Carbohydrate Levels and Photoassimilate Export from Leaves of *Phaseolus vulgaris* Exposed to Excess Cobalt, Nickel, and Zinc[1]

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ABSTRACT

Exposure of white bean seedlings to phytotoxic burdens of Co, Ni, or Zn reduced the export of ^{14}C-photoassimilates from the nearly fully expanded unifoliate leaves. Little ^{14}C reached the major sink areas, the young trifoliate leaves and the root tips, of seedlings exposed to metal. The unifoliate leaves accumulated sucrose, reducing sugars, and starch. These effects were evident within 1 or 2 days.

The most general symptoms of metal toxicity in plants are stunting and chlorosis (7). Yield reductions could occur by excess metal directly or indirectly inhibiting either assimilate production in source leaves, translocation from source to sink, utilization in sink regions, or several of these. Various metal ions have adverse effects on respiration and photosynthesis in intact organisms, ranging from algae and lichens to vascular plants, as well as on isolated mitochondria and chloroplasts (5, 10, 16, 19, 22). In this context, it is particularly noteworthy that carbohydrates accumulate in shoots of barley (1) and starch accumulates in leaves of white beans (23) on exposure of seedlings to excess Co, Ni, or Zn. Agarwala *et al.* (1) suggested that the carbohydrate accumulations were due to metal interferences with carbohydrate metabolism. Rauser (23) gave the alternative interpretation that starch accumulations reflected reduced translocation out of leaves which normally export carbon.

The aim of this work was to determine the levels of sucrose and reducing sugars in relation to starch in seedlings of *Phaseolus vulgaris* exposed to individual phytotoxic burdens of Co, Ni, and Zn. In addition, we assessed the rate of export of ^{14}C-photoassimilates from unifoliate leaves and their pattern of distribution in bean seedlings exposed to excess metal.

MATERIALS AND METHODS

Plant Material and Hydroponic Culture. White beans (*Phaseolus vulgaris* L., var. Kentwood) were sown in equal parts of Vermiculite and Perlite. Five or 6 days later uniform seedlings were transplanted to hydroponic cultures. Half-strength complete Hoagland solution No. 2 (13) with iron supplied as Fe-EDTA at 22 μM was made up with deionized H_2O and adjusted to pH 5.0 before use. One-liter beakers lined with polyethylene bags contained the nutrient solution for two seedlings held in Styrofoam lids (13 × 13 × 2.5 cm). The hydroponic cultures were aerated continuously. Ten days after seeding, the plants were exposed to individual excesses of metal. The nutrient solution was replaced with fresh solution containing 400 μM Co, 200 μM Ni or Zn as the

sulfate salts. Excess Zn was superimposed over that already present in the nutrient solution. These concentrations of excess metals cause early and reproducible development of visual toxicity symptoms in roots and shoots. After 4 days of exposure to excess metals shoot dry matter production is depressed a little whereas root yields are at least 66% of controls (23).

Plants were grown in a growth room at 22 to 24 C and an 18-h photoperiod. Daylight fluorescent tubes and incandescent bulbs provided a mean irradiance of 42 w m^{-2} and mean PAR (400–700 nm) of 140 μmol m^{-2} s^{-1} at plant height.

Sucrose, Reducing Sugars, and Starch Contents. Unifoliate leaves were harvested between 1200 and 1300 h and quickly dropped into 40 ml boiling 13.7 mol l^{-1} ethanol in water and refluxed for 30 min. After reextraction with 40- and 20-ml portions of aqueous ethanol, the leaves were completely decolorized. The three alcohol extracts of each leaf were combined and evaporated nearly to dryness by gently warming. The residue was taken up in 20 to 30 ml deionized H_2O filtered quantitatively through a glass fiber filter, and the filtrate made to a total volume of 50.0 ml with water. A 10.0-ml subsample of the filtrate was pipetted into a 15-ml centrifuge tube containing 1 ml each of swollen Dowex 1-X8 anion exchange resin (200–400 mesh, Cl$^-$ form) and of Dowex 50-W cation exchange resin (100–200 mesh, H$^+$ form). The contents were mixed thoroughly and centrifuged at 1,500g for 2 to 3 min. The supernatant was decanted and the resin mixture was extracted once with 4 ml water. Both supernatants were combined and made to a total volume of 15.0 ml.

