Effects of Heavy Metal Concentrations in Waste Water on Rice, Spinach and Earthworm

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Abstract

Heavy metals contaminating soils in areas near Ho Chi Minh City include Mn, Cu, Pb, Zn, Fe and Cd. These metals occur in different concentrations in river water, in the water of canals and in 'soil-contained' water. Heavy metals accumulate in earthworms at very high concentrations and are directly correlated with the concentration of these metals in the soil. Earthworms therefore can be used as an indicator for heavy metal contamination in soils. In vitro experiments show that the influence of Pb^{2+} (0. 63 – 0.75 mg l^{-1}) and Cd^{2+} (0.32-0. 43 mg l^{-1}) can be measured after the 7th day of growth and that after 21 days 100% of the test population is dead. It is further observed that the influence of Cd^{2+} on the growth of rice is stronger than the influence of Pb^{2+}. Morning Glory adapts itself to an environment polluted by Pb but at concentrations of 5.0 mg l^{-1} roots of the plant turn to black and the plant becomes necrotic after one week. At higher concentrations growth is stalled. Cd^{2+} kills Morning Glory at concentrations of 2.5 mg l^{-1}.

Introduction

Little research has been carried out on contamination by heavy metals near HCM City. This paper reports on the results obtained from some experiments aimed at identifying heavy metals in rivers and canals near the city and on the effects of heavy metals contamination on the growth of rice, morning glory and earthworms.

Methodology

In vitro, greenhouse and field experiments were carried out following the standard methods for the examination of water and wastewater of the American Public Health Organization (1992.). Correlation graphs and correlation coefficients were established using the method of Rumxki (Moscow, 1972.). The following main experiments were carried out:

Effects of Pb^{2+} and $Pb(NO_3)_2$ on the yield of rice downstream from Nha Be

Rice variety: VN95.2R.
Control Treatment 1: Waste water from Ho Chi Minh City to Nha Be and IRRI nutrient solution.
Control 2: Pb^{2+} = 0 mg kg^{-1}.
7 Treatments: 1/ Pb_0= 0.2 mg kg^{-1}, 2/ Pb_1= 0.5 mg kg^{-1}, 3/ Pb_2= 0.7 mg kg^{-1}, 4/ Pb_3= 1.0 mg kg^{-1}, 5/ Pb_4= 1.2 mg kg^{-1}, 6/ Pb_5= 1.8 mg kg^{-1}, 7/ Pb_6= 2.3 mg kg^{-1}.

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Effects of Cd^{2+} and $CdSO_4$ on the yield of rice downstream from Nha Be
Rice variety VN95.2R
Control Treatment 1: Waste water from Ho Chi Minh City to Nha Be and IRRI nutrient solution. Control 2: Cd^{2+} = 0 mg kg^{-1}.
7 variants: 1/ Cd_0= 0 mg kg^{-1}, 2/ Cd_1= 0.025 mg kg^{-1}, 3/ Cd_2= 0.315 mg kg^{-1}, 4/ Cd_3= 0.345 mg kg^{-1}, 5/ Cd_4= 0.535 mg kg^{-1}, 6/ Cd_5= 1.5 mg kg^{-1}, 7/ Cd_6= 1 mg kg^{-1}.

Effects of Pb^{2+} and $Pb(NO_3)_2$ on the growth of Morning Glory
Morning Glory from Rach Dia and Nha Be.
Control Treatment 1: Wastewater from HCM City to Nha Be and IRRI nutrient solution. Control 2: Pb^{2+} = 0 mg kg^{-1}
6 Treatments: 1/ Pb_0= 0 mg kg^{-1}, 2/ Pb_1= 1.00 mg kg^{-1}, 3/ Pb_2= 2.5 mg kg^{-1}, 4/ Pb_3= 5.0 mg kg^{-1}, 5/ Pb_4= 7.5 mg kg^{-1}, 6/ Pb_5= 10.00 mg kg^{-1}

Effect of Cd^{2+} and $CdSO_4$ on the growth of water morning glory
Control Treatment 1: Wastewater from HCM city to Nha Be and IRRI nutrient solution.
Control 2: Cd^{2+} = 0 mg kg^{-1}

Table 1. Chemical characteristics of waste water from Ho Chi Minh City (mg l^{-1})

Elements	Fe^{2+}	NH^+_4	NO_3^-	Cl^-	Cu^{2+}	Zn^{2+}	Cd^{2+}	Pb^{2+}	Hg^+	As^{2+}	pH	EC (mS/cm)
Concentration	20.5	3.6	5.8	568	0.03	0.06	0.01	0.08			6.93	3.48

Results

Heavy metal pollution in the Saigon River and canal systems down stream

Table 2. Heavy metal pollution in the Saigon River.

