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The Effect of Heavy Metals on Plants

II. Net Photosynthesis and Transpiration of Whole Corn and Sunflower Plants Treated with Pb, Cd, Ni, and Tl

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Corn and sunflower plants were grown in hydroponic culture and treated with various levels of Pb, Cd, Ni, and Tl salts. Net photosynthesis, transpiration and toxic metal ion concentration of leaf material and total plant biomass was measured. Tl was found to be the most toxic to net photosynthesis and growth of both species followed in order by Cd, Ni, and Pb.

INTRODUCTION

Anthropogenic sources of lead, cadmium and nickel in the environment include stack emissions of fossil fuel burning power plants (Natusch *et al.*, 1974) and automobile emissions (Lagerwerff and Specht, 1970). Thallium occurs as a trace element in certain fossil fuels, and like Pb, Cd, and Ni, seems to be preferentially concentrated in the smaller particle sizes of stack emissions (Natusch *et al.*, 1974). The consequences are particularly insidious since these small particles not only pass easily through conventional emission control equipment but also may be carried large distances from their source. Except for Pb these heavy metals are highly water soluble and may therefore be readily absorbed by plants.

Pb, Cd, Ni, and Tl have all been shown to cause reduction in photosynthesis and transpiration of detached leaves of corn and sunflower (Bazzaz *et al.*, 1974a, b). These results have also shown that (1) stomatal opening may be inhibited when heavy metals are introduced quickly into leaves or epidermal cells, (2) the effectiveness of heavy metals increases with ion concentration, and (3) a Pb effect on physiological processes similar to that of other heavy metals can be measured for excised plants. The present study examines the uptake and toxicity of these heavy metals in whole plants of sunflower and corn. Whole plant studies are an important corollary to studies with excised plants since the latter measures potential damage while the former measures the realized damage of heavy metals to physiological processes taking place in the leaves of intact plants.

MATERIALS AND METHODS

Corn (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) were germinated from seed and grown hydroponically in aerated Hoagland solution (5 mM

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$Ca(NO_3)_2 \cdot 4H_2O$, 5 mM KNO_3, 2 mM $MgSO_4$, 1 mM KH_2PO_4, 0.09 mM $FeC_6H_5O_7$ and 1 ml/liter of micronutrient stock solution containing 46 mM H_3BO_3, 9.1 mM $MnCl_2 \cdot 4H_2O$, 0.81 mM $ZnCl_2$, 0.29 mM $CuCl_2 \cdot H_2O$ and 0.11 mM $NaMoO_4 \cdot 2H_2O$). These plants were transplanted to 12-liter plastic tubs, at a density of 4–6 plants per tub, containing fresh Hoagland solution when they reached a height of 30–45 cm. Aliquots of concentrated heavy metal solutions were added 3–5 days later to achieve treatment levels of 0, 1, 2, 5, and 10 ppm in solution. Chloride salts were used for Pb, Cd, and Ni while the sulfate salt was used for Tl. The plants were grown during the summer months in a glasshouse supplemented with fluorescent lighting. Due to space limitations in the glasshouse and the time required for gas exchange measurements a full set of concentrations, one tub per concentration, for only three of the four heavy metals could be run at any one time. The four heavy metals were changed in successive experiments such that each was replicated three times and each pair repeated twice. For each experiment net photosynthesis and transpiration of individual mature leaves was measured for a full set of plants on each of 3 consecutive days beginning 4 or 5 days after the addition of heavy metals.

Gas exchange measurements were made according to techniques described by Bazzaz and Boyer (1972). Environmental conditions within the assimilation chamber were maintained at 1200 $\mu e\ m^{-2}\ sec^{-1}$ PAR (400–700 nm photosynthetically active radiation), 25°C air temperature, 70% relative humidity, 1.5 m sec^{-1} windspeed and 300–310 μl/liter CO_2 in the air around the leaves. The leaf material used for these measurements was then harvested, oven dried, and analyzed for heavy metal content by the Heavy Metals Analytical Laboratory at the University of Illinois. Whole plant biomass was measured at the end of each experiment. Measurement of the effect of heavy metal ions on stomatal movement of epidermal peels followed that described previously (Bazzaz *et al.*, 1974a).