Variable volumes of the resin-treated supernatants were used to determine reducing sugars (18). Other subsamples were digested with 11 units of invertase (220 units mg^{-1}, Sigma Chemical Co.) for 2 h at 50 C. The volumes of supernatant used were always taken such that the reducing sugar content before and after invertase action was below 0.6 μmol glucose. The reducing sugars liberated by invertase activity were used to calculate the amount of sucrose. The resin treatment removed inhibitors of invertase activity from the extracts, thus allowing complete hydrolysis of sucrose under the conditions specified. It is recognized that the sugar mono- and diphosphates will be adsorbed to the resin leading to a small underestimation of reducing sugars. In sunflower leaves *e.g.* these components represented about 7% of the soluble carbohydrates or about 5% of total carbohydrates (4).

Starch estimates were made on the leaves that had been extracted with aqueous ethanol. The starch was digested with amyloglucosidase and the glucose liberated was measured with glucose oxidase (23).

Analysis of variance and Duncan's multiple range test were applied to the data. Statistical tests on percentage data were done after their transformation to the arcsin $\sqrt{percentage}$ (25).

Translocation of ^{14}C-Photoassimilates. Plants were exposed to [14]CO_2 between 1130 and 1200 h in a growth room identical to the one used for growing the plants. One unifoliate leaf of up to 14 plants was placed in an elongated acrylic plastic chamber (7.18 dm^3 volume) with the petioles extending through slots on one side

of the chamber. The petioles were sealed into the chamber with silicone grease. $^{14}CO_2$ was released into the chamber by injecting 60 μCi NaH^{14}CO$_3$ (50 mCi mmol^{-1}, New England Nuclear) onto a few crystals of citric acid in a flask connected to the chamber. The $^{14}CO_2$ was distributed and recirculated around the leaves and flask with a pump at a flow rate of 0.11 to 0.13 m^3 h^{-1}. The leaves were allowed to photosynthesize in $^{14}CO_2$ for 15 min after which the air in the chamber was passed through 1 M NaOH for 5 min. The petioles and leaves were then released from the exposure chamber and the plants were allowed to translocate ^{14}C-photoassimilates for an additional 2 h in the growth room.

At the end of the translocation period, the petioles subtending the leaves exposed to $^{14}CO_2$ were excised at their extremities, dropped into liquid N$_2$, and stored frozen until analyzed for callose (21). The remainder of the plants were cut at the root-shoot junction. The roots, shoots, and the ^{14}C-exporting leaves were oven-dried at 100 C for 24 h, weighed, and ground separately into fine powders with a mortar and pestle. Forty-mg samples of the powders were combusted in a Packard model 305 Tri-Carb oxidizer and counted in a Nuclear-Chicago Isocap 300 liquid scintillation spectrometer. The radioactivity in the plant excluding the source leaf as a percentage of radioactivity in the whole plant was considered as the percentage of ^{14}C-photoassimilate exported through the petiole.

Autoradiography of Entire Plants. Immediately following the labeling and translocation periods described above, the plants were cut and separated into unifoliate leaves, shoot, and roots. These parts were pressed between paper and oven-dried at 100 C for 24 h. Each plant was reconstructed on a herbarium sheet and was exposed to Kodak SB-5 x-ray film for 4 days.