Fe (µg l^{-1})	Mn (µg l^{-1})	Zn (µg l^{-1})	Cu (µg l^{-1})	Cd (µg l^{-1})
2	0.9	1.7	0.9	10

In river water

Zn (mg l^{-1})	Cu (mg l^{-1})	Cd (mg l^{-1})	Ni (mg l^{-1})	Cr (mg l^{-1})
172	35	1.76	61	114

In suspended organic matter

Table 3. Tidal fluctuations of heavy metal pollution in the Saigon River and canal systems

Place	time	pH	EC-25^{0}C (µS/cm)	Mn (mg l^{-1})	Cu (mg l^{-1})	Zn (mg l^{-1})	COD (mg l^{-1})
Phuoc Loc							
River	17h15	6.5	1602.54	0.30	0.030	0.246	6.4
Field	17h15	6.6	1689.54	0.38	0.024	0.126	5.6
River	1h15	6.7	1688.67	0.80	0.048	0.314	7.2
Field	4h40	6.7	1725.21	0.24	0.008	0.126	6.4
River	5h15	6.9	1733.04	0.28	0.030	0.218	6.8
Field	11h40	6.6	1660.83	0.78	0.022	0.114	6.0
Muong Chuoi							
River	16h	6.3	990.88	0.58	0.046	0.230	15.6
Field	16h	6.3	968.88	0.32	0.042	0.388	6.4
River	23h	6.1	1075.36	0.66	0.034	0.116	6.6
Field	5h30	6.7	948.64	0.24	0.040	0.274	6.8
River	5h30	6.6	955.68	0.20	0.032	0.144	5.6
Field	10h20	6.2	1021.68	0.42	0.036	0.102	6.0

Note: High tide: 6h and 17 h. Low tide: 23h 30 and 11h20.

The fluctuations of heavy metal pollution in the Saigon River and in the downstream canal system are significant. These dynamics depend on the flow of the river, as influenced by tidal fluctuations, the concentration of metals in wastewaters and sampling location. On the fields the concentration is always higher than in the river and in the canal system.

Heavy metal contamination in sediment and soil

Table 4 shows, that while the concentration of heavy metals in itself is not very high the effective exchange between environments is high. It could mean that pollution in this environment is caused by cation exchange between organic matter and hydroxyl in water.

Table 4. Concentration of heavy metals in sediment and soil

		Humic acid					Fulvic Acid				
Material	Site	Acid	Cu	Zn	Fe	Al	Acid	Cu	Zn	Fe	Al
		(%)	(mg kg^{-1})				(%)	(mg kg^{-1})			
Sediment	RO	0.026	10.6	23.5	23.5	34.9	0.186	52.9	19.1	31.4	229.3
	MC	0.015	0.6	9.7	9.7	Trace	0.059	34.8	5.3	5.6	Trace
Soil	RO	0.067	3.2	13.0	13.0	17.9	0.016	2.0	1.4	17.2	12.1
	MC	0.184	5.6	9.2	9.2	23.9	0.023	25.2	6.8	6.0	111.0

Note: RO: Rach Ong canal; MC: Muong Chuoi canal

Table 5. Concentration of heavy metals in 2 soil horizons and in earthworms.

location	Sample	OM (%)	Pb ($mg\ kg^{-1}$)			Mn ($mg\ kg^{-1}$)			Cd ($mg\ kg^{-1}$)		
			2 acid Extraction	activity	Total	2 acid Extraction	activity	Total	2 acid Extraction	activity	Total
Long Thoi	Earthworm			9.0		14.3			0.7		
	A horizon	4.03	0.7	45.0	53.5	24.0	182.2	175.3	0.3	0.5	1.5
	B horizon	3.86	0.7	51.0		48.2			0.2		
Rach Ñia	Earthworm			4.2		10.8			0.0		
	A horizon	4.00	1.3	48.0	59.0	24.2	88.6	65.7	0.1	0.5	1.0
	B horizon	3.62	1.0	32.5		23.4			0.0		

