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ABSTRACT

The effects of heavy metal stress on leaf reflectance of sugar maple seedlings (*Acer saccharum* Marsh) were examined. It was found that sugar maple seedlings treated with anomalous amounts of heavy metals in the rooting medium exhibited an increased leaf reflectance over the entire range of investigated wavelengths, from 475 to 1650nm. These results conform to those of a previous investigation in the visible wavelengths from 475 to 660nm, but tend to contradict the previous study in the near infrared wavelengths from 1000 to 1650nm. The differences may possibly be due to different water regimes in the two investigations.

INTRODUCTION

The investigation described in this paper was conducted to provide data on plant response to heavy metal stress. Specifically, this paper examines the effects of heavy metal stress on the leaf reflectance of sugar maple seedlings (*Acer saccharum* Marsh). An understanding of the changes in leaf reflectance in response to heavy metal toxicity may be useful for geobotanical exploration with remote sensing.

Previous field investigations studied the reflectance of plant leaves and plant canopies growing in areas of heavy metal mineralization, and compared these observations with data collected on similar vegetation growing in areas with background levels of soil minerals (Canney et al., 1970; Howard et al., 1971; Yost and Wenderoth, 1971; Lyon, 1975; Collins et al., 1979). These investigations have often found differences in vegetation reflectance in certain spectral regions in comparisons of the background and mineralized sites. The investigations, however, were generally limited to spectral observations in the visible wavelengths or to a few fixed wavebands. A further limitation of field investigations is that the observed vegetation may be under the influence of labile environmental conditions. The plant response observed in the field may be the result not only of heavy metal stress, but the recorded plant response may be influenced by transient environmental factors completely unrelated to the superabundance of soil minerals. Laboratory investigations have consequently been undertaken to elucidate the fundamental botanical effects of toxic but sub-lethal doses of heavy metals.

A recent laboratory investigation by Horler et al., (1980) was designed to assess plant response to toxic metals while other environmental factors were held as constant as possible. They reported statistically significant ($p < 0.05$) changes in the spectral reflectance of four investigated plant species in response to heavy metal stress. Furthermore, they concluded that the leaf hemispherical reflectance of heavy metal stressed plants increased in the visible region from 475 to 660 nm, and that leaf reflectance generally decreased in the near infrared wavelengths from 850 to 2200 nm.

Horler et al. (1980) also reported results of a field investigation which compared the reflectance of oak leaves from trees in a mineralized area with the leaf reflectance of the same species from an area of background mineralization. Results showed significant ($p < 0.05$) differences in reflectance between trees from the mineralized and background sites when compared to the within tree variations at 1650 and 2200 nm. Furthermore, the investigation found a strong negative correlation between soil metal concentration and reflectance at 1650 and 2200 nm, suggesting that an increase in soil metal concentration is associated with a decrease in leaf reflectance at these wavelengths, in agreement with the results of their laboratory investigations.

A recent field investigation by Labovitz et al. (1981) compared the reflectance of oak leaves from mineralized and background areas in a waveband from 1550 to 1750 nm. They found a statistically significant increase ($p < 0.05$) in the reflectance of samples collected from the mineralized site, when compared with samples of the same species collected from a background site, in apparent conflict with the results obtained by Horler et al. (1980).

The present investigation was designed to study the effects of heavy metal stress on the spectral reflectance of greenhouse-grown sugar maple seedlings with particular attention directed to the effects of heavy metal stress on leaf reflectance in the near infrared. The results of the present investigation are employed to help resolve the apparent conflicts in the previously mentioned investigations.

EXPERIMENTAL

Three and four year old sugar maple seedlings of uniform size (approximately 20 cm tall) were collected in the spring of 1980 from two locations on the forest floor in the easternmost section of the Keweenaw Peninsula in Michigan's Upper Peninsula. Half of the seedlings were taken from an area with background concentrations of copper in the B soil horizon, and half of the seedlings were collected from an area which exhibited anomalously high concentrations of copper in the soil. The seedlings were transplanted to pots containing clean silica sand and randomly assigned to one of three treatments: a control treatment, a copper treated group (30, 60, and 90 ppm copper as copper sulfate), and a manganese treated group (500, 1000, and 5000 ppm manganese as manganese sulfate). The plants were placed on a greenhouse bench and watered daily until the fall of 1980, a total of approximately 150 days. Sand surfaces in pots were covered with perlite to help reduce evaporative losses. Nutrient solutions (half strength modified Hoagland's solution) were added weekly to prevent deficiencies of essential elements.

Due to time constraints, leaves were harvested from each of the three treatments on September 3, 5, 8, and 12 of 1980. Leaf reflectance measurements were collected with a Beckman DK-2A spectrophotometer using a barium sulfate standard; the leaves were mounted on a black plate to reduce backscatter. The spectrophotometer measures hemispherical reflectance from a small (approximately 3 cm^2) sample and records the measurement as per cent reflectance versus wavelength. In this paper any reference to leaf reflectance specifically refers to leaf hemispherical reflectance.