RESULTS

Sucrose, Reducing Sugar, and Starch Contents. Phytotoxic concentrations of Co, Ni, and Zn all caused abnormal accumula-

tions of sucrose, reducing sugars, and starch in the unifoliate leaves of white beans (Table I). Contents of sucrose and reducing sugars were 2.5- to 3-fold greater than those of controls following 1 day of exposure to excess Co or Ni. Exposure to excess Zn for 1 day caused a significant increase in reducing sugars. Starch contents of leaves of seedlings exposed to excess metal for 1 day ranged from 1.6- to 2.0-fold greater than those of controls. The accumulations of the three types of carbohydrates increased with time, particularly for seedlings exposed to excess Ni and Zn. This pattern was not as clearly evident in two other trials of this same experiment where somewhat different carbohydrate contents were found. Significant accumulations of sucrose, reducing sugars, and starch were evident in some cases after 1 day, and in all cases after 2 days of exposure to either Co, Ni, or Zn.

In control plants the total of the three classes of carbohydrates and the proportion of each as a percentage of the total, remained essentially constant over the 4-day period of the experiments (Table II). The total carbohydrate level increased 1.7-fold for Ni and Zn treatments and 2-fold for Co treatment for 1 day. With time, more carbohydrates accumulated in the leaves of the seedlings exposed to metal, so that after 4 days, the increases were 5.6-fold for Co, 5.7-fold for Zn, and 8.0-fold for Ni. Exposure to each of the three metals for 1 day caused significant shifts in the proportions of the total carbohydrate found as either sucrose, reducing sugar, or starch. From the 2nd to the 4th day of exposure to excess Zn, the unifoliate leaves had essentially the same proportions of each class of carbohydrate as did the controls, despite the marked accumulation of carbohydrate in the tissues. Exposure to excess Co for 2 days or longer caused a preferential accumulation of starch with proportionately little as reducing sugars. The leaves of seedlings exposed to excess Ni had proportionately higher contents of sucrose and reducing sugars than of starch.

Expansion of Unifoliate Leaves. The transition of dicotyledonous leaves from being net carbohydrate importers to net exporters occurs when leaves are about 50 to 60% fully expanded, depending

Table I. Contents of sucrose, reducing sugar and starch in unifoliate leaves of bean seedlings exposed to excess metal.

Ten day old seedlings were exposed to 400 μM cobalt and 200 μM nickel or zinc. Sucrose, reducing sugar and starch contents were determined one to four days after exposure to metal. The data represent the mean ± SE of six replicates.

Treatment	Day 1	Day 2	Day 3	Day 4
	Sucrose (μmol g^{-1} fresh wt)			
Control[1]	3.88 ± 0.16	3.89 ± 0.41	3.19 ± 0.21	2.55 ± 0.21
Cobalt	10.54 ± 0.38*	8.67 ± 0.45*	11.20 ± 0.91*	15.60 ± 1.53*
Nickel	9.69 ± 0.47*	17.40 ± 1.73*	36.69 ± 3.48*	44.29 ± 7.00*
Zinc	3.56 ± 0.18	8.03 ± 0.89*	8.41 ± 0.26*	13.75 ± 1.29*
	Reducing sugar (μmol glucose g^{-1} fresh wt)			
Control[1]	2.53 ± 0.15	2.89 ± 0.16	2.55 ± 0.12	2.62 ± 0.31
Cobalt	7.52 ± 0.44*	4.65 ± 0.29*	4.44 ± 0.39*	5.45 ± 0.18*
Nickel	6.86 ± 0.58*	19.51 ± 1.19*	36.43 ± 2.61*	41.29 ± 3.73*
Zinc	5.38 ± 0.31*	9.51 ± 1.07*	14.91 ± 0.31*	18.01 ± 1.49*
	Starch (μmol glucose g^{-1} fresh wt)			
Control[1]	16.85 ± 1.08	17.15 ± 1.08	14.34 ± 0.57	14.57 ± 0.52
Cobalt	27.66 ± 1.38*	74.30 ± 2.11*	70.54 ± 4.42*	88.66 ± 2.74*
Nickel	21.60 ± 0.64*	39.45 ± 3.28*	38.53 ± 1.18*	48.77 ± 5.22*
Zinc	33.89 ± 1.45*	47.83 ± 4.23*	63.99 ± 5.35*	84.27 ± 3.75*

[1] Day 0 control values: sucrose 2.75 ± 0.05; reducing sugar 3.09 ± 0.19, starch 18.45 ± 0.74.

