conclude that the experiment provides evidence of real differences in the adsorption behavior of the tested materials.

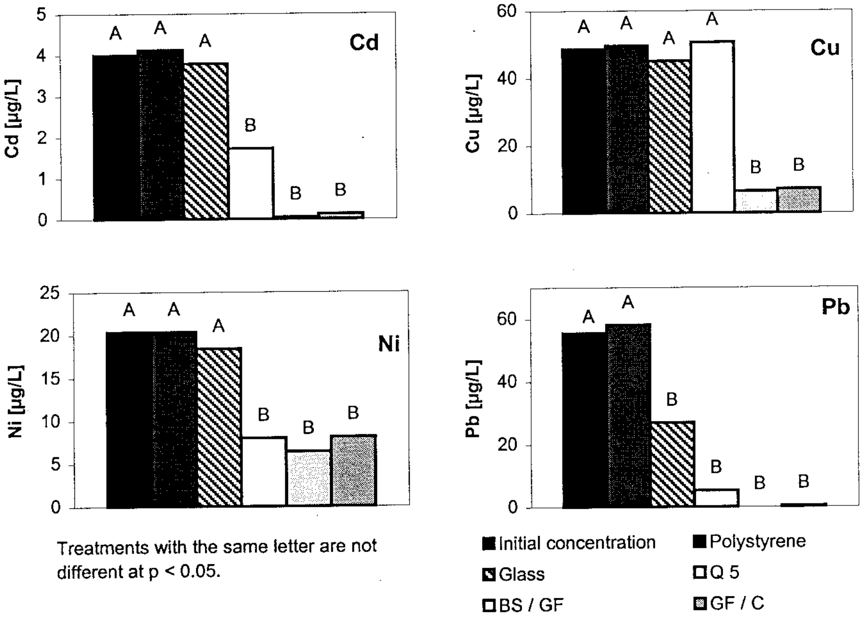
Table 1. Results of one-way ANOVA

	F value	Pr > F
Cd	95.00	0.0001
Cu	153.11	0.0001
Ni	22.45	0.0001
Pb	524.82	0.0001

Differences between the initial and the final trace element concentrations of the test solution were evaluated by a post hoc Dunnett's One-tailed T test using the initial concentration as the control. The same test using glass as the control was applied to evaluate differences between the adsorption behavior of the glass petri dishes and the holder materials. The results confirmed the statistics of the Dunnett's T test using the initial concentration as the control.

Only the Fisherbrand ® polystyrene petri dishes were effective in maintaining Cd, Cu, Ni and Pb in solution at pH 4.2. Glass petri dishes removed more than 50 % of Pb from solution but not Cd, Cu or Ni which confirms the results that can already be found in the literature for other glass containers. Isaaq and Zielinski (1974) have observed losses up to 60 % of Pb in Pyrex ® and Kimax ® glass containers within 60 min due to surface adsorption. Struempler (1973) has reported a 60 % loss of Pb at pH 4 over a period of 10 days in borosilicate glass bottles.

All the tested seed holder materials have significant adsorption capacities for Cd, Ni and Pb. The concentrations of Cd, Ni and Pb were reduced to less than 60 % of the initial metal concentrations by the seed holder materials. The glass fiber materials, BS/GF and GF/C, removed more than 80 % of Cu from the test solution. No adsorption of Cu to the seed holder material Q5 was observed.



Treatments with the same letter are not different at $p < 0.05$.

Figure 1. Initial and final concentrations

An essentially complete adsorption of Cd and Pb (> 98.5 %) occurred when the glass fiber material, BS/GF, was tested. The actual adsorption capacity of glass petri dishes and the seed holder BS/GF for Cd and Pb might even be higher which confirms the affinity of Pb for oxide surfaces (McBride, 1989).

In a study of different membrane filters, Menzies et al. (1991) observed that all the evaluated filter elements exhibited an adsorption behavior. Although they have evaluated different materials than we did, their and our results indicate that measurable changes in the concentration of solutions could occur in small samples due to surface adsorption of filter materials. Our results are contradictory to the report of Pellenbarg and Church (1978). They did not observe a sorptive loss of dissolved Cu in seawater to Gelman A/E glass fiber filter, a filter that is similar to the glass fiber materials BS/GF and GF/C. Pellenbarg and Church (1978) have tested seawater in large volumes. Larger volumes of test solution might have minimized the adsorption effect in their study. Furthermore, seawater is more complex than the solution we have tested. The effect of surface adsorption to filter or seed holder materials could be less marked for each metal in more complex solutions as competition with other cations for adsorption sites might occur.

Nevertheless, the suggestion of Chiang et al. (1989) to use borosilicate filter materials for specific analysis procedures of trace metal contaminated soil or soil solution samples needs reconsideration when samples in small sizes are being analyzed.

Our results seem to indicate that surface adsorption of trace metals at environmentally effective concentrations is an important issue for developing and using toxicity test protocols. Thus, the adsorptive behavior of lab materials used in tests needs to be considered to optimize the sensitivity of phytotoxicity tests and to calculate EC 50 values and for any complex bioassessment.

Using Fisherbrand ® polystyrene petri dishes as test containers is therefore found to be effective in minimizing sorptive losses of Cd, Cu, Ni and Pb in test solutions at pH 4.2. Pyrex ® glass petri dishes are not desirable for bioassay procedures if Pb is being tested. Except for the combination of Cu and paper Q 5, all of the tested holder materials removed significant amounts of Cd, Cu, Ni and Pb from the test solution. If holder material has to be used in bioassay procedures, soaking in the test solution (Ratsch, 1983) might be useful in minimizing adsorption. Alternate systems have been proposed that may avoid the problem of changing solution concentrations; see, for example, Parker et al., 1998.

It was also clear from this study that one can not assume that the nominal concentration of the test solution is the concentration in the solution during the test or the effective concentration that induces the biological response. The concentration of trace metals must be measured before and after the test to obtain reliable results.

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