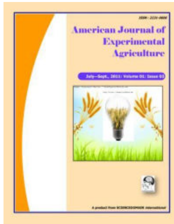

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Evaluation of Heavy Metal Toxicity on Radish: Comparison between Soil and Floating Hydroponics Systems

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Research Article

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ABSTRACT

Two plant growing methods, namely use of soil and a floating hydroponic system, were compared in order to determine whether the response of radish to Cd, Cu, Ni and Zn was affected by the cultural system. Seed germination, plant biomass (root, shoot, hypocotyl), and heavy metal accumulation in different parts of the radish plants were monitored. The presence of heavy metals in the germination medium had no effect on seed germination, while the biomass of plants grown in hydroponics was negatively affected by the presence of metals in the nutrient solution. In both cultural systems, the amount of metals in roots, hypocotyls and shoots increased significantly with the increasing metal concentration in the growth media; the highest concentrations of heavy metals were found in the roots. The bioconcentration factor (BCF) and translocation factor (TF) values for the four metals were found to be considerably different between both growing systems. The different responses of plants suggest that in contaminated soils the plants are in the presence of lower quantities of available metals than those found in the corresponding substrates of the hydroponic system.

Keywords: Floating hydroponics system; soil system; radish; heavy metals; BCF, TF, correlation test.

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1. INTRODUCTION

Most studies on the toxicity of heavy metals on higher plants have been based on hydroponic cultures, while fewer studies have been undertaken using soil as the growth substrate. This is mainly due to the technical difficulties of measuring the effective metal bioavailability precisely (Astolfi et al., 2004): the bioavailability of metals is evaluated through the use of specific extracting solutions (Brun et al., 1998; Brun et al., 2001).

The choice of the extracting solution is linked to three complex parameters: soil physical and chemical properties, the chemistry of each element, the different responses to the absorption of the plant species (Izza et al., 1994). In recent decades, to prove that the floating hydroponic system and soil are exchangeable techniques a number of comparative studies were performed. Cox et al. (1996), studied the effect of As on canola and noted that the plant is sensitive to As if grown in soil, but moderately tolerant to As when grown in hydroponics. Astolfi et al. (2004) studied the effects of Cd on oats cultured in soil and hydroponic system, and reported that both field and hydroponic conditions represent suitable systems for investigating Cd effects on oat growth and metabolism. Weiss et al. (2006) studied the effect of heavy metals present in stormwater on plant grown in soil and a hydroponic system; they noted that plants grown hydroponically with stormwater accumulated higher levels of heavy metals than plants grown in soils. Therefore he recommended hydroponics as an alternative remediation technique of stormwater contaminated with heavy metals. Grispen et al. (2006) evaluated the phytoextractive ability of different varieties of *Brassica napus* and suggested that metal accumulators should be selected under standard and repeatable conditions using hydroponics. Kashem et al. (2008) also studied the phytoextractive ability of three plants grown in hydroponics; they reported that hydroponics provided potential to examine metal tolerance and magnitude of metal accumulation in plant species with greater precision than soil studies. In a comparative study on the effects of Cd on corn and sunflower grown both in hydroponics and soil culture, Pritsa et al. (2008) compared in the hydroponic and soil systems the effect of various Cd levels in corn and sunflower and reported that the ratio of the hydroponic-soil pollutant rate that produced a comparable effect on plant growth was about 1:5. The factor that links the above works is the different concentrations of the metals in the compared growth systems. In our opinion, the comparison is experimentally correct if, and only if, the concentration of metals is the same in both growing systems. Hence for the floating hydroponic system we used three nutrient substrates with concentrations of Cd, Cu, Ni and Zn equal, or presumed equal, to those present in the available fraction (soil solutions) of the three tested soils.