* Significantly higher than the control value of that day at the 0.05 level.

Table II. Total carbohydrate content and relative proportions between sucrose, reducing sugars and starch in unifoliate leaves of bean seedlings exposed to excess metal.

The sucrose contents shown in Table I were converted to glucose equivalents and added as such to the glucose found for the reducing sugars and starch to give total carbohydrate (μmole glucose equivalents g^{-1} fresh wt). The relative proportion of each type of carbohydrate is given as a percentage of the total. The data represent the mean or mean $\pm$ SE of six replicates.

	Day 1	Day 2	Day 3	Day 4
Control				
Sucrose	28.7	27.8	27.3	22.8
Reducing sugar	9.3	10.5	11.0	11.4
Starch	61.9	61.7	61.7	65.9
Total	(27.13 $\pm$ 1.45)	(27.81 $\pm$ 1.69)	(23.25 $\pm$ 0.76)	(22.39 $\pm$ 0.75)
Cobalt				
Sucrose	37.5	18.0	23.1	24.7
Reducing sugar	13.4	4.8	4.6	4.3
Starch	49.0	77.2	72.3	70.9
Total	(56.26 $\pm$ 1.61)*	(96.28 $\pm$ 2.78)*	(97.38 $\pm$ 4.55)*	(125.30 $\pm$ 3.54)*
Nickel				
Sucrose	40.5	36.9	48.7	48.5
Reducing sugar	14.2	21.0	24.5	23.9
Starch	45.3	42.1	26.8	27.6
Total	(47.79 $\pm$ 2.03)*	(93.74 $\pm$ 6.43)*	(149.00 $\pm$ 8.12)*	(178.64 $\pm$ 20.29)*
Zinc				
Sucrose	15.4	21.8	17.8	20.9
Reducing sugar	11.6	12.9	15.9	13.8
Starch	73.1	65.3	66.3	65.3
Total	(46.38 $\pm$ 1.90)*	(73.40 $\pm$ 6.86)*	(95.55 $\pm$ 5.43)*	(131.11 $\pm$ 4.72)*

*Significantly higher than the control value of that day at the 0.01 level.

Table III. The export of ^{14}C-photoassimilates out of unifoliate leaves of bean seedlings exposed to excess metal.

Ten day old seedlings were exposed to 400 μM cobalt and 200 μM nickel or zinc. One to four days after, one of the unifoliate leaves was exposed to $^{14}CO_2$ for 15 min. The values are the amounts of ^{14}C in roots and shoots as a percentage of the total in the plant following two hours of translocation. Means of three replicates, the numbers in brackets are the means $\pm$ SE of the arcsin transformation.

	% ^{14}C translocated			
	Day 1	Day 2	Day 3	Day 4
Control	45.3	53.5	48.7	51.1
	(42.30 $\pm$ 0.63)	(47.01 $\pm$ 0.30)	(44.27 $\pm$ 0.87)	(45.65 $\pm$ 2.19)
Cobalt	0.5	13.0	5.5	4.0
	(3.97 $\pm$ 0.60)*	(19.30 $\pm$ 6.51)*	(13.41 $\pm$ 1.28)*	(11.32 $\pm$ 1.78)*
Nickel	18.8	0.8	6.6	0.9
	(25.52 $\pm$ 2.48)*	(5.20 $\pm$ 0.46)*	(11.15 $\pm$ 7.35)*	(5.32 $\pm$ 1.11)*
Zinc	5.4	9.8	20.6	9.9
	(11.88 $\pm$ 4.61)*	(18.16 $\pm$ 1.06)*	(26.98 $\pm$ 0.56)*	(18.31 $\pm$ 1.10)*

* Significantly less than the controls of the day at the 0.01 level.

on the species (9). Repetitive measurement of leaf lamina lengths indicated that at the time the white bean seedlings were routinely exposed to excess metal, the unifoliate leaves had expanded to at least 89% of the final lamina length attained by controls. Excess metal did not significantly reduce lamina expansion during the 4-day experimental period.