Location	Sample	OM (%)	Cr ($mg\ kg^{-1}$)			Cu ($mg\ kg^{-1}$)		
			2 acid Extraction	activity	Total	2 acid Extraction	activity	Total
Long Thoi	Earthworm		6.3			43.1		
	A horizon	4.03	0.4	12.5	107.0	3.0	33.0	58.5
	B horizon	3.86	0.6			2.5		
Rach Ñia	Earthworm		3.2			36.1		
	A horizon	4.00	0.5	15.5	99.5	5.5	28.0	60.5
	B horizon	3.62	0.3			1.0		

Location	Sample	OM (%)	Zn ($mg\ kg^{-1}$)			Ni ($mg\ kg^{-1}$)		
			2 acid Extraction	activity	Total	2 acid Extraction	activity	Total
Long Thoi	Earthworm		62.5			6.9		
	A horizon		17.1	144.5	69.5	3.1	9.5	42.0
	B horizon		16.6	138.0		3.9		
Rach Ñia	Earthworm		60.2			3.2		
	A horizon	4.00	23.4	161.5	383.0	4.5	12.5	34.0
	B horizon	3.62	10.5	74.0		1.9		

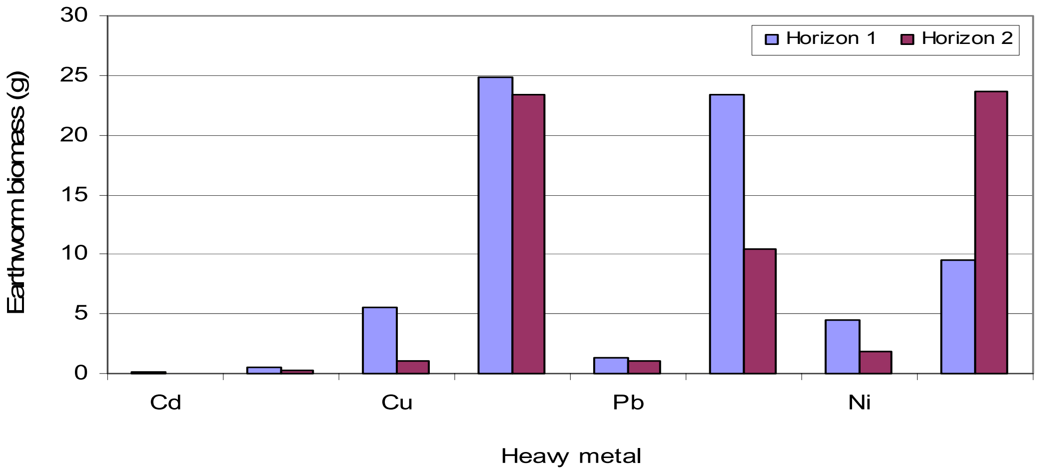
Note: Long Thoi is 25km and Rach Ñia is 34km from HCM City center

Table 5 shows that heavy metal concentration in the A horizon of the soil (0-5 cm) is always higher than in the B horizon (5-25 cm), indicating the surface accumulation of heavy metals.

Accumulation of heavy metals in earthworms

The total cumulative accumulation of heavy metals in the earthworm is about 600 - 650 mg kg^{-1} comprised of Zn 62.5 mg kg^{-1}, Cu 43.1 mg kg^{-1}, Mn 14.3 mg kg^{-1}, Pb 9.0 mg kg^{-1}, Ni 6.9 mg kg^{-1}, Cr 6.3 mg kg^{-1} and Cd 0.7 mg kg^{-1}.

Figure 1. Relationship between heavy metal and biomass of the earthworm



Effect of Heavy metal toxicity on the growing of rice
The results of these experiments are shown in the following tables.