2. MATERIALS AND METHODS

2.1 Soil Samples

For the experiment we used an agricultural soil (soil A or the control) sampled at the slopes of Vesuvius (Torre del Greco) and two artificially polluted soils (B and C), obtained from the contamination of soil A. Soil C is a mix of soils used in the period February 2006 and December 2009 for other experiments. The various mix fractions were individually polluted, adding solutions containing various concentrations of $Cd(NO_3)_2$, $Cu(NO_3)_2$, $Ni(NO_3)_2$ and $Zn(NO_3)_2$ to soil A. Soil B was obtained by mixing, in equal parts, soils A and C. Soils A, B and C were individually remixed and stabilized in a greenhouse for four weeks at ambient temperature. During the "stabilization period", in accordance with Brun et al. (2003), the soil samples were thoroughly mixed and slightly moistened several times.

2.2 Soil Analysis

Texture, pH, cation exchange capacity (CEC), organic matter (OM)) and total heavy metal concentrations in soil were determined according to Italian Standard Methods (MiPAF, 2000). Soil field capacity (FC) and permanent wilting point (PWP) were determined by Richards' plates (PR) following the procedures described by Dane and Hopmans (2002) (Table 1a).

Table 1a. Generals physicochemical properties of the soil A

Parameters	Unit	Values
Sand (0.2-0.02 mm)	%	79
Silt (0.002-0.002mm)	%	14
Clay (<0.002 mm)	%	7
$CaCO_3$	g/kg	*trace*
pH		6.5±0.02
Organic Matter	(%)	5.01±2.15
CEC	(meq)	19.2±3.27
FC	%	30.2
PWP	%	15.2
AW (available water)	ml/kg	150

Soil-available metals were evaluated by three extraction methods: a) aqueous extract using a 1:5 soil to water ratio (Ernst, 1996); b) NH_4OAc 1 M (Brun, 2003); c) a mixture of 5 mM DTPA, 10 mM $CaCl_2$ and 100 mM triethanolamine (TEA) at pH 7.3 (Lindsay and Norvell, 1978). Total and available concentrations of Cd, Cu, Ni and Zn were determined with a Perkin Elmer Lambda 3 spectrophotometer by flame atomic absorption spectroscopy (AAS flam) or AAS graphite (HGA 700) depending on the heavy metal concentrations. Total metal concentration and available metal concentration of the three soils are presented in Table 1b.

Soil moisture was determined with a cylindrical transparent plastic probe (Ǿ 15 mm). The probe was inserted horizontally into pots through small holes throughout the root zone. Then the samples were immediately weighed (approximately 3 g), oven-dried at 105° C for 30 minutes and reweighed. The moisture was expressed as % of weight on dry soil. Plant-available water (PAW) was obtained by the difference between the soil water content at field capacity (FC) and the soil water content at the permanent wilting point (PWP) (Hillel, 1998).

2.3 Plant Growth Conditions

Seeds of *Raphanus sativus* L. (cv cardinale) were sterilized in 10% Na hypochlorite solution for 20 min under slow agitation in order to prevent fungal growth and washed several times with distilled water. In plastic pots (55x17x15cm) containing 10 kg of dry soil, previously brought to field capacity, six seeds were sown. Each soil/plant combination was replicated eight times; five replicates were used to determine plant dry weight and metal content, whereas the others were employed to measure soil water content during the experiment. The plants were kept for 50 days in a growth chamber (25/20° C day/night temperature, 14 h photoperiod irradiance at 200 µmol $m^{-2}s^{-1}$ PAR at leaf level), with a completely randomized design.

Table 1b. Total and extractable heavy metals concentrations (mg/kg) in soil

Metals	Extractants	Soil		
		A	B	C
Cd	H_2O	Nd	0.012	0.036
	NH_4OAc	**Nd**	**0.087**	**0.152**
	DTPA	Nd	5.20	13.2
	Total	Nd	7.91	19.5
Cu	H_2O	Nd	0.072	0.016
	NH_4OAc	**0.051**	**0.188**	**0.423**
	DTPA	11.20	30.10	90.2
	Total	29.9	159	285
Ni	H_2O	Nd	0.011	0.024
	NH_4OAc	**Nd**	**0.177**	**0.375**
	DTPA	Nd	0.18	0.38
	Total	5.84	95.70	219
Zn	H_2O	Nd	0.250	0.448
	NH_4OAc	**0.101**	**0.93**	**2.08**
	DTPA	12.88	66.90	112
	Total	90.11	274	475