Export of ^{14}C-Photoassimilates. Translocation of photoassimilates out of leaves was severely reduced in white bean seedlings exposed to excess Co, Ni, and Zn (Table III). In control plants 45 to 53% of the total ^{14}C present was transported out of unifoliate leaves during 2 h. In contrast, exposure of seedlings to excess Co, Ni, or Zn for 1 to 4 days caused dramatic, significant declines in

the percentage of ^{14}C-photoassimilates exported from unifoliate leaves. There was no recognizable trend in the reduction of photoassimilate translocation by increasing the duration of exposure from 1 to 4 days. In contrast to controls, seedlings exposed to excess metal showed larger variations in the pecentage of assimilate transported. Such variation is attributable in part to differing rates of metal absorption and translocation between individual seedlings, leading to variable metal burdens in the tissues.

In control plants ^{14}C-photoassimilate reached the growing apex, the expanding trifoliate leaves, the roots, and root tips in particular (Fig. 1A). In the seedlings exposed to excess metal the major sink areas contained less radioactivity (Figs. 1, B–D). ^{14}C-Photoas-
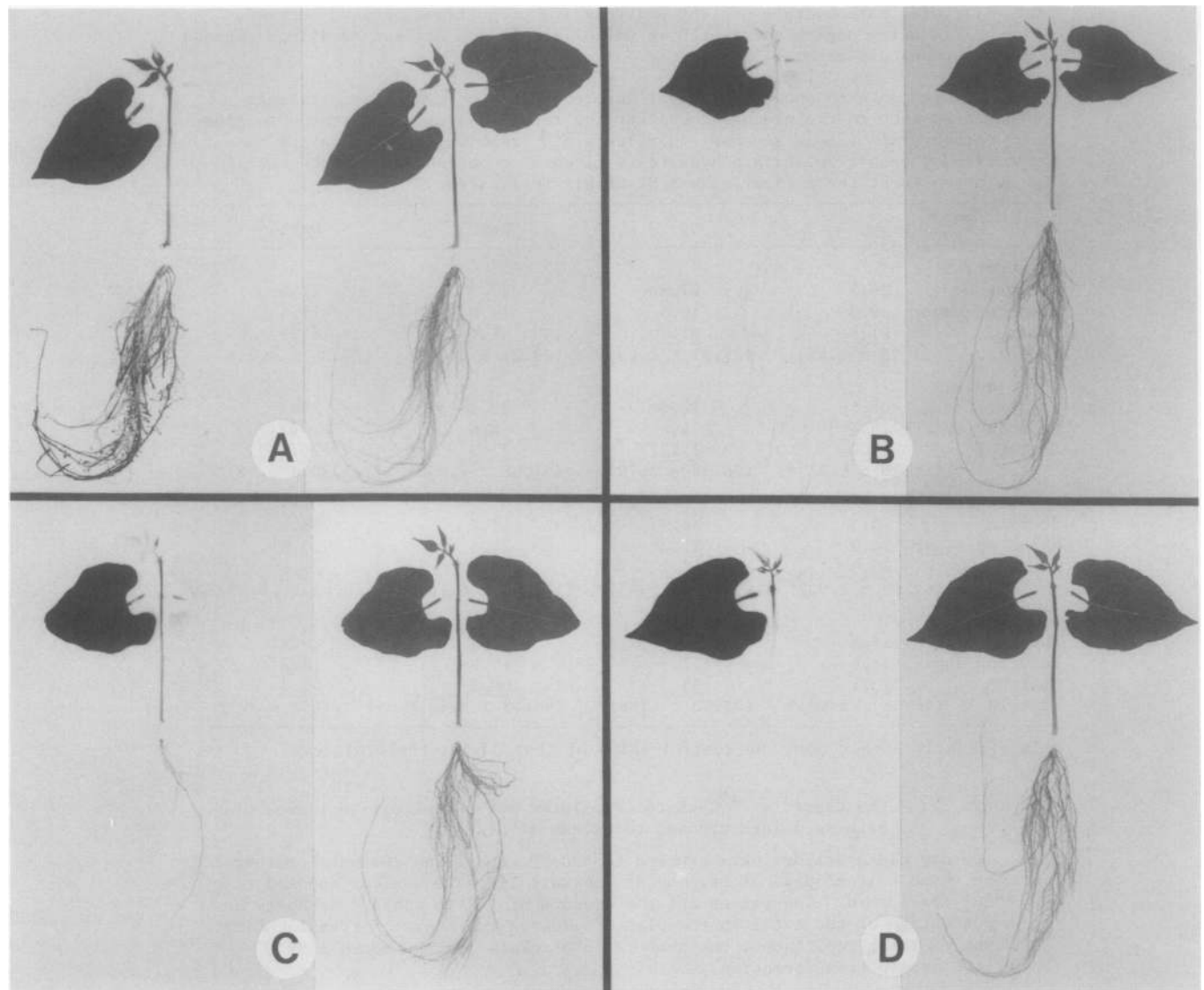
FIG. 1. Distribution of ^{14}C-photoassimilates after 2 h of translocation in 11-day-old white bean seedlings: control seedling (A) and seedlings exposed to 400 μM Co, (B), 200 μM Ni (C), or 200 μM Zn (D) for 1 day. Autoradiograms corresponding to the plants are on the left.

