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Heavy metals (Cd, Ni and Pb) pollution effects on cassava (*Manihot esculenta* Crantz)

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The response of crops to heavy metals is important to plant breeders working on marginal environments. Environmentalists need such knowledge for environmental quality assessment. Consequently, a study was carried out to determine the effect of heavy metals (Cd, Ni and Pb) stress on cassava in a greenhouse. The treatments comprised five improved cassava varieties (TME 419, TMS 98/0505, TMS 98/0510, TMS 98/0581 and TMS 97/2205), three heavy metals (Cd, Ni, Pb) and four heavy metal application rates (0, 100, 200 and 300 mg kg^{-1}). Heavy metals Cd, Ni and Pb reduced cassava sprouting at four weeks after planting (WAP). The stem girth, root length, root weight and dry matter yield of cassava were also negatively affected by the heavy metals at 12 WAP. The extent of the influence of the heavy metals on cassava was found to be dependent on the specific heavy metal and cassava variety. Generally, the ability of the heavy metals to affect the growth indices studied was found to be in the order: Ni > Cd > Pb. Of the five improved cassava varieties studied, TMS 98/0505 was found to be the most tolerant to the three heavy metals combined.

Key words: Cassava, nickel, cadmium, lead, effect, pollution.

INTRODUCTION

Cadmium, nickel and lead belong to the group of elements called heavy metals. They are toxic pollutants of soils and are frequently accumulated by crops grown on soils polluted with such heavy metals. When crops are grown on heavy metal-polluted soils, these metals can enter the food chain with a significant potential to impair animal and human health (John et al., 2009). Anthropogenic activities, such as indiscriminate fertilizer application, as prevalent in Nigeria, industrial waste and sewage sludge disposals on land, have led to accumulation of heavy metals in soils (Alloway, 1990; Naidu et al., 1997), with eventual increase in its concentration in food crops. Some of these heavy metals, when ingested even at a very low concentration, may lead to undesirable consequences. The FAO/WHO recommended maximum tolerable intake of Cd is 70 µg/day (John et al., 2009). With the estimated half-life of Cd in soil varying between 15 and 1100 years (Kabata-Pendias and Pendias, 1992), its accumulation in the environment and its entry into the food chain are of great concern. Another source of concern arises from recent observations that heavy metals adversely affect plant physiological indices (Doganlar et al., 2012). It has been reported that Pb induced proline accumulation and oxidative stress and reduction in chlorophyll content and plant biomass in rice plants (Zeng et al, 2007). Lead has also been reported to cause a reduction in yield of sugar beet (*Beta vulgaris*). Peralta et al. (2000) reported that Ni caused reduction in root length of alfalfa.

The sensitivity of plants to heavy metals have been reported by John et al. (2009) to be dependent on an interrelated network of physiological and molecular

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mechanisms that are plant- and even variety- dependent and include (i) uptake and accumulation of metals binding to extracellular exudates and cell wall constituents (ii) efflux of heavy metals from cytoplasm to extranuclear compartments, including vacuoles (iii) complexation of heavy metal ions inside the cell by various substances, for example, organic acid, amino acids, phytochelatins and metallothioneins; (iv) accumulation of osmolytes and osmoprotectants and induction of antioxidative enzymes; and (v) activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures. So, the effect of heavy metal on crop will depend on the crop and the crop variety. Little information exists on the effect of heavy metals on cassava (*Manihot esculenta* Crantz) that is, a crop consumed by >300 million people in sub-Saharan Africa. Many high yielding, disease-resistant varieties of cassava have been developed by National Root Crops Research Institute, Umudike, Nigeria and their international collaborators. The aim of this work was to test the reaction of these improved varieties to heavy metal stress.

MATERIALS AND METHODS

A 5 x 3 x 4 factorial experiment, arranged in a randomized complete block design and replicated three times, was used to study the effect of heavy metals (Cd, Ni and Pb) stress in a green house on five cassava varieties. About 200 kg of surface soil (0 to 25 cm) [sandy loam soil (*Typic Paleudult*)] obtained from the research farm of National Root Crops Research Institute, Umudike, Nigeria was air dried and passed through a 2 mm-sieve. The soil pH in water was 5.5, organic carbon content was 0.12%, effective cation exchange capacity was 5.20 cmol kg^{-1}, available P was 15.0 mg kg^{-1} and total N was 0.08%. One kilogram of the dried and sieved soil was placed in each of 180 plastic bags. Inorganic fertilizer NPK (20:10:10) equivalent to 450 kg ha^{-1} was applied to each of the bags.