Table 6. Effect of soil heavy metal contamination on rice root growth

Treatments	**Root length (cm)**
7Th day	
Control	6.60
Contaminated soil	2.40***
Fresh sand	5.20
Original soil	3.70
21st day	
Control	7.20
Contaminated soil	Die***
Fresh sand	7.60
Original soil	5.16
27th day	
Control	13.29
Contaminated soil	17.10
Fresh sand	13.10

Table 7. Effect of soil Cd^{2+} on the growth of rice leaves and stems (cm)

Days after growing	Control		Heavy metal contamination		Sand		Beginning	
	leaves	height	leaves	height	leaves	height	leaves	height
1	-	1.0	-	-	-	1.00	-	1.0
3	-	6.0	-	2.0	-	4.75	-	4.5
5	5.0	10.0	-	5.5	4.5	9.50	3.5-4.5	8.5-9
7	6.0	17.0	3.0-3.5	6.0	4.0-4.5	15.0	4.0-5.0	15.5

Effect of heavy metals (Pb^{2+} and Cd^{2+}) in the nutrient solution of young rice

When the concentration of Pb^{2+} > 0.5 mg l^{-1} the impact on rice growth increases by more than 50% (Table 8). With Cd^{2+} this effect is stronger, with Cd^{2+} > 0.25 mg l^{-1} affecting over 60% of young rice plants (Table 9). There is apparently a limited toxic influence of these two metals, with the LC_{50} of Pb^{2+} being 0.31 mg l^{-1}, and the LC_{50} for Cd^{2+} being 0.121 mg l^{-1}.

Table 8. Effect of Pb^{2+} in the nutrient solution on rice growth (%)

Treatments	Number of plants			Pb^{2+} (mg kg^{-1})		
	1 week	2 week	3 week	1 week	2 week	3 week
Pb_0	80	80	80	0.0	0.0	0.0
Pb_1	80	80	50	0.0	0.0	0.5
Pb_2	80	60	55	0.0	0.3	0.4
Pb_3	60	55	50	0.3	0.4	0.5
Pb_4	50	40	30	0.5	0.6	0.7
Pb_5	45	36	25	0.8	0.9	1.0
Pb_6	30	27	22	1.0	1.1	1.2

Table 9. Effect of Cd^{2+} in the nutrient solution on rice growth (%)

Treatments	Number of plants			Cd^{2+} (mg kg^{-1})		
	1 week	2 week	3 week	1 week	2 week	3 week
Cd_0	80	80	80	0.000	0.000	0.000
Cd_1	80	80	50	0.000	0.000	0.500
Cd_2	40	38	35	0.005	0.105	0.205
Cd_3	35	30	28	0.015	0115	0.215
Cd_4	28	20	18	0.025	0.125	0.225
Cd_5	20	17	15	0.400	0.500	0.600
Cd_6	30	27	22	0.500	0.600	0.700

Note: The number plants at the beginning of the test was 200

Table 10. Effect of Pb^{2+} in the nutrient solution of rice 21 Days After Planting (DAP).

Treatments	$Pb(NO_3)_2$ (mg l^{-1})	Pb^{2+} (mg l^{-1})	Rice growth (%)
Pb_0	0.00	0.00	good
Pb_1	0.50	0.31	good
Pb_2	0.40	0.25	good
Pb_3	0.50	0.31	Good 50%
Pb_4	0.70	0.44	100% death
Pb_5	1.00	0.63	100% death
Pb_6	1.20	0.75	100% death

Table 11. Effect of Cd^{2+} in the nutrient solution of rice 21 DAP.

Variants	$CdSO_4$ (mg l^{-1})	Cd^{2+} (mg l^{-1})	Rice growth (%)
Cd_0	0.000	0.000	good
Cd_1	0.025	0.014	good
Cd_2	0.205	0.110	good
Cd_3	0.215	0.120	good
Cd_4	0.225	0.121	Dead 50%
Cd_5	0.600	0.320	Dead 100%
Cd_6	0. 700	0.380	Dead 100%

There is an increase of biomass at 7 DAP with $Pb^{2+} < 0.500$ mg l^{-1} and $Cd^{2+} < 0.025$ mg l^{-1}. Similarly, at 21 DAP an increase in biomass is observed with $Pb^{2+} < 0.600$ mg l^{-1} and $Cd^{2+} <$ 0.225 mg l^{-1} (Table 12).