2.4 Floating Hydroponic System

An innovative technique of hydroponic cultivation was used: it is a system of production in a static liquid medium in which plants are grown on polystyrene panels 50x15x4cm with alveoli partly filled with vermiculite, floating in tanks 55x17x10cm, filled with nutrient solution (1.5 l of a modified Hoagland and Arnon (1950) solution) with the addition of Cd, Cu, Ni and Zn in the form of nitrates. Each container had six alveoli; on each alveolus 250 mg of vermiculite were placed and a radish seed sterilized according to the above-described method.

These containers were kept for 50 days with a completely randomized design in a growing chamber (25/20°C day/night temperature, 14 h photoperiod irradiance at 200 µmol m-2s-1PAR at leaf level), until the plants reached their marketable maturity. Oxygen for root respiration was provided through a bubbler insufflating air. Metal concentrations were as follows: A (Cd 0.0, Cu 0.333, Ni 0.0, Zn 0.668 mg/l); B (Cd 0.579, Cu 1.250, Ni 1.180, Zn 6.22 mg/l); C (Cd 1.0152, Cu 2.820, Ni 2.50, Zn 13.88 mg/l). Each treatment was replicated five times, for a total of 30 seeds per treatment.

Hoagland solution was buffered at pH 6.5 with 2 mM MES (Grispen et al., 2006). In order to reduce the variation in the composition of the nutrient buffers, the hydroponic media were replaced every week.

2.5 Plant Analysis

Plants were harvested at marketable maturity and divided into root, hypocotyl and shoot. The three plant parts were first thoroughly washed with tap water in order to remove soil or vermiculite particles. Then they were rinsed twice with deionized water (Cox et al., 1996) and oven dried at 70°C until constant weight was obtained. The weight of the three plant parts was utilized to assess biomass production. Dry root, hypocotyl and shoot were ground in a mill; 0.5 g samples were placed in a Teflon container with high-purity HNO_3 and H_2O_2 (6:1 v/v) and mineralized in a Mega FKV microwave oven. The mineralized sample was brought to 50 ml with bi-distilled water, and determination of metals content was carried out with a Perkin Elmer Lambda 3 model atomic absorption spectrophotometer with AAS-flame or AAS-graphite furnace HGA 700 depending on the heavy metals.

2.6 Statistical Analysis

Plant dry weight was analyzed by 2-way ANOVA with the growing system (hydroponic vs soil) and substrate (A, B, C) as main factors. The means of sub-groups were compared by Newman test at the 0.05 significance level. Metal concentration in plant tissues was analyzed on a first step by 4-way ANOVA applied to the whole population of treated plants, with metals (Cd, Cu, Ni, Zn), growing system, substrate and plant part (root, hypocotyl, shoot) as the main factors. Four separate 3-way ANOVA were then conducted for each of the four metals, with growing system, substrate and plant part as the main factors.

3. RESULTS AND DISCUSSION

3.1 Comparison Set-Up

To compare the two growing systems, it was supposed that the same quantity of element absorbed by hydroponically grown plants may equally be taken up by plants grown in a soil with the same concentration of the same element in its available fraction. Given that in hydroponics all the elements present in the substrate are totally available to the plant, while in the soil the total quantity of elements deviates considerably from that effectively available, three nutrient substrates were prepared for the float system (hydroponics) with concentrations of Cd, Cu, Ni and Zn equal to those present in the available fraction (circulating solutions) of the three tested soils.

To carry out the above, it is necessary: a) to use the same system for measuring the available elements in both growth substrates; b) to determine the concentration of available metals in the three tested soils to obtain the concentrations of the mix of metals to add to Hoagland in the floating system; c) to maintain constant in both growth systems the concentrations of available metals during the experiments.