Treatment

The treatment comprised: 1. Five cassava varieties (TME 419, TMS 98/0505, TMS 98/0510, TMS 98/0581 and TMS 97/2205); 2. Three heavy metals (Cd, Ni, Pb); 3. Four rates of the heavy metals (0, 100, 200 and 300 mg kg^{-1} soil)

The heavy metals were applied to the soil in the bags as solutions of their nitrates and the soil was watered to field capacity with distilled water and allowed to equilibrate for two days. Thereafter, a 25 cm long cassava stake was planted in each bag. The bags were arranged in the greenhouse in a split-split plot with three replications. Throughout the experiment, the soil was maintained at 80% field capacity.

One week after planting (WAP), cassava sprout count was taken. Percentage of sprouted cassava (for a particular treatment) was taken as the number of cassava stakes of that treatment that sprouted divided by the total number of cassava stakes planted (for that treatment) multiplied by 100. At 4 WAP, the number of leaves per plant was recorded whereas stem girth was measured with calipers at 12 WAP. The cassava plants were harvested at 12 WAP and the plants separated into roots and shoots. The root length was then measured. Dry matter of roots and shoots was weighed after washing them with distilled water and dried to a constant weight in an oven. The dry matter yield was taken as the sum of the weights of the roots and the shoot. The data obtained were analyzed using GENSTAT. Significant treatment means were separated using F-LSD at 5% level of probability.

RESULTS AND DISCUSSION

Cassava sprouting

The effect of heavy metals (Cd, Ni and Pb) on percentage sprouting of cassava at one week after planting is shown in Table 1. Heavy metals, rate of application and heavy metals x rate of application significantly ($p<0.05$) reduced percentage sprouting of cassava. Application of 100 mg Cd kg^{-1} led to lower percentage sprouting of cassava over the control; however, when the rate of cadmium was increased to 200 mg kg^{-1}, no significant effect was observed on the percentage sprouting over the control. Increasing the rate of Cd further to 300 mg kg^{-1} gave a significantly lower sprouting when compared with the control. All the three rates of Ni and Pb studied lowered percentage sprouting over the control. Peralta et al. (2000) reported that seed germination and growth of alfalfa were negatively affected by heavy metals (Cd, Cr, Cu and Ni). Azmat et al. (2009) also reported that seed germination of *Lens culinaris* was inhibited by Pb. Claire et al. (1991) reported that Ni caused a reduction in seed germination of cabbage, lettuce and millet. Heavy metals have been reported to physiologically block the absorption of water by seeds and plants (Azmat et al., 2006). Water is necessary for both sprouting and germination. So, the reduction in percentage sprouting of cassava by the heavy metals could be because of the ability of the heavy metals to inhibit the uptake of water by the cassava stakes. The effect of heavy metal on cassava sprouting differed with cassava variety. TME 419, TMS 98/0581 and TMS 98/0505 were less affected as compared to TMS 98/0510 and TMS 97/2205 (Table 1).

Cassava leaves

Application of heavy metals (Cd, Ni and Pb) led to the production of lower number of leaves by the cassava plants at 4 WAP when compared with the control. Increasing the rate of application of either Cd or Ni from 100 to 300 mg kg^{-1} caused a consistent decrease in the number of leaves produced by the cassava plants; however, increasing the rate of application of Pb from 100 to 300 mg kg^{-1} did not lead to a further decrease in the number of leaves produced by the cassava plants (Table 2). At 4 WAP, cassava varieties TMS 98/0505 and TMS 98/0581 had significantly higher number of leaves than TME 419, TMS 98/0510 and TMS 97/2205. Cassava variety TME 419 had more leaves than TME 98/0510 and

Table 1. Effect of heavy metals (Cd, Ni and Pb) on percentage sprouting of cassava at 1 WAP.