Table 12. Effect of Pb^{2+} in the nutrient solution on rice biomass (Dry Weight)

Treatments	7 DAP		21 DAP	
	control	Pb^{2+}(mg l^{-1})	control	Pb^{2+} (mg l^{-1})
Pb_0	171.8	0.0	145.0	0.0
Pb_1	176.8	0.0	122.0	0.5
Pb_2	194.7	0.0	235.7	0.6
Pb_3	280.6	0.3	193.0	0.7
Pb_4	292.0	0.5	154.6	0.8
Pb_5	194.4	0.8	158.6	1.0
Pb_6	121.4	1.0	153.0	1.2
Cd_0	171.8	0.0	145.0	0.000
Cd_1	175.7	0.0	150.0	0.025
Cd_2	176.7	0.005	176.9	0.205
Cd_3	244.2	0.015	196.3	0.215
Cd_4	246.3	0.025	232.6	0.225
Cd_5	179.3	0.400	144.1	0.600
Cd_6	*209.8*	*0.500*		*0.800*

From other experiments it can be concluded that at equivalent concentrations of 0.6 mg l^{-1} Cd^{2+} is always more phyto-toxic that Pb^{2+}. This effect is stronger on rice stems as compared with the leaves and roots of the rice plant (Table 13).

Table 13. The biomass increase of straw, leaves and roots of rice plants as affect by concentrations of $Pb^{2+}$and Cd^{2+}.

Pb^{2+}					Cd^{2+}				
Treatment	mg l^{-1}	straw	Leaves	Roots	Treatment	mg l^{-1}	straw	Leaves	roots
Pb_4	0.6	30.2	27.1	4.10	Cd_6	0.600	28.1	31.0	8.60
Pb_5	0.9	38.2	32.3	14.8	Cd_2	0.110	31.3	27.1	14.3
Pb_6	1.1	34.1	39.0	22.0	Cd_1	0.008	34.4	28.0	22.6

Effect of Pb^{2+}, Cd^{2+} on growth of water spinach (water morning glory)

Wastewater has no effect on roots and the length of water spinach or on the biomass.

Table 14. Effect of city wastewater on the biomass of water spinach (mg)

Days after growing	Control	Waste water
7	980	990
14	2550	2545

Effect of Pb^{2+} and Cd^{2+} on the dry biomass of water spinach

Table 15. Effect of Pb^{2+} on the biomass of water spinach (Dry Weight)

Pb^{2+} Treatments (mg l^{-1})	Days after growing	
	7	14
Control	750	2600
1.0	740	2500
2.5	750	2550
5.0	750	1950
7.5	745	1800
10.0	750	1550

Table 16. Effect of Cd^{2+} on the biomass of water spinach (Dry Weight)

Cd^{2+} Treatments (mg l^{-1})	DAP	
	7	14
Control	750	2550
1.0	745	2600
2.5	750	2650
5.0	750	2400
7.5	745	600
10.0	600	500

Table 17. Survival rate of water spinach in nutrient solution with Pb^{2+} (%)

Pb^{2+} Treatments (mg l^{-1})	DAP	
	7	14
1.0	100	100
2.5	100	100
5.0	100	80
7.5	85	50
10.0	90	30

Table 18. Survival rate of water spinach in nutrient solution with Cd^{2+} (%)

Cd^{2+} Treatments (mg l^{-1})	DAP	
	7	14
1.0	100	100
2.5	100	100
5.0	100	95
7.5	100	45
10.0	70	20

Table 19. Length (cm) of water spinach in control and wastewater solutions

Treatment	Time of growing (days)															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	4.9	6.2	7.4	8.8	9.6	10.2	10.7	11	11.4	11.8	12.3	13.6	14.2	15.7	16.6	17.2
Waste water	5	6.8	8	9.3	9.9	11	12	13	13	15	16	17	17	18	18	19

Table 20. Length (cm) of water spinach in control and Cd^{2+} solutions

Cd^{2+} Treatment (mg l^{-1})	Time of growing (days)															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	4.9	6.2	7.4	8.8	9.6	10.2	10.7	11	11.4	11.8	12.3	13.6	14.2	15.7	16.6	17.2
0.1	5	5.8	7.3	9	9.6	10.3	11.4	12	13.7	14.5	15.2	16.6	17.8	18.0	19.2	19.7
0.5	4.9	6.3	7.6	8.4	9.8	10.6	11.7	12.4	14.3	14.6	15.7	16	17.3	18.2	19	19.8
1	4.9	6	6.7	8.6	9.3	9.7	10.2	11.3	11.7	12.6	13.8	15	16.7	17	17.2	17.5
2.5	5	5.7	6.8	8.3	9.4	10.7	11.3	11.5	11.7	12.3	15					
5	4.8	6.2	7.4	8	8.6	10.2	10.6									