RESULTS

Increasing treatment levels of all heavy metals except Pb caused a reduction in net photosynthesis (NPs) of both corn and sunflower (Fig. 1). Photosynthesis decreased most rapidly and to a lower level in Tl-treated plants than in plants treated with the other heavy metals. Cadmium was found to be less toxic to NPs than Tl but generally more toxic than Ni. Lead did not cause a statistically significant reduction in NPs at these treatment levels. Additional experiments were conducted with both corn and sunflower in which the treatment level was raised up to 500 ppm (2410 μM) Pb. Rates of NPs at this as well as intermediate concentrations were found also not to be statistically lower than that of the control plants. In general, the reduction in NPs for both species, in terms of treatment concentration, followed the same pattern with Pb having little or no effect, Ni causing reductions of 5–25%, Cd causing slightly greater reductions of 20–30% and Tl lowering NPs by 50–75%.

Sunflower accumulated significantly more Cd and Ni by the end of 4 to 7 days than did corn at all treatment levels (Fig. 2). The concentration of Cd and Ni in photosynthetic tissue reached 3.5–4.1 μmole/g at treatment concentrations of 80

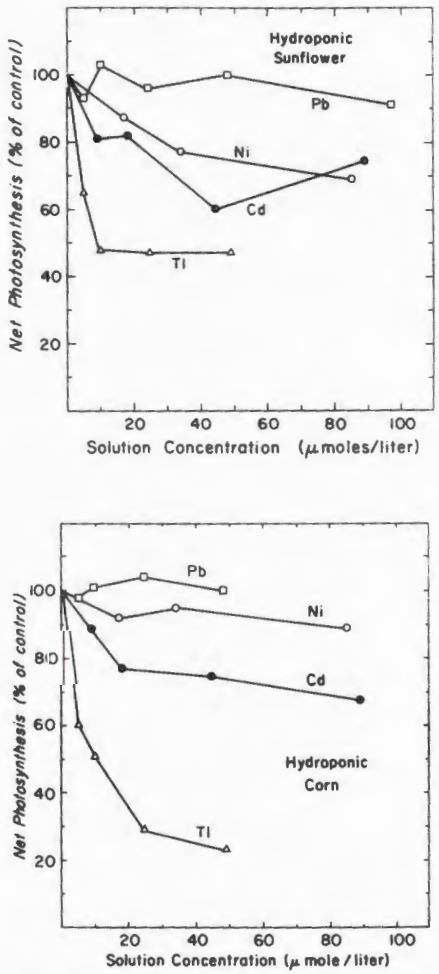

FIG. 1. Relationship between net photosynthesis and heavy metal treatment concentration for plants grown in hydroponic culture. Net photosynthesis is expressed as a percent of the rate for untreated control plants.

to 90 μM while that of corn leaves reached levels of only 0.9–1.3 μmole/g. The accumulation of Tl in both species was similar to the relatively low values observed for Ni in corn. The amount of Pb in leaves of both sunflower and corn treated with up to 500 ppm Pb averaged 0.02 μmole/g (4 ppm) and was not statistically different from the Pb content of leaves from untreated plants.

Thallium caused a 50% reduction in NPs at a leaf content of less than 0.4 μmole/g (82 ppm) for both sunflower and corn (Fig. 3). A 50% reduction in NPs of Cd treated plants occurred at a leaf content of 1.4 μmole/g (160 ppm) for corn and 3.0 μmole/g (340 ppm) for sunflower. Sunflower and corn plants treated with Ni exhibited a 50% reduction at leaf concentrations of 5 to 6 μmole/g