similate was particularly absent from several of the major roots and generally from the root tips. The autoradiograms of plants exposed to each metal for 2 to 4 days showed essentially the situation as depicted in Figure 1, B through D. Indeed, some seedlings showed even less translocation than found for an exposure of 1 day. In making the autoradiograms the exposure to x-ray film was constant for all, but deliberately excessive for control plants in order to foster some image development in the roots, stems, and young leaves of the metal-treated seedlings.

DISCUSSION

The concentrations of excess metal used in our study were phytotoxic as judged by the specific visible symptoms but the effect on yield was relatively little. Over the 4-day experimental period, shoot dry matter production was depressed slightly; however, root yield was reduced to about 66% of control (23). The experiments using $^{14}CO_2$ feeding clearly showed that white bean seedlings exposed to these phytotoxic burdens of Co, Ni, or Zn had impaired phloem translocation. The source-to-sink concept of movement of materials in the phloem of higher plants is supported by many experiments. In the present work, the bean seedlings

were 10 days old when they were exposed to excess metal. At this stage the unifoliate leaves were at least 89% fully expanded and would have acquired all of the characteristics of assimilate exporters (15). Control plants exported about 50% of their photoassimilates within 2 h (Table III), with the growing roots and shoots acting as major sinks (Fig. 1A).

The observed reduction in export of photoassimilate in beans exposed to excess metal might be due to a lack of translocatable carbohydrate. This view seems to be contradicted by the observed abnormal accumulations of sucrose in unifoliate leaves (Table I). Sucrose is the major translocated photoassimilate in P. vulgaris (8, 15). Despite the accumulation of sucrose in leaves of metal-treated plants, the sucrose may be diverted into a storage pool, thus making it unavailable for translocation. Geiger (9) has postulated the existence of sucrose storage pools and of sucrose translocation pools in leaves. A detrimental metal effect on photosynthetic CO_2 fixation is another possibility. The plants used in the translocation study contained 30 to 40% less ^{14}C than did the controls. This amount of fixed carbon does not reflect the real photosynthetic carbon fixation, since losses due to respiration and photorespiration were not part of the measurements. It is possible that carbohydrate accumulation in the leaves causes a feedback inhibition

of further CO_2 fixation. However, carbon fixation can not have been impaired drastically in these metal-treated seedlings since only continued synthesis could lead to the total carbohydrate accumulations observed (Table II). It is necessary to perform a complete carbon balance sheet in order to distinguish between the possibilities posed.