Heavy metal	Rate of application (mg kg^{-1} soil	Percentage sprouting of cassava (%) Cassava variety TME 419	TMS 98/0505	TMS 98/0510	TMS 98/0581	TMS 97/2205	Mean (%)	SE
Cd	100	100.0	66.7	100.0	66.7	66.7	80.0	8.60
	200	66.7	100.0	100.0	100.0	100.0	93.3	20.36
	300	66.7	100.0	66.7	100.0	66.7	80.0	40.82
Ni	100	100.0	33.3	33.3	100.0	66.7	66.7	14.66
	200	100.0	100.0	0.0	100.0	66.7	73.3	28.93
	300	100.0	100.0	33.3	100.0	0.0	73.3	46.59
Pb	100	66.7	100.0	33.3	100.0	33.3	60.0	14.66
	200	100.0	100.0	66.7	100.0	0.0	80.0	28.93
	300	100.0	66.7	33.3	100.0	33.3	66.7	44.64
Control	0	100.0	100.0	100.0	100.0	100.0	100.0	18.26
Mean		90.01	86.67	56.7	96.7	56.7		
SE		5.08	7.37	11.17	3.33	11.33		
LSD (0.05)		Rate (R) = 13.07; cassava variety (V) = 15.89; R x V = 28.16, Heavy metal = 18.32						

Table 2. Effect of heavy metals (Cd, Ni and Pb) on number of cassava leaves plant^{-1} at 4 WAP.

Heavy metal	Rate of application (mg kg^{-1} soil)	Number of cassava leaves plant^{-1} Cassava variety TME 419	TMS 98/0505	TMS 98/0510	TMS 98/0581	TMS 97/2205	Mean	SE
Cd	100	1.00	7.00	3.00	5.00	0.00	3.20	17.71
	200	1.00	5.00	3.00	6.00	2.00	3.40	35.90
	300	3.00	3.00	2.00	4.00	0.00	2.40	54.34
Ni	100	3.00	6.00	2.00	4.00	5.00	4.00	17.54
	200	4.00	2.00	0.00	3.00	1.00	2.00	36.15
	300	1.00	3.00	0.00	2.00	0.00	1.20	54.55
Pb	100	4.00	5.00	3.00	6.00	1.00	3.80	17.58
	200	4.00	4.00	4.00	6.00	2.00	4.00	35.79
	300	2.00	6.00	2.00	8.00	4.00	4.40	53.98
Control	0	4.00	7.00	4.00	6.00	7.00	5.60	1.19
Mean		2.70	4.80	2.3	5.00	2.20		
SE		0.42	0.55	0.45	0.56	0.76		
LSD (0.05)		Rate (R) = 1.00; cassava variety (V) = 1.00; R x V = not significant, Heavy metal = 1.00						

Table 3. Effect of heavy metals (Cd, Ni and Pb) on mean stem girth of cassava 12 WAP.

Heavy metal	Rate of application (mg kg^{-1} soil)	Stem girth plant^{-1} (cm) Cassava variety					Mean (cm)	SE
		TME 419	TMS 98/0505	TMS 98/0510	TMS 98/0581	TMS 97/2205		
	100	0.100	0.467	0.267	0.233	0.000	0.213	18.219
Cd	200	0.200	0.400	0.300	0.433	0.100	0.287	36.463
	300	0.100	0.333	0.167	0.133	0.000	0.147	54.746
	100	0.267	0.267	0.100	0.200	0.233	0.213	18.218
Ni	200	0.167	0.000	0.000	0.200	0.000	0.073	36.501
	300	0.000	0.100	0.000	0.000	0.000	0.020	54.769
	100	0.300	0.333	0.200	0.433	0.100	0.273	18.208
Pb	200	0.267	0.200	0.233	0.267	0.100	0.213	36.476
	300	0.133	0.467	0.233	0.533	0.167	0.307	54.716
Control	0	0.467	0.467	0.400	0.500	0.467	0.460	0.085
Mean		0.200	0.303	0.190	0.293	0.117		
SE		0.042	0.051	0.040	0.055	0.046		
LSD (0.05)		Rate= 0.070; cassava variety (V) = 0.082; heavy metal = 0.135 R x V =not significant,						

TMS 97/2205. Ryser and Sauder (2006) reported lowering of leaf production rate in *Hieracium piloselloides* by heavy metals.

Cassava stem girth

The effect of heavy metals on mean cassava stem girth at 12 WAP is shown in Table 3. Application of Cd, Ni or Pb resulted in the reduction in stem girth of cassava. Increasing the rate of application of Cd, Ni or Pb consistently led to a significant ($p<0.05$) reduction in cassava stem girth when compared with the control. The reduction of stem girth of cassava by these heavy metals could partly be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis by these metals (Padmaja et al., 1990).