As regards to point (a) above, given that the concentration of available elements in a soil is usually expressed in mg/kg, and in hydroponics as mg/l, it was possible to express in mg/l the concentration of available elements in both growth systems by determining the volume of the circulating solution in the soil. The volume of the soil solution (PAW) is the difference between field capacity (FC) and the permanent wilting point (PWP). In relation to (b), the availability of metals in the soil was determined experimentally by dosing the amount of available metal, extracted with specific extractant solutions. Currently the choice of the extractant, in determining the real phytoavailability of metals in a soil, is made according to the higher or lower correlation existing between the quantity of metal contained in the plant and the quantities of the same metal, extracted from the soil with one or more extractants (Wang et al., 2004). In this work, according to the methods most widely proposed in the literature for determining available metals in a soil, H_2O, DTPA and NH_4OAc were used as extractant agents. The best extractant agent proved to be acetate: only for acetate did the correlation test show a significant and positive correlation between the concentrations of Cd, Cu, Ni and Zn in the radish plants and the available concentrations in soils (table 2). Once the availability to plants of metals in the soils was established, three solutions were prepared for the float hydroponic system with the same concentration of each metal element as in the available fractions of the corresponding soils.

Table 2. Correlation coefficient between the extracted metal concentration in soil and metal concentrations in radish plant

Extractants	Metals			
	Cd	**Cu**	**Ni**	**Zn**
H_2O	0.218	0.369	0.403	0.326
DTPA	0.463	0.508	***0.708***	0.634
NH_4OAc	***0.670***	***0.608***	***0.872***	***0.705***

Bold italic Pearson correlation coefficient are significant at P<0,05

Finally, as regards to point (c), the concentrations of metals throughout the experiment were maintained constant in both systems in the following way: in hydroponics, through periodic substitution of the substrate and/or the addition of known quantities of water; in the soil, by keeping constant the quantity of water available for the plant. To do this, vegetation-free soil was brought to FC and the corresponding relative soil water content was determined. The frequent measurement of soil water content (alternate days) enabled to rapidly ascertain when the level of soil water content was below the maximum level of available water and to adjust this by adding water. The soil water quantity was thus kept constant in time, hence also the concentration of available metals. Lastly, it should be stressed that during the trial the content of available metals in soils A, B and C remained almost unchanged (data not shown).

3.2 Heavy Metal Toxicity

The parameters analyzed were: 1) germination percentage; 2) shoot biomass; 3) concentrations of heavy metals in radish shoots, hypocotyls and roots. The different parts of the radish were analyzed separately to obtain considerable data to process in order to make the comparison more reliable. In agreement with previous findings (Di Salvatore et al., 2008), the presence of metals (Cd, Cu, Ni and Zn) in the substrates did not affect seed germination. In fact, in both growth systems after 72 hours, the seed germination percentage was almost constant (>90%) and the differences between the germination percentages of the controls and the respective treatments were in no case statistically significant (data not shown). Table 3 reports data on the effect of metals on root, hypocotyl and shoot biomass, in hydroponically grown plants (nutrient solutions A, B and C) and in soil-grown plants (soil solutions A, B and C). The table shows that addition of the metals to the Hoagland solution had a significant effect on radish growth: the dry weight of the different plant parts, in hydroponics, significantly decreased with the increasing concentration of the metals in substrates A, B and C. In contrast, contamination of the soils had no effect on the growth of the tested species: the increasing concentration of metals in the soil did not result in any significant difference in biomass in any part of the plant. Table 3 also shows that there were no significant differences in dry weight between control plants grown either in soil or the floating system. Thus, under equal metal concentrations in both growing systems, the inhibiting effect was manifested only for plants grown in hydroponics.

