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BRIEF COMMUNICATION

Determination of metal interactions on root growth of *Sinapis alba* seedlings

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Abstract

Root growth of *Sinapis alba* seedlings was used to determine the interactions (antagonism, synergism, and indifference) among Cu^{2+}, Ni^{2+}, Mo^{6+}, Mn^{2+} and V^{5+} (each to each). For majority of combinations the antagonistic or indifferent effect was observed. The synergism was confirmed only between Ni and V, Mo and V, and Cu and V. V and Mn had reciprocal antagonistic effect each other, and Mn was like as Mo and Cu the antagonist to Ni. Molybdenum came up reciprocal indifference with regard to Cu and Mn. Mn and Ni did not influence unfavourable effect of Cu on *S. alba* root growth.

Additional key words: root growth inhibition, metal-metal interaction, copper, nickel, manganese, molybdenum, vanadium, mustard.

The majority of toxicity studies involve only one tested substance. However, the results outcoming from multiple toxicants can have a dramatically varying influence on an ecosystem or organism compared to the individual components alone (Ebbs and Kochain 1997, Miadoková *et al.* 1998). Elements with similar physico-chemical properties compete for absorption, transport and accumulation in living cells. They replace one another in enzymatic pathways and receptor proteins (Friberg *et al.* 1986).

In the present study, mustard seedlings were used as a model. As soil-less cultures are recommended in toxicological testing (Adema and Henzen 1989), hydroponic cultivation of mustard was used. Toxicity of metals was evaluated in their

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combinations and compared with the toxicity of individual metals (Fargašová 1998). Interactions were expressed in terms of synergism, indifference, and antagonism.

The seeds of mustard (*Sinapis alba* L.) were germinated in Petri dishes (diameter 14 cm) on plastic net (the holes diameter 2 mm) for 3 d at temperature of 23 ± 1 °C. After 3 d the plastic nets with germinated seeds were transferred into plastic containers filled with 500 cm^3 of modified Knop solution which contained [mg dm^{-3}]: $Ca(NO_3)_2$ 0.8; KH_2PO_4 0.2; KNO_3 0.2; $MgSO_4.7\,H_2O$ 0.2; KCl 0.2; $FeSO_4$ 0.01 (pH = 5.2). The solution was supplemented with metals in the form of following compounds: Cu^{2+} - $CuSO_4.5\,H_2O$; Ni^{2+} - $NiSO_4.7\,H_2O$; Mn^{2+} - $MnSO_4.1\,H_2O$; Mo^{6+} - $(NH_4)_6Mo_7O_{24}.4\,H_2O$; V^{5+} - V_2O_5 (*Merck*, Darmstadt, Germany) and following combinations: V^{5+} + Ni^{2+}; V^{5+} + Mo^{6+}; V^{5+} + Cu^{2+}; V^{5+} + Mn^{2+}; Ni^{2+} + Mo^{6+}; Ni^{2+} + Cu^{2+}; Ni^{2+} + Mn^{2+}; Mo^{6+} + Cu^{2+}, Mo^{6+} + Mn^{2+} and Mn^{2+} + Cu^{2+}. Each metal was tested in the concentration equal to the determined EC_{50} value (median effective concentration) for root growth inhibition (Fargašová 1998): Cu^{2+} 4.3; Mn^{2+} 13.0; Mo^{6+} 6.1; Ni^{2+} 11.5; V^{5+} 15.8 mg dm^{-3}. Plants were grown under natural sunlight for next 8 d and the root length was measured. All experiments were set up in a completely randomized design with 3 replicates and significant difference between the treated and control samples as well as between the samples with single metals and metal-metal combinations were analyzed by *t*-test.

Table 1. Inhibitory and stimulatory (+ indicates stimulation) effects of individual metals and metal-metal combinations on root growth of *Sinapis alba* (means ± SD, $n = 3$). I_1 - percentage of inhibition in comparison with the control; I_2 - percentage of inhibition in comparison with the first metal in combination; I_3 - percentage of inhibition in comparison with the second metal in combination; * - no significant difference; ** - significant difference ($P < 0.05$); *** - highly significant difference ($P < 0.01$).