Excessive amount of Cd decreased uptake of nutrient elements, inhibiting various enzyme activities, and inducing oxidative stress, including alterations in enzymes of the antioxidant defense system in the cassava plants, leading to reduction in cassava plant stem girth. This has also been observed by Sandalio et al. (2001) in pea plant and John et al. (2009) in *Brassica juncea* L . The effect of the heavy metals on cassava stem girth was found to be dependent on the cassava variety. The ability of these heavy metals to reduce the cassava stem girth is in the following order: TMS 98/0510 = TMS 97/2205 > TMS 98/0581 > TMS 98/0505 = TME 419.

Cassava root length and weight

The effect of heavy metals on mean cassava root length at 12 WAP is shown in Table 4. Heavy metals led to a reduction in cassava root length. All the cassava varieties were equally affected. The ability of the heavy metals to reduce cassava root length is in the order: Ni > Cd > Pb. At 300 mg Cd kg^{-1}, none of the cassava varieties produced roots. Cadmium has been reported to inhibit root growth (di Toppi and Gabrielli 1999).

There was no significant difference between the cassava root length at 100 mg Pb kg^{-1} and the control; however, when the rate was increased to 200 mg Pb kg^{-1} and above, the cassava root length was significantly ($p<0.05$) reduced. John et al. (2009) reported reduction in root length of *B. juncea L*. by Cd and Pb. Reduction in root length of alfalfa by Ni was also reported by Peralta et al. (2000). Oncel et al. (2000) reported reduction in root length of wheat by Cd. The reduction in the growth of the cassava roots was caused by the suppression of the elongation growth rate of cells, because of an irreversible inhibition exerted by the heavy metals on the proton pump responsible for the root elongation process (Aidid and Okamote, 1993).

Heavy metals resulted in a reduction of the cassava root weight (Table 5). Cassava is grown mostly for tuberous roots. As the rate of application of the heavy metals increased, mean cassava root weight decreased. Highest mean cassava root weight was obtained with TMS 98/0505 whereas TMS 97/2205 had the least root

Table 4. Effect of heavy metals (Cd, Ni and Pb) on mean cassava root length 12 WAP.

Heavy metal	Rate of application (mg kg^{-1} soil)	Cassava root length plant^{-1} (cm)					Mean (cm)	SE
		Cassava variety						
		TME 419	TMS 98/0505	TMS 98/0510	TMS 98/0581	TMS 97/2205		
Cd	100	1.27	8.50	4.47	5.63	0.00	3.97	17.58
	200	1.30	4.17	5.83	5.00	0.00	3.26	35.93
	300	0.00	1.17	1.83	2.13	0.00	1.03	54.59
Ni	100	10.83	6.00	0.00	2.50	4.50	4.77	17.46
	200	0.00	0.00	0.00	2.33	0.00	0.47	36.43
	300	0.00	0.00	0.00	0.00	0.00	0.00	54.77
Pb	100	17.67	16.67	11.00	12.33	10.33	13.60	15.83
	200	9.67	10.33	12.50	11.17	3.13	9.36	34.84
	300	9.00	8.33	6.00	9.33	4.33	7.40	53.43
Control	0	12.50	10.50	17.77	21.77	21.83	16.87	3.72
Mean		6.22	6.57	5.94	7.72	4.41		
SE		2.05	1.70	1.92	2.08	2.20		
LSD (0.05)		Rate (R) = 5.21; cassava variety (V) = 3.36, heavy metal = 4.06 R x V = not significant,						

Table 5. Effect of heavy metals (Cd, Ni and Pb) on mean cassava root weight 12 WAP.

Heavy metal	Rate of application (mg kg^{-1} soil)	Cassava root weight (g plant^{-1})					Mean (g)	SE
		Cassava variety						
		TME 419	TMS 98/0505	TMS 98/0510	TMS 98/0581	TMS 97/2205		
Cd	100	0.105	0.380	0.198	0.467	0.000	0.230	18.216
	200	0.110	0.335	0.398	0.677	0.000	0.304	36.459
	300	0.000	0.148	0.288	0.068	0.000	0.101	54.754
Ni	100	0.352	0.455	0.000	0.087	0.189	0.217	18.218
	200	0.000	0.000	0.000	0.179	0.000	0.036	36.508
	300	0.000	0.000	0.000	0.000	0.000	0.000	54.772
Pb	100	0.185	1.739	0.148	0.342	0.069	0.497	18.169
	200	0.122	0.124	0.108	0.223	0.060	0.127	36.492
	300	0.040	0.150	0.156	0.520	0.106	0.194	54.737
Control	0	0.587	0.797	0.264	1.369	0.354	0.674	0.215
Mean		0.150	0.413	0.156	0.393	0.078		
SE		0.059	0.166	0.043	0.128	0.037		
LSD (0.05)		Rate = 0.253; cassava variety (V) = 0.259; R x C = 0.521, heavy metal = not significant						

weight (Table 6). Gerakis et al. (1980) also reported differential effect of Pb on the root weight of sugar beet (*Beta vulgaris* L.) cultivars.

