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Along-vein necrosis as indicator symptom on water spinach caused by nickel in water culture

En-Jang Sun[1] and Fen-Yi Wu

Department of Plant Pathology and Entomology, National Taiwan University, Taipei, Taiwan, Republic of China

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Abstract. Water spinach (*Ipomoea aquatica* Forsk, cv. Bamboo-Leaf) grown in aerated nutrient solution in greenhouse was treated with various levels of nickel, copper, zinc, chromium, cadmium, manganese, arsenic, aluminum, and lead. Injury symptoms caused by these metals were compared with each other. Among these metals, only nickel produced specific symptoms of along-vein necrosis on leaves and stems. Leaf yellowing or chlorosis also appeared on the plants treated with nickel, zinc, chromium, cadmium, and manganese, but not on those treated with copper, arsenic, aluminum, and lead. The copper caused only stunting and small leaf symptoms, while the arsenic and aluminum caused only wilting symptoms. Some of these nine elements produced browning or necrotic symptoms on roots, but the symptoms were not specific. All of tested metals except Pd resulted in visible symptoms on leaves or roots for the tested concentrations. The toxicity threshold concentrations for Ni, Cu, Zn, Cr, Cd, Mn, As, and Al were 2.5, 5, 5, 1.25, 1, 20, 2, and 50 ppm, respectively. These results indicate that water spinach has potential for use as an indicator plant of Ni in the environment.

Keywords: Bioindicator; Heavy metal; *Ipomoea aquatica*; Nickel; Water spinach.

Introduction

Heavy metals from the effluent of factories are of priority concern by agriculturists and environmentalists owing to their potential to cause severe damage to crops or the ecosystem; through related products, they may pose serious risks to humans as well (Foy et al., 1978). Eight of these metals were listed as major pollutants by Taiwan's Environmental Protection Administration (Environmental Protection Administration, 1991; Wang, 1987): mercury (Hg), cadmium (Cd), arsenic (As), copper (Cu), zinc (Zn), nickel (Ni), chromium (Cr), and lead (Pb). All of them are highly toxic to plants and animals. The first three—Hg, Cd, and As—have received more public attention as they were shown to be associated with some widely spread human diseases. However, plant injuries caused by these three species were not as frequent as those caused by the other five species—Cu, Zn, Ni, Cr, and Pb (Sun, 1994; Wang, 1987).

Toxic metals may also originate from mining wastewater, solid waste, soil additives, fertilizers, pesticides, and even air pollutants (Cox and Hutchinson, 1979; Rhoads et al., 1989; Sun, 1994), thereby making the problems more complicated. Once reaching the soil, heavy metals are difficult to remove, and remediation is very costly or even impossible (Lee and Liao, 1992b; Wang, 1987). Detection of these elements in soil is heavily dependant on special instruments, although some approaches use bioindicators for indexing or detecting unique elements (Cox and Hutchinson, 1979; Peterson, 1981).

Both Cu and Zn are essential elements for plants; however, unnecessarily high concentrations will be phytotoxic (Foy et al., 1978; Rhoads et al., 1989; Savage et al., 1981). Ni, Cr, Pb, Hg, Cd, and As are not essential elements. Characteristic symptoms caused by many heavy metals may be similar to one another (Foy et al., 1978; Peterson, 1981). For instance, symptoms caused by copper toxicity include stunting, leaf chlorosis similar to iron deficiency, and root deformity such as the club-like root of citrus (Brams and Fishell, 1971; Foy et al., 1978; Rhoads et al., 1989; Savage et al., 1981). Zn, Ni, Cr, Pb or Cd may produce similar leaf chlorosis symptoms on plants (Cary et al., 1977; Foy et al., 1978; Hunter and Vergnano, 1952; 1953). Only a few characteristic symptoms caused by these metals were found to be specific as reviewed by Foy et al. (1978). For instance, the characteristic necrosis on oat leaf was reported to be an ideal indicator of Ni toxicity (Foy et al., 1978; Hunter and Vergnano, 1953), while purple pigment on pulpinus of soybean leaf was mentioned to be a good indicator of Cd toxicity (Foy et al., 1978).