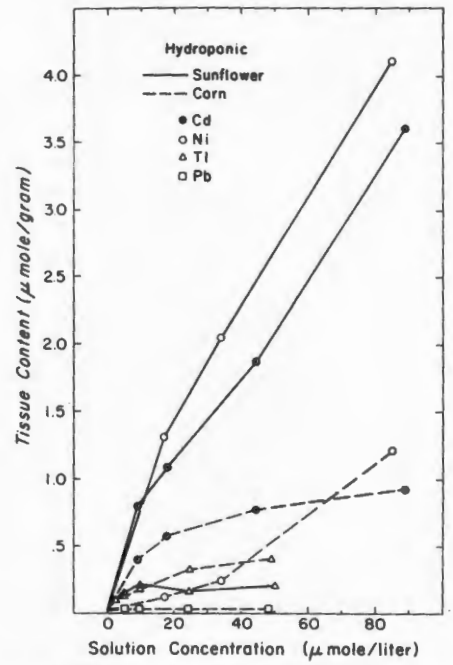

FIG. 2. Leaf accumulation of heavy metals in plants grown in hydroponic culture. Leaf content is expressed in micromoles per gram of oven dried tissue.

(290–350 ppm). The Pb content of leaves from plants treated with this heavy metal was not significantly greater than the control plants, thus precluding the development of a regression relating the effect of tissue Pb content to the rate of net photosynthesis.

The rate of transpiration for Pb- and Ni-treated plants of both sunflower and corn was found to be highly correlated with that of NPs at all treatment levels. This is indicated by the values of the NPs/Ts (net photosynthesis-transpiration) ratio as shown in Fig. 4. The NPs/Ts ratios of sunflower and corn are less than unity for Tl-treated plants indicating a more severe decrease in photosynthesis than transpiration at any given treatment level for this heavy metal. An indication of a similar response was observed for the effect of Cd on sunflower. The opposite effect, i.e., a more severe reduction in the rate of transpiration than in net photosynthesis, was observed for corn plants treated with Cd.

The results of experiments designed to examine the direct effects of toxic cations on stomatal opening by treating epidermal strips with solutions of heavy metals are presented in Table 1. Comparable trends in stomatal opening occurred at various concentrations of Pb, Cd, and Ni. Only in the case of Tl did results differ significantly from these three heavy metals. Thallium caused a 50% reduction in stomatal opening of corn at 1/10 the concentration required for the other heavy metals.