Table 21. Length (cm) of water spinach in control and Pb^{2+} solutions

Pb^{2+} Treatment ($mg\ l^{-1}$)	Time of growing (days)															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control	4.9	6.2	7.4	8.8	9.6	10.2	10.7	11	11.4	11.8	12.3	13.6	14.2	15.7	16.6	17.2
0.1	4.8	6	7.4	8.5	9	10.7	11.3	11.5	12	12.4	12.5	13.2	14.2	15.2	16.7	16.2
0.5	5	6.4	7.5	8.3	9.2	10.3	10.8	11.4	12.6	12.8	13	13.7	14.8	15.1	16.9	17
1	4.9	6	7.6	8.7	9.3	9.7	11.3	12.4	12.6	13	13.5	13.6	13.7	13.8		
2.5	4.9	6.3	7.2	8	9.1	10.3	11.4	12.6	13.1	13.4	13.4	13.5	13.5	13.5		
5	4.8	5.7	6.8	7.6	8.2	9.3	11.5	12.2	12.4	12.6	12.7					

Table 22. Length (cm) of root of water spinach in control and waste solution

Days after growing	Control	Waste water
7	4.7	4.8
14	6.7	7.1

Table 23. Length (cm) of root of water spinach in control and Pb^{2+} solutions ($mg\ l^{-1}$)

Time of growing	Control	1	2.5	5	7.5	10
7	4.5	4.3	5.6	5.2	5.2	5.6
14	6.2	6	6.7	4	4.3	5.7

Table 24. Length (cm) of root of water spinach in control and Cd^{2+} solutions ($mg\ l^{-1}$)

Time of growing	Control	1	2.5	5	7.5	10
7	4.7	5	4.2	4.9	4.1	4.8
14	6.3	6.1	5.6	4.1	3.9	2.3

Heavy metal accumulation in water spinach (water Morning Glory)

Table 25. The accumulation of Pb and Cd in water spinach plants after 15 days

Concentration ($mg\ l^{-1}$)	Pb			Cd		
Experimental solution	1.0	5.0	10.0	0.5	2.5	5.0
Accumulation	1.63	1.90	2.67	0.20	0.37	0.41

Pb^{2+} and Cd^{2+} do not have a major effect during the first week of the experiment except when Cd^{2+} equals or is greater than 5.0 mg l^{-1}. However, during the second week the effect is very obvious. In general, water spinach (Morning Glory) is more robust at withstanding the effects of Pb^{2+} as compared to Cd^{2+}.

Conclusions

Wastewater from Ho Chi Minh City causes heavy metals pollution downstream from the city near Nha Be at a distance of 25-30km. The polluting heavy metals include in order of descending concentrations, Zn (3575 mg kg^{-1}) > Mn (120 mg kg^{-1}) > Cr (100 mg kg^{-1}) > Cu (60 mg kg^{-1}) >Pb (555 mg kg^{-1}) > Ni (37 mg kg^{-1}) > Cd (1.3 mg kg^{-1}). Significant heavy metal uptake is found in earthworm, so much so, that it is believed that this can be used as an indicator species.

The effects of Pb^{2+} (0.63 mg kg^{-1} to 0.75 mg kg^{-1}) and Cd^{2+} (0.32 to 0.43 mg kg^{-1}) concentrations in rice growth experiments become significant after 7 DAP, with an LC_{50} of Pb^{2+} of 0.31 mg kg^{-1}, and for Cd^{2+} of 0.121 mg kg^{-1}. The influence of Cd^{2+} on the growth of rice is stronger than the influence of Pb^{2+}.

Water Spinach also known as Morning Glory is able to adapt itself to an environment where water is polluted by Pb. However when the concentration of Pb exceeds 5.00 mg l^{-1} the roots of water spinach turn black and the plant becomes necrotic. At lower concentrations spinach can grow but when concentrations are increased to < 5.00 mg l^{-1} growth is stalled. In contrast, Cd^{2+} causes the death of water spinach plants at a concentration of 2.50 mg l^{-1}. The uptake of heavy metals in Morning Glory can reach 1.63 to 2.70 mg kg^{-1} for Pb and 0.25 to 0.5 mg kg^{-1} for Cd.

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