As the specificity of a symptom is critical for diagnosing an injury or identifying cause-effect, we started this investigation in 1990, with the aim of establishing a systemic diagnosis technique in Taiwan (Sun and Chuang, 1992). Because different plant species may demonstrate different sensitivities and display different symptoms for each element, we have attempted to screen some special plant species for use as specific bioindicators of heavy metals. Herein, we summarize the results of some studies attempting to differentiate the symptoms caused by nine metals (Ni, Cu, Zn, Cr, Cd, Mn, As, Al, and Pb) on water spinach (*Ipomoea aquatica* Forsk). The results in this

[1]Corresponding author. Telephone and Fax: 02-2392-5622.

study provide valuable information in diagnosing or identifiying cause-effect in the future.

Materials and Methods

Test Plants

Water spinach (*Ipomoea aquatica* Forsk cv. Bamboo-leaf) was grown by a standard hydroponic procedure developed by Taiwan Fertilizer Company in a greenhouse. In each hydroponic tank (l × w × h = 40 × 30 × 20 cm), 30 liters of water were added with the nutrient solution developed by Taiwan Fertilizer Company that made the final concentrations of the elements (mg/l) as N 240, P 40, K 360, Ca 200, Mg 65, Fe 4, Mn 0.7, Zn 0.1, Cu 0.04, Cl 10, B 0.6, and Mo 0.02. The pH of the solution was maintained at 6.5 ± 0.1. A perforated polystyrene foam (polyloam) plate was hung above the solution to support all the plants. In addition, they were continuously aerated with a small blower.

Plant seeds were sown on perforated ceramic granules in 5-cm plastic pots on the tank surface plate then covered with a layer of vermiculite. Seven days after germination, they were thinned to one plant per pot. When they were about 20 cm in height, the plastic pots including the medium were carefully removed, leaving the roots freely suspended in the solution. A sponge block of about 5 × 5 × 4 cm was used to support the plants at the stem base and keep them upright. Two days after transplanting, they were treated with heavy metals.

Heavy Metal Treatments

Nine metals (each with four to six concentration levels) were applied to the water spinach seedlings in the water culture as described above. Five to six plantlets were used for each concentration. Chemical compounds representing these nine elements Ni, Cu, Zn, Cr, Cd, Mn, As, Al, and Pb were $NiSO_4 \cdot 6H_2O$, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $K_2Cr_2O_7$, $Cd(NO_3)_2 \cdot 4H_2O$, $MnSO_4 \cdot 4H_2O$, $NaAsO_2$, $AlCl_3 \cdot 6H_2O$, and $Pb(NO_3)_2$, respectively; however the concentrations were calculated on the basis of metal ion portion. Chemicals used in the experiments were of reagent analytical grade. Desired amounts of heavy metal were added to water culture tank in the beginning and the plants were grown in the solution for two weeks to observe the symptoms and to compare yields. The water loss due to evaporation or transpiration was compensated for by adding the water without nutrient or the test metal. The experiment was performed three times.

The preliminary symptoms on leaves or roots were generally recorded after treatments for three days or longer. The plants were harvested after two weeks of incubation in the aerated solution after the plant height was measured, and the toxic threshold concentration was determined according to appearance of visible symptoms or significant plant height reduction.

Results

Symptomological Studies

The symptoms caused by nickel were more specific than those caused by the other eight elements, as the newly developed leaves displayed the necrotic lesions of irregular shape along the veins (along-vein necrosis) (Figure 1A) about a week after treating with 5–15 ppm Ni. A necrotic lesion also occurred on the stem (Figure 1B), another type of along-vein necrosis. Occasionally, the leaf midrib showed the symptoms of vein yellowing,