The reduced rate of translocation of photoassimilates in beans exposed to excess metal could be due to the inhibition of one or more of the three major steps in the process, namely: (a) loading at the source region; (b) translocation along the pathway; and (c) unloading at the sink region. Separate studies are required to evaluate the contribution of each of these processes and to determine whether the metal is acting directly or indirectly.The incidence of callose on sieve plates and the effect of ^{14}C-photoassimilate translocation through petioles are reported in the following paper (21).

B and K deficiencies also cause accumulations of carbohydrates in leaves (6, 20). Both nutrient elements have also been implicated in the translocation of sugars, since their deficiencies for 1 week or longer cause reduced transport of photoassimilates (2, 3, 11, 12, 14, 17). The effects reported here for beans were evident within 1 day after exposure to excess metals. It is unlikely that metals induce general K or B deficiencies within 1 day under our nutrient culture conditions. However, metal interference with these elements at critical sites in the translocation process cannot be ruled out at present.

The accumulation of sucrose, reducing sugars, and starch in leaves of the dicotyledon *P. vulgaris* exposed to excess metal (Table I) are responses shared to a degree with the monocotyledon *Hordeum vulgare* (1). In beans the accumulations occur earlier and at lower concentrations of metal in the nutrient solution than in barley. Even though each metal resulted in the common effect of carbohydrate accumulation, metal-specific alterations in carbon metabolism occurred (Table II). In seedlings exposed to excess Ni, carbohydrate metabolism was altered toward the accumulation of sucrose and reducing sugars, exposure to Co caused the preferential accumulation of starch, whereas little difference in the complement of the three carbohydrates occurred between controls and plants exposed to excess Zn. If one assumes that carbohydrate metabolism in leaves is regulated, it is clear that exposure of seedlings to excess Co or Ni causes shifts from the normal regulation of carbon metabolism. Whether these changes are due to metal ions acting directly in leaf cells or due to metal-induced changes in the leaves requires detailed study. Exposure of white bean seedlings to excess Cd caused similar visual toxicity symptoms as those for excess Co, Ni, and Zn (23, 24). We found no definite and reproducible accumulation of reducing sugars, sucrose, or starch in bean seedlings exposed to 6 μM Cd.

The reduction of photoassimilate transport in beans exposed to phytotoxic burdens of metals, shown here directly for the first time, is of practical and fundamental importance. The yield reductions usually observed in plants exposed to excess metals, particularly those of roots (23), may in part be accounted for by reduced phloem translocation, which over time would cause decreased mass transfer of photosynthate to the sink regions. Investigation of the mode of inhibition may help clarify our understanding of phloem translocation as well as indicate some possible avenues for guarding against occurrence of the effect. We suggest that the reduction in phloem translocation documented here is an example of an "essential-to-life" process (7) which has failed due to metal phytotoxicity.