Metal	Root length [cm]	I_1 [%]	I_2 [%]	I_3 [%]
Control	2.2 ± 0.08			
Mn^{2+}	1.1 ± 0.03	50.0 ***		
V^{5+}	1.1 ± 0.03	50.0 ***		
Cu^{2+}	1.2 ± 0.04	45.5 ***		
Ni^{2+}	1.0 ± 0.03	50.0 ***		
Mo^{6+}	1.2 ± 0.04	45.5 ***		
V^{5+} + Ni^{2+}	0.8 ± 0.03	63.6***	27.3**	20.0**
V^{5+} + Mo^{6+}	0.8 ± 0.18	63.6***	27.3**	33.3**
V^{5+} + Cu^{2+}	1.0 ± 0.02	54.5***	9.1**	16.6**
V^{5+} + Mn^{2+}	1.5 ± 0.17	31.8**	+ 36.4**	+ 36.4**
Ni^{2+} + Mo^{6+}	1.3 ± 0.05	40.9**	+ 30.0**	+ 8.3*
Ni^{2+} + Cu^{2+}	1.2 ± 0.11	45.5***	+ 20.0**	0.0*
Ni^{2+} + Mn^{2+}	1.2 ± 0.14	45.5***	+ 20.0**	+ 9.1*
Mo^{6+} + Cu^{2+}	1.2 ± 0.04	45.5***	0.0*	0.0*
Mo^{6+} + Mn^{2+}	1.2 ± 0.10	45.5***	0.0*	+8.3*
Mn^{2+} + Cu^{2+}	1.0 ± 0.06	54.5***	9.1*	16.6**

All combinations of metals significantly inhibited root growth of *S. alba* seedlings in comparison with the control (Table 1). In comparison with the metals alone, no significant differences were found in combinations Mo + Cu and Mo + Mn. Therefore, for both these combinations the reciprocal indifferent effect can be concluded. Indifferent effect was also confirmed for Ni to Mo, Cu and Mn, and for Mn to Cu (Table 1). According to Ouzounidou (1995), Cu (as $CuSO_4$ in concentration 5.8 and 10.16 mg dm^{-3}) suppresses the effect and uptake of Mn and V and enhances that of Mo and Ni. This is in contrast with our results where Cu reduced the unfavourable effect of Ni on root growth and enhanced that of Mn. Effects of single metals and their combined pairs are usually associated with their uptake. According to our previous results (Fargašová and Beinrohr 1998), Cu (as $CuSO_4.5\ H_2O$ in concentration 4.3 mg dm^{-3}) decreased the accumulation of Mn, Mo, Ni and V both in the roots and in the cotyledons of *S. alba* but all these metals increased Cu accumulation in the roots. However, as concerns root growth, Cu suppressed only the effect of Ni and enhanced that of Mn and V (Table 2).

Table 2. Metal-metal interactions: S - synergism (the interactive ion increased the inhibitory effect of metal introduced in the first column); A - antagonism (the interactive ion decreased the inhibitory effect of metal introduced in the first column); I - indifference (the interactive ion did not influence the inhibitory effect of metal introduced in the first column). Interactive metals - metals which influence the effect of the main metal.

Main metal	Effect	Interactive metals	Main metal	Effect	Interactive metals
V^{5+}	S	Ni^{2+}, Mo^{6+}, Cu^{2+}	Mn^{2+}	S	Cu^{2+}
	A	Mn^{2+}		A	V^{5+}
	I			I	Mo^{6+}, Ni^{2+}
Ni^{2+}	S	V^{5+}	Cu^{2+}	S	V^{5+}
	A	Mo^{6+}, Mn^{2+}, Cu^{2+}		A	
	I			I	Mn^{2+}, Mo^{6+}, Ni^{2+}
Mo^{6+}	S	V^{5+}			
	A				
	I	Mn^{2+}, Cu^{2+}, Ni^{2+}			

Reciprocal stimulation of root growth was confirmed for V + Mn. Mo, Cu, and Mn had strong antagonistic effect to Ni and in combinations with this metal the significant stimulatory effect was observed. In opposite Ni had indifferent effect to Mo, Cu, and Mn. Reciprocal synergism was confirmed only in the combinations V + Ni, V + Mo, and V + Cu when each metal enhanced inhibitory effect of the other. However, V had reciprocal antagonistic effect toward Mn. Cu indicated synergistic effect only in combinations with V and Mn. In other tested combinations, the root length was either not changed or enhanced (Table 2). Ebbs and Kochian (1997) and Tomasik *et al.* (1995) also described similar effects. What kind of interactions will appear depends both on the plant species, observed parameter, and compounds used in combinations (Tomasik *et al.* 1995, Fargašová and Beinrohr 1998).

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