Total biomass for roots as well as plant tops of both species treated with

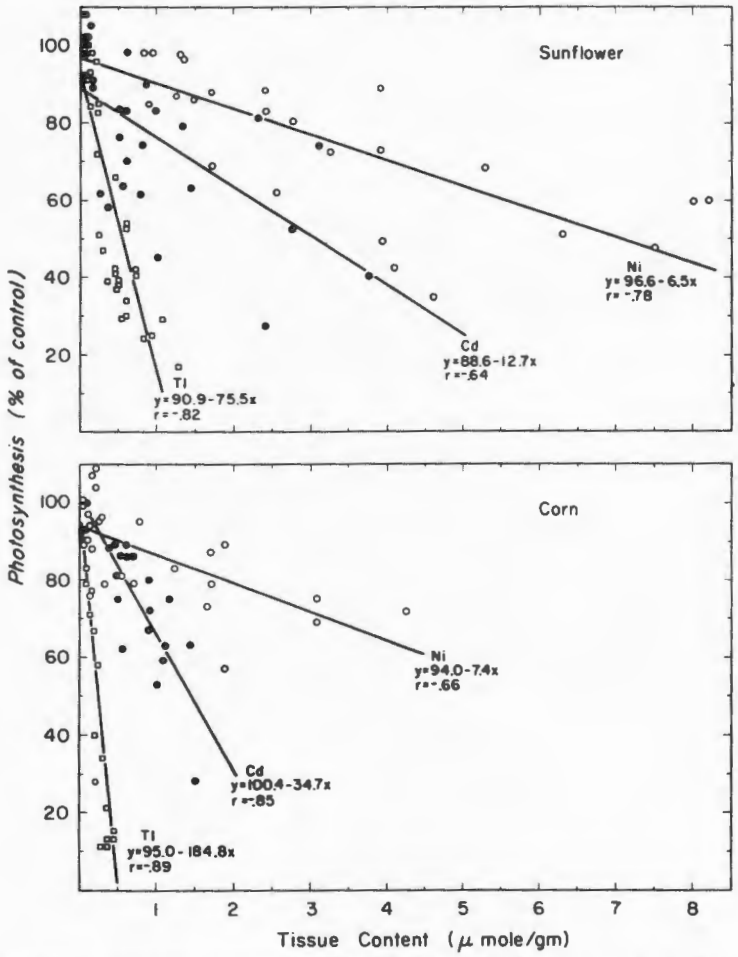

FIG. 3. Relationship between photosynthesis and leaf content of heavy metals for plants grown in hydroponic culture. Photosynthesis is expressed as a percent of the rate for untreated control plants and leaf content in micromoles per gram of oven dried tissue.

various levels of Cd, Ni or Pb were not significantly different from that of the control plants. However, in corn, treatment with Tl severely reduced top and root biomass to 50–60%. Although the biomass of sunflower plants treated with Tl was less than that observed for any of the other heavy metals, the reductions of up to 50% at the higher treatment levels were not significantly lower statistically than untreated sunflower due to the wide variation exhibited by the control plants.

Foliar symptoms of leaf yellowing developed at the higher treatment levels for all heavy metals tested except Pb. The intensity and pattern of chlorotic development varied between heavy metals. Chlorosis in plants treated with Cd began between veins at a treatment concentration of 20 μM and progressed to over

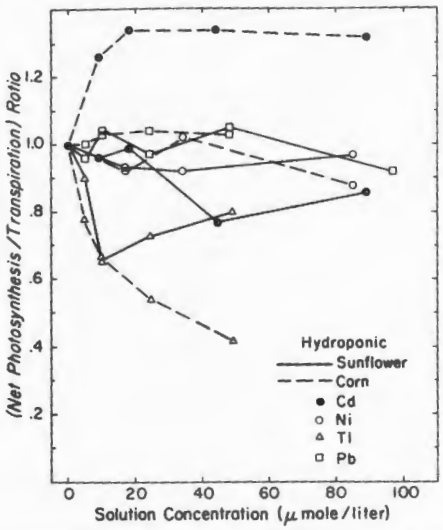

FIG. 4. Ratio of net photosynthesis to transpiration for plants treated with various concentration of heavy metals in hydroponic culture. The ratio is calculated from rates of photosynthesis and transpiration expressed as a percent of untreated controls for each treatment concentration.

50% of the leaf area at high treatment levels. Symptom development in Ni-treated plants was similar to but less severe than that observed for plants treated with Cd. Leaf yellowing began along the veins in plants treated with 5 μM Tl and extended into interveinal areas at higher concentrations.

DISCUSSION

Although leaf tissue accumulated less Tl than the other heavy metals, it caused larger reductions in NPs and growth. Thallium accumulated to higher levels in corn than in sunflower but any given leaf concentration reduced NPs

TABLE 1
EFFECT OF HEAVY METALS ON STOMATAL OPENING ON SUNFLOWER AND CORN[a]

	Heavy metal concentration μmole/liter	Pb	Cd	Ni	Tl
Sunflower	1	90	92	88	70
	10	68	63	63	13
	100	50	50	41	5
Corn	1	87	89	92	75
	10	59	65	73	52
	100	48	55	59	23

[a] Epidermal peels were floated on solutions of heavy metals for 4 hours in the dark at ambient CO_2 levels followed by 4 hours in the light at lowered CO_2 concentrations. Stomatal opening, scored at the end of the light period, is expressed as a percent of control samples run concomittently.

more in corn. Similarly, at any given treatment level, both root and shoot biomass of corn was reduced more than that of sunflower.

Thallium also inhibits stomatal opening more than any of the other heavy metals tested. Since Tl appears to mimic the shuttle of potassium between guard and subsidiary cells which occurs during stomatal movement in corn (Pallaghy, 1972; Maercker, 1965), a possible mechanism may exist in which Tl is accumulated in the stomatal apparatus. Since the ratio of NPs/Ts is less than unity for both corn and sunflower plants treated with Tl we conclude that Tl inhibits NPs not only due to stomatal closure but also through reduction of the biophysical and biochemical steps in CO_2 assimilation.