LITERATURE CITED

1. AGARWALA SC, SS BISHT, CP SHARMA 1977 Relative effectiveness of certain heavy metals in producing toxicity and symptoms of iron deficiency in barley. Can J Bot 55: 1299–1307
2. AMIR S, L REINHOLD 1971 Interaction between K-deficiency and light in ^{14}C-sucrose translocation in bean plants. Physiol Plant 24: 226–231
3. ASHLEY DA, RD GOODSON 1972 Effect of time and plant K status on ^{14}C-labeled photosynthate movement in cotton. Crop Sci 12: 138–143
4. ATKINS CA, DT CANVIN 1971 Photosynthesis and CO_2 evolution by leaf discs: gas exchange, extraction, and ion-exchange fractionation of ^{14}C-labeled photosynthetic products. Can J Bot 49: 1225–1234
5. BAZZAZ FA, RW CARLSON, GL ROLFE 1975 Inhibition of corn and sunflower photosynthesis by lead. Physiol Plant 34: 326–329
6. DUGGER WM JR, TE HUMPHREYS, B CALHOWN 1956 The influence of boron on starch phosphorylase and its significance in translocation of sugars in plants. Plant Physiol 31: S-17
7. FOY CD, RL CHANEY, MC WHITE 1978 The physiology of metal toxicity in plants. Annu Rev Plant Physiol 29: 511–566
8. FREGEAU J 1976 The effect of growth regulators on ^{14}C assimilate transport in *Phaseolus vulgaris*. MSc thesis. Univ Guelph, Guelph
9. GEIGER DR 1975 Phloem loading. *In* MH Zimmerman, JA Milburn, eds, Encyclopedia of Plant Physiology, New Series Vol 1 Chap 17. Springer-Verlag, Berlin, pp 395–431
10. GREENFIELD SS 1942 Inhibitory effects of inorganic compounds on photosynthesis in *Chlorella*. Am J Bot 29: 121–131
11. HAEDER HE, K MENGEL, H FORSTER 1973 The effect of potassium on translocation of photosynthates and yield pattern of potato plants. J Sci Food Agric 24: 1479–1487
12. HARTT CE 1969 Effect of potassium deficiency upon translocation of ^{14}C in attached blades and entire plants of sugarcane. Plant Physiol 44: 1461–1469
13. HOAGLAND DR, DI ARNON 1950 The water culture method for growing plants without soil. Calif Agric Res Stn Circ 347
14. ILL'YASHUK EM, AS OKANENKO 1970 Effect of potassium on translocation of photosynthetically assimilated $^{14}CO_2$ in sugar beets. Sov Plant Physiol 17: 361–366
15. KOCHER H, OA LEONARD 1971 Translocation and metabolic conversion of ^{14}C-labeled assimilates in detached and attached leaves of *Phaseolus vulgaris* L. in different phases of leaf expansion. Plant Physiol 47: 212–216
16. LEE KC, BA CUNNINGHAM, KH CHUNG, GM PAULSEN, GH LIANG 1976 Lead effects on several enzymes and nitrogenous compounds in soybean leaf. J Environ Qual 5: 357–359
17. MENGEL K, M VIRO 1974 Effect of potassium supply on the transport of photosynthates to the fruit of tomatoes (*Lycopersicon esculentum*). Physiol Plant 30: 295–300
18. NELSON N 1944 A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 153: 375–380
19. OVERNELL J 1975 The effect of heavy metals on photosynthesis and loss of cell potassium in two species of marine algae, *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*. Marine Biol 29: 99–103
20. PANDY PM 1969 Effects of potassium deficiency on the carbohydrate content in the cotton plant at various stages of its development. Sov Plant Physiol 16: 9–16
21. PETERSON CA, WE RAUSER 1979 Callose deposition and photoassimilate export in *Phaseolus vulgaris* exposed to excess cobalt, nickel, and zinc. Plant Physiol 63: 1170–1174
22. PUCKETT KJ 1976 The effect of heavy metals on some aspects of lichen physiology. Can J Bot 54: 2695–2703
23. RAUSER WE 1978 Early effects of phytotoxic burdens of cadmium, cobalt, nickel, and zinc in white beans. Can J Bot 56: 1744–1749
24. SAMARAKOON AB 1978 The effects of excess zinc, nickel and cobalt on the export of photoassimilates in *Phaseolus vulgaris*. MSc thesis. Univ Guelph, Guelph
25. SNEDECOR GW 1956 Statistical Methods, Ed 5 Chap 11. Iowa State College Press, Ames, pp 316–320