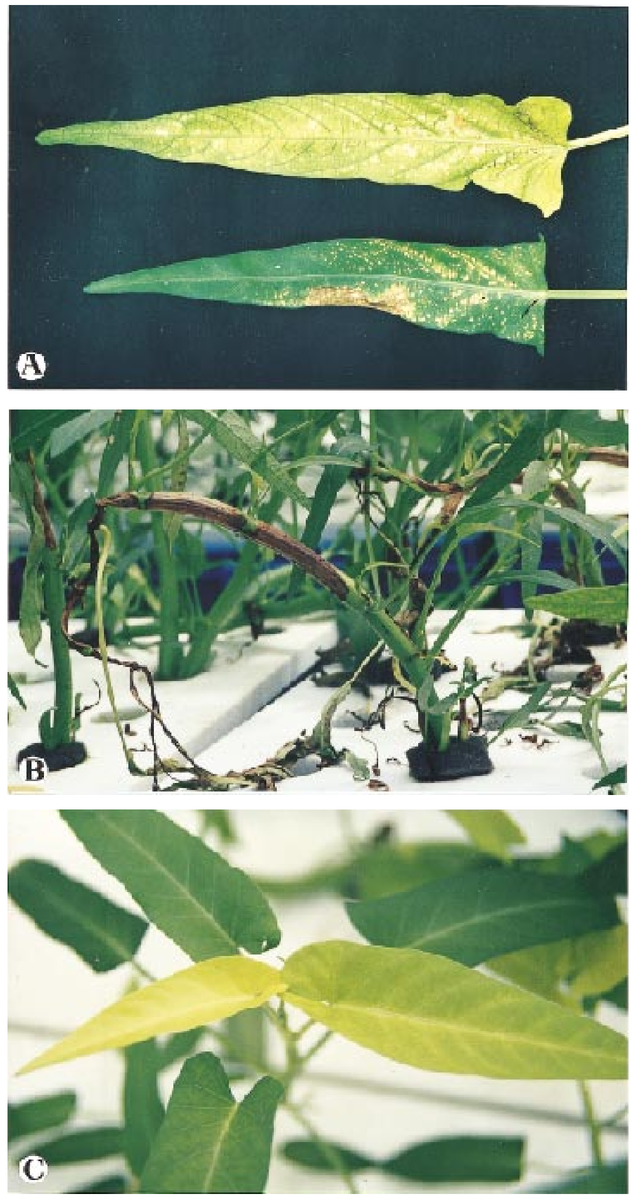

Figure 1. Symptoms on water spinach caused by nickel as compared with other heavy metals. (A) Along-vein necrosis on water spinach leaves after treatment with 5 ppm nickel in water culture. (B) Stem necrosis on water spinach as affected by 5 ppm nickel in water culture. (C) Conspicuous yellowing of newly developed water spinach leaves as affected by 5 ppm zinc in water culture.

vein browning, or vein necrosis. The plants were severely stunted. However, at 20 ppm the entire plant was weak and stunted, thereby accounting for why the leaf symptoms did not appear.

Among the nine elements, four species produced leaf yellowing or chlorosis symptoms on water spinach. Zinc at a level exceeding 5 ppm produced conspicuous yellowing symptoms on the newly developed leaves (Figure 1C). The leaves that had developed before the metal treatments were unaffected. The severe chlorosis might turn into necrosis and the injured leaf might drop. Chromium produced leaf chlorosis only at certain levels of concentration. Namely only 1.25 ppm caused the chlorosis on newly developed leaves of water spinach. At concentrations exceeding 1.25 ppm, particularly 5 and 10.0 ppm, the growth was severely suppressed. The root showed severe necrosis, leaving the leaves wilting, dark green with slight thickening. The tiller number was also reduced. Cadmium at levels exceeding 1 ppm produced slight yellowing symptoms on water spinach leaves, but conspicuous yellowing symptoms were displayed on newly developed leaves when the concentrations exceeded 5 ppm. The manganese produced marginal and interveinal chlorosis at levels exceeding 20 ppm. The yellowing symptoms caused by Zn, some levels of Cr, and Cd were not differentiable from each other. The yellowing made by Mn was different from that of Zn, Cr, and Cd, but was not differentiable from that caused by other nutritional deficiency.

Water spinach displayed other types of symptoms when treated with the other three elements: Cu, As and Al. The seedlings treated with over 5 ppm copper expressed symptoms like stunting, reduced tiller, and root necrosis. The leaves were smaller at higher concentrations. However, no chlorosis appeared on the leaves or stems. Plants at arsenic levels of 2 ppm displayed rapid wilting symptoms with root decay, finally dying in a few days. Plants at aluminum levels exceeding 50 ppm also displayed wilting but no characteristic symptoms on the leaves. At lower concentrations the plants were entirely unaffected.