Table 3. Effect of heavy metals on biomass yield (mg, DW) of radish, cultivated in hydroponic culture and soil

Substrate	Root		Hypocotyl		Shoot	
	Hydroponic	Soil	Hydroponic	Soil	Hydroponic	Soil
A	16.0 a	17.2 a	311 a	316 a	279 a	289 a
B	13.7 b	16.8 a	86 b	362 a	98 b	284 a
C	11.0 c	17.6 a	51.0 c	354 a	58 c	276 a

Significant differences in biomass yield among systems are indicated by different letters ($P<0.05$); values represent the means ± SD of the five replicates

Table 4a and 4b report the concentrations of Cd, Cu, Ni and Zn in roots, hypocotyls and shoots of radish plants growing in hydroponics (nutrient substrates A, B and C) and in soil (A, B and C) and results of three-way ANOVA. In both growing systems, the concentrations of metals in the three plant parts increased significantly with the increasing metal concentration in the substrate. In the tissues of plants grown in hydroponics, the content of all the tested elements was found to be significantly higher than in tissues of plants grown in the corresponding soil. In both growing systems, according to Cox et al. (1996), the roots are the plant part most suited to accumulating the metals tested, showing significantly higher metal concentrations than the hypocotyl or the shoot: the sequence of accumulation of metals in the different plant parts follows the order: root > shoot > hypocotyls.

3.3 BCF and TF

The Response of Radish to the Heavy Metals was also evaluated in terms of two important factors, the Bioconcentration Factor (BCF) and the Translocation Factor (TF).

The BCF provides an estimate of the ability of a plant to absorb metals from growth substrate. In the soil it is defined as the ratio of metal concentration in the shoots to the NH_4OAc extractable metal concentrations of the soil (Vogel-Mikus et al., 2005); in hydroponic floating system, the BCF means the ratio of metal concentration in the shoots to metal concentration in nutrient solution (Zacchini et al., 2009).

The TF estimates the ability of a plant to transfer metals from root to shoot: it is defined as the ratio of metal concentrations in the shoots to the roots (Marchiol et al., 2004a; Zacchini et al., 2009). Though BCF and TF are used by many researchers (Marchiol et al., 2004a; Marchiol et al., 2004b; Benzarti et al., 2008; Bose and Bhattacharyya, 2008) to evaluate phytoextraction capability, in this work these parameters were used to characterize the behaviour of radish with respect to the four metals tested.

Table 4a. Heavy metals concentrations (µg/g, DW) in root, hypocotyl and shoot of radish

Available metals (mg/l)			Root		Hypocotyl		Shoot	
			Hydroponic	**Soil**	**Hydroponic**	**Soil**	**Hydroponic**	**Soil**
A		nd	0.20±0.10	0.21±0.06	0.066±0.04	0.33±0.08	0.23±0.15	0.38±0.11
B	Cd	0.5789	95±13	16.4±2.4	22.8±5.9	0.42±0.26	71±7	3.25±0.86
C		1.0150	185±19	24.2±2.9	43±8	1.00±0.91	98.2±11.1	7.11±1.21
A		0.333	10.79±5.32	17.0±5.7	3.01±0.90	2.70±1.83	5.63±0.71	4.81±0.55
B	Cu	1.250	286±73	55±9	16.9±6.9	8.3±3,0	16.8±4.3	11.4±3.6
C		2.820	420±73	95±4	29±6	16±2	29.1±9.3	35.0±50.8
A		nd	0.34±0,08	0.36±0.18	0.66±0.31	0.43±0.13	0.18±0.04	0.41±0.018
B	Ni	1.180	191±50	57±13	32±4	3.99±1.99	33.0±3.3	3.45±1.66
C		2.500	373±138	93±1	48±11	10.5±3.4	64±27	10.1±3.8
A		0.668	689±10	68.7±30	125±15	34±6	357±41	87±8
B	Zn	6.22	721 ±162	136±38	124±15	40±4	454±55	150±50
C		13.88	1135±271	205±4	197±45	54±20	536±43	245±39

Values represent the means ± SD of the five replicates

Table 4b. Results of 3-way Anova applied to radish plant subjected to varying levels of pollution by four heavy metals (Cd, Cu, Ni, Zn), with growing system (hydroponic vs soil), part of plant (root, hypocotyl and shoot) and substrate (A, B, C) as main factors.