The amount of Cd accumulated in sunflower was two to three times that accumulated in corn probably because the actual rate of Ts per unit leaf area of sunflower averaged more than twice that of corn for all treatment levels. However, at any given leaf content Cd depressed NPs in corn to a value which is approximately half that observed for sunflower. Thus, when Cd is equally available in the root medium, NPs will be inhibited to the same degree in both species due to differential uptake. In corn, Cd caused a greater reduction in Ts than NPs while the opposite response was found in sunflower. Apparently Cd has an inhibitory effect on water movement through the roots and into the leaves of corn that is not exhibited in sunflower. The effect of Cd on stomatal opening was found to be nearly identical for both species indicating that differences in stomatal opening between corn and sunflower are probably not contributing to the dissimilarity in NPs/Ts ratios.

In Ni-treated plants the relationship of NPs to leaf content of sunflower is nearly identical to that of corn. However the amount of Ni accumulated by sunflower at low treatment levels is more than 10 times that accumulated by corn leaves. This value decreased to a twofold increase at higher treatment levels. This means that when Ni is equally available in the root medium, sunflower will accumulate a greater amount per unit time and thus NPs will be inhibited more in sunflower than in corn.

The ratio of NPs to Ts of both sunflower and corn plants treated with Ni is close to unity (average 0.94) at all treatment levels. This suggests that stomatal opening may be an important factor in the influence of Ni on gas exchange in both species.

The amount of Pb that accumulated in leaves of plants treated with this heavy metal, even at concentrations up to 500 ppm, was not significantly greater than that found in untreated control plants. Neither were the rates of NPs or Ts of Pb-treated plants significantly different from that of control plants. These results suggest that Pb is not translocated in appreciable quantities from hydroponic media through the root system and into leaf tissue of intact plants during short term experiments. However, leaves of tree species grown for 90 days in soil containing 75 to 600 ppm Pb do accumulate significant amounts of Pb (Rolfe, 1973). A reduction in Pb accumulation occurred when the phosphorus level of the soil was increased indicating that the presence of free phosphate in the root medium causes reduced Pb uptake by plants. The phosphate concentration of the hydroponic media used in the present study is 1 mM or 400 times the highest Pb con-

centration examined. It is quite possible therefore that most if not all the Pb precipitated as $PbPO_4$ and not available for plant uptake.

When the stems of excised plants are placed in solutions of Pb salts, Pb translocated into leaf tissue and gas exchange is decreased (Bazzaz *et al.*, 1974 b). Similarly, in vitro experiments with both sunflower and corn indicate that I inhibits stomatal opening to a degree that is similar to that caused by Cd or N This suggests that if Pb were present in the vicinity of the stomatal apparatus concentrations similar to that of other heavy metals it would inhibit stomat movement and therefore gas exchange to the same degree. Since Pb may b deposited on leaves in aerosol form (Lagerwerff, 1971), an alternative pathwa might exist for Pb contamination of leaf tissue in which the root system is n included.

Of the four heavy metal ions used in these experiments, Tl was found to b the most toxic to NPs and growth of whole plants followed in order by Cd, N and Pb. This is true for both species when expressed either as a function of lea content or treatment concentration. When expressed in terms of leaf conten NPs is reduced more in corn than sunflower by Tl and Cd but to approximatel the same level in both species by Ni. However, since the rate of accumulation o Cd and Ni is much higher in sunflower than corn, an alternative ranking is in dicated based on treatment concentration with Ni having a greater toxicity t sunflower and Cd effecting both species to the same degree.

Measurements of stomatal opening and transpiration support the hypothesis that stomatal movement may be an important factor in the effect of these heavy metals on NPs. In addition, Cd has an inhibitory effect on water uptake in corn and Tl reduces NPs to a level below that expected on the basis of stomatal closure alone.

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