In this study, lead was found to be non-toxic to water spinach for the range 5–25 ppm. Neither leaves nor roots had any symptoms. The plant height and growth of treated plants were like those of healthy control.

Toxicity Threshold Concentration of Tested Metals

The toxicity threshold concentration of each tested metal was determined according to the appearance of visible symptoms or significant plant height reduction. Except for lead, which did not produce any visible effects, the threshold concentrations of the other eight species were determined from Table 1.

In comparison, Cd, Cr and As exhibited the lowest threshold concentration of 1, 1.25 and 2 ppm, respectively; followed by Ni, Cu and Zn each for 5 ppm, Mn for 20 ppm, and Al for 50 ppm.

Discussion

Water spinach originates from Mainland China and is a popular vegetable for Chinese people. This *Ipomoea* species is used to growing in ponds or paddies, thereby making it susceptible to the effects from any kind of water pollution, similar to rice plants. However, the effects of heavy metals on this species were rarely studied in the past. Chui et al. (1988) reported an injury case of water spinach caused by Ni and Cu from a recycling factory for scrap plastic in Hong Kong, finding an accumulation of Ni and Cu in the tissue. In another case in Thailand, this species was reported to accumulate mercury (Hg) near a caustic soda factory that released Hg (Suckcharoen, 1978). In Taiwan, heavy metals such as Hg, Cd, Cu and Pb at hundreds of ppm levels were reported to inhibit the seed germination and seedling growth of *Miscanthus* species (Hsu and Chou, 1992). For water spinach, the

Table 1. Responses of water spinach seedlings in water culture to nine heavy metals at various concentrations.

Metal concentration	Plant responses[a]								
(ppm)	Ni	Cu	Zn	Cr	Cd	Mn	As	Al	Pb
0	–	–	–	–	–	–	–	–	–
0.5				–	–				
1	–				+		–		
1.25				+					
2							+		
2.5	+	–	–	+					
3					+				
5	+	+	+	+	+	–	+		–
10	+	+	+	+		–	+	–	–
15	+	+	+			–		–	–
20	+	+	+			+		–	–
25			+			+		+	–
30						+		+	
50						+		+	

[a] –: No visible symptoms; +: Showing visible symptoms.

effect of soil-contaminated Cu on yield during the reclamation period had been investigated (Lee and Liao, 1992a; 1992b). Copper was found to depress the growth, reduce the number of tiller and retard the extension of roots. However, previous literature has not fully addressed the plant symptoms caused by various heavy metals. Therefore, this study first recorded and compared the various symptoms caused by nine major metals so that the characteristic, specific, or indicative symptoms could be used to establish a diagnostic technique for these pollutants in the future. Since mercury is seldom found in the field in Taiwan, it was excluded in this study.

The symptomological comparisons in this study revealed that nickel produced more specific symptoms than the other eight elements. The along-vein necrosis (Figure 1A) and stem lesions (Figure 1B) contrasted with those in the previous literature of metal toxicity. These symptoms were also different from those caused by major air pollutants or other nutrient deficiency found in the literature (Bould et al., 1983; Fridlund, 1989; Jacobson and Hill, 1970). Nickel also produced the symptoms of stunting, leaf chlorosis, and occasionally vein necrosis and vein yellowing; however, these were not differentiable from those caused by other metals, herbicides or other factors (Fridlund, 1989). Hunter and Vergnano (1952; 1953) reported that the characteristic necrosis on oat leaves could be used as an indicator of Ni pollution. Results presented herein suggest that water spinach is also an adequate indicator of Ni.

The results in Table 1 suggested that water spinach displayed different sensitivities to these nine metals in water culture. Except for Pb, Al and Mn, all metals are toxic to this species at the level of 1–5 ppm, indicating the high sensitivities of this species to the heavy metals. Since this species is very easy to grow and very popular, it has high potential as a means of evaluating water pollution in the field.

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