Source	*df*	Cd		Cu		Ni		Zn	
		F	*Sig.*	*F*	*Sig.*	*F*	*Sig.*	*F*	*Sig.*
Hydroponic - soil culture	1	779.0	***	70.1	***	42.0	***	259.3	***
Root - hypocotil - shoot	2	175.3	***	135.5	***	49.6	***	100.0	***
A - B - C solutions	2	351.4	***	55.1	***	35.5	***	19.4	***

*Significance level is denoted by the number of stars following the treatment (P<0.001: ***).*

Table 5. Biocentration factor [BCF= $C_{shoot}/C_{available\ fraction}$ and Traslocation factor [TF (C_{shoot}/C_{root}] calculated for *Raphanus sativus*

Available metals (mg/l)			BCF		TF	
			Hydroponic	Soil	Hydroponic	Soil
A	Cd	nd	–	–	–	–
B		0.5789	124.08	5.62	0.76	0.20
C		1.0150	96.74	7.01	0.53	0.29
A	Cu	0.333	16.92	14.45	0.52	0.28
B		1.250	13.47	9.12	0.06	0.21
C		2.820	10.31	12.41	0.07	0.37
A	Ni	nd	–	–	–	–
B		1.180	27.95	2.92	0.17	0.06
C		2.500	25.68	4.03	0.17	0.11
A	Zn	0.668	535.26	130.56	0.52	1.27
B		6.22	73.06	24.22	0.63	1.10
C		13.88	38.64	17.62	0.47	1.20

In table 5, BCF and TF values are reported for radishes grown in hydroponics in substrates A, B and C and in the corresponding A, B and C soils. The table shows that both BCF and TF values for the four metals were quite different in the two growing systems. The same table shows that, in agreement with the published literature (Benzarti et al., 2008; Vogel - Mikus et al., 2005), the values of BCF in hydroponics decreased with increasing concentration of pollutants in the substrate, with the exception of BCF values for copper. In soils only for Zn, the increasing the concentration of the metal resulted in a lower BCF value, whereas for the other elements the trend of BCF was variable. Finally, in the same table, it is to be noted that in both growing systems, both BCF and TF values for Zinc, at equal concentrations, proved considerably higher than the other elements.

4. CONCLUSIONS

The different responses in terms of biomass, metal absorption, BCF and TF, in the two growing systems suggest that the differences encountered may stem either from the growing system or from the evaluation of the elements available in the tested soils. The first hypothesis may be discarded, given that there are no significant differences between the dry weight of the hydroponically-grown plants (nutrient solution A) and that of the control plants grown in soil (soil solution A). The second hypothesis seems plausible insofar as the larger biomass of radish and lower presence of metals in the various parts of the plants grown in contaminated soils, with respect to the same grown in the corresponding substrates of the float hydroponic system, suggest that in contaminated soils there is a lower quantity of available metals than those found in the corresponding substrates of the hydroponics. Indeed, inhibition of plant growth is only manifested in hydroponically-grown plants, where the concentration of the mix of metals is probably far higher than that encountered in the corresponding soils. The indirect proof of the presence, in the two growing systems, of different quantities of tested metals suggests an incorrect estimate of the concentration of metals available in the soil. The availability of metals in the soil gives an indication of the metal fraction potentially available for root absorption and is determined experimentally by applying the metal at the rate to be assimilated, extracted with specific solutions. The choice of the extracting solution is linked to three complex parameters: soil chemical and physical properties, the chemistry of each element, and the different responses to absorption of each plant species. The correct relationship between the parameters and the extracting solution is indispensable to avoid transferring into the solution, due to unsuitable chemical agents, a percentage of the element normally unavailable for crops. Currently, the phytoavailability of metals in soils to plants is generally determined by correlating the concentrations of metals in plants with that in soils. Nevertheless, we believe should be emphasized that such correlation test provides suitable information about the linearity of the responses measured, but it doesn't allow to affirm, a priori, that the extracted amounts of metals do really correspond to the available fraction in the soil, since points out that metal accumulation by plants is proportional to the amount of metal extracted in the soil.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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