Cassava dry matter

Cadmium, Ni, and Pb significantly ($P<0.05$) decreased

Table 6. Effect of heavy metals (Cd, Ni and Pb) on cassava dry matter 12 WAP.

Heavy metal	Rate of application (mg kg^{-1} soil)	Cassava dry matter (g $plant^{-1}$) Cassava variety					Mean (g)	SE
		TME 419	TMS 98/0505	TMS 98/0510	TMS 98/0581	TMS 97/2205		
Cd	100	0.422	1.567	0.997	1.272	0.000	0.852	18.104
	200	0.782	1.422	1.186	2.273	0.190	1.171	36.302
	300	0.047	0.771	1.092	0.599	0.000	0.502	54.681
Ni	100	1.248	1.474	0.273	0.895	0.347	0.847	18.104
	200	0.258	0.000	0.000	0.815	0.000	0.215	36.476
	300	0.000	0.220	0.000	0.000	0.000	0.044	54.764
Pb	100	1.231	3.030	1.012	1.917	0.575	1.553	17.978
	200	1.303	1.064	0.980	1.720	0.353	1.084	35.317
	300	0.319	1.433	0.900	2.469	0.605	1.145	54.564
Control	0	1.740	3.202	1.831	3.058	1.931	2.352	0.516
Mean		0.735	1.418	0.827	1.502	0.400		
SE		0.193	0.330	0.182	0.301	0.186		
LSD (0.05)		Rate (R) = 0.602; cassava variety (V) = 0.522; heavy metal = 0.625 R x V = not significant						

cassava dry matter yield over the control (Table 6). Application of 100 mg each of Cd, Ni and Pb resulted in a decrease of 36.2, 36.0 and 66.0%, respectively, of the dry matter yield of the control (Table 6). There was no significant difference between the cassava dry matter yields obtained with 100 and 200 mg Cd kg^{-1}. However, when the rate of application of Cd was increased to 300 mg kg^{-1}, a further decrease in dry matter was obtained. Increasing the rate of Ni from 100 to 300 mg kg^{-1} consistently resulted in a decrease in cassava dry matter yield over the control. In the case of Pb, the dry matter obtained with 100, 200 or 300 mg kg^{-1} were similar. The ability of the heavy metals to reduce dry matter yield of cassava was in the order: Ni > Cd > Pb. Zeng et al. (2007) reported reduction in rice biomass by Pb. Cassava variety TMS 98/0581 and TMS 98/0505 significantly out-yielded TME 419, TMS 98/0510 and TME 97/2205. The effect of the heavy metals can, therefore, be said to be dependent on the cassava variety. Differential response to Pb by sugar beet cultivars has also been reported (Gerakis et al., 1980).

The reduction in shoot growth and, hence, shoot weight in plants by heavy metals was attributed to the ability of the heavy metals to reduce water potentials and Fe concentrations in plants (Chatterjee and Chatterjee, 2000). The reduction in biomass was also attributed to inhibition of chlorophyll synthesis and photosynthesis by heavy metals (Oncel et al., 2000). Cadmium was found to cause decreased uptake of nutrient elements, inhibition of various enzyme activities, induction of oxidative stress, including alterations in enzymes of the antioxidant defense system (Sandalio et al., 2001; Wu et al., 2009; Sanita di Toppi and Gabbrielli, 1999), all of which will lead to reduction in crop biomass.

Conclusion

Heavy metals (Cd, Ni and Pb) had inhibitory influence on cassava sprouting and reduced root elongation and root weight in cassava. These metals also reduced stem girth and total plant biomass of cassava. The effect, however, depends on the metal and cassava variety. The ability of the heavy metals to negatively affect the growth indices of cassava was in the order: Ni > Cd > Pb. Of the five improved cassava varieties studied, TMS 98/0505 was found to be the most tolerant to the three heavy metals combined. There is need to consider variety responses to heavy metals-polluted soils in selection schemes for cassava improvement in areas where soil pollution is prevalent.

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