Spectral hemispherical reflectance values were read directly from the curves at 475, 550, 660, 700, 775, 1000, 1475, and 1650 nm. Figure 1 shows* the location of these wavelengths with respect to typical leaf reflectance curves. These wavelengths were chosen for the following reasons: 475 nm is approximately the center of a broad chlorophyll and carotenoid absorption band, 550 nm is approximately the center of the leaf reflectance peak in the green wavelengths, 660 nm is near the center of the chlorophyll absorption band in the red region, 700 nm is located on the steep rise in reflectance between the chlorophyll absorption band and the region of high leaf reflectance in the near infrared, and 775 nm is an arbitrarily chosen wavelength from the region of high leaf reflectance in the near infrared wavelengths. A second wavelength, 1000 nm, was arbitrarily chosen for study from the region of high leaf reflectance in the near infrared wavelengths. The 1475 nm wavelength was included because it is near the center of one of the prominent water absorption bands in the

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*All figures and tables are located at the end of this report.

near infrared. This wavelength was included for the sake of thoroughness, but it is unsuitable for remote sensing purposes because of atmospheric attenuation of radiation at this wavelength. The wavelength at 1650 nm was chosen because it is near the center of the Thematic Mapper band 5. This wavelength is an atmospheric radiation absorption minimum located between two prominent bands of radiation absorption due to water. Thus radiation at this wavelength passes relatively freely through the atmosphere. In addition, this wavelength may be useful in detecting water stress in vegetation (Tucker, 1980). The reflectance in the region from 450 to 800 nm was measured with a photomultiplyer detector, and reflectance data from about 900 to 2100 nm were collected with a lead sulfide detector on the Beckman spectrophotometer.

RESULTS

The data were divided into four general groups: a group from the control treatment, the copper treatment, the manganese treatment, and a group composed of dead leaves. The dead leaf group apparently arose in response to the compound stresses of transplanting the seedlings, growing them in a greenhouse environment, and treating some of them with heavy metals. These stresses resulted in a certain amount of leaf mortality in each of the three treatment groups. The dead leaves were readily delineated as a population of individual leaves which exhibited a spectral reflectance greater than 44% at 1450 nm. In general a dead leaf was dried out, appeared brown in color, and exhibited anomalously high reflectance at most other investigated wavelengths.

In the copper treated group a series of t-tests disclosed no significant differences between the leaf reflectance of plants collected from forest sites which contained anomalous concentrations of heavy metals in the B soil horizon, when compared with the leaf reflectance of plants collected from regions with background levels of soil metals ($p > 0.20$ at all investigated wavelengths). Similar results were obtained in t-tests of the control group, with one exception. It is likely that this one exception was due to chance because the probability of finding one statistically significant difference where none exists in the investigated populations becomes large when a large series of statistical tests is conducted. No corresponding tests were conducted on the manganese treated group because only 2 of the 13 samples from this group were taken from the collection site which exhibited anomalously high levels of soil minerals. However, given the results of t-tests in the control and copper treated groups it appears likely that, at a given wavelength in a given treatment group, no significant differences exist between the recorded leaf reflectance from either of the two collection sites. Furthermore, a closer examination of the two treatment groups found no significant differences ($p > 0.05$) in the leaf reflectance of plants treated with 30, 60, or 90 ppm copper or for plants treated with 500, 1000, or 5000 ppm manganese at any of the investigated wavelengths. Thus it was decided to pool the copper data into a single copper treated group, and to pool the manganese data into a single manganese treated group for all subsequent analyses.

The values of per cent spectral reflectance for all investigated wavelengths for all leaves are presented in Table 1. A univariate one-way analysis of variance was performed to compare the mean reflectance of the control group with each other group (copper treated, manganese treated, and dead leaf group) at each of the previously mentioned wavelengths. The results of these tests are presented in Table 2. Note that two leaves were omitted from statistical treatment in the dead leaf group. These two individual leaves were dry and brittle, but also quite green, and probably belong to a separate population.

Figure 1 illustrates the whole leaf hemispherical reflectance from 400 to 1650 nm of a typical sample from the control, copper treated, and dead leaf groups. A group's typical leaf reflectance curve is defined as that sample which comes as close as possible to the group's mean reflectance at all investigated wavelengths. The leaf reflectance curve from the manganese treated group was not included in this figure because, as Table 2 indicates, the mean reflectance of the manganese treated group is statistically different ($p < 0.05$) than the mean reflectance of the control group at only two of the eight investigated wavelengths.

DISCUSSION

As previously mentioned in the section on experimental methods, the data analyzed in this paper were collected on four separate dates with an interval of nine days between the first and final observations. This procedure has almost certainly increased the variability of observed leaf reflectance. This increased variability may have prevented the detection of some differences in mean leaf reflectance among the investigated groups. However, increased variability should not affect the interpretation of the data in those cases where significant differences are found between two groups.

The results presented in Figure 1 and Table 2 indicate that the average leaf reflectance of the copper treated plants is significantly greater ($p < 0.05$) than the average leaf reflectance of the control group over all tested wavelengths from 475 to 1650 nm. Table 2 indicates that at a given wavelength the differences between the mean spectral reflectance of the manganese treated group and the controls are generally not significant at the 5% level. At 660 and 700 nm, however, the mean leaf reflectance of the manganese treated group *is* significantly greater than the mean reflectance of the control group ($p < 0.05$). *In no instance* is the leaf reflectance of the copper or manganese groups significantly less than the reflectance of the control group at any of the wavelengths investigated.

This study suggests that in all investigated wavelengths the leaf hemispherical reflectance of sugar maple seedlings does not decrease as a result of heavy metal stress, when compared with a control treatment. The reconciliation of these findings with those of Horler et al. (1980) may possibly be explained in light of plant physiological response to heavy metal stress, and a consideration of the differences in experimental design between the two investigations.

Excess heavy metals in the rooting medium inhibit root growth by affecting cell division and elongation (Turner, 1973; Wainwright and Woolhouse, 1975). Actively growing roots normally produce cytokinins, and inhibition of root growth by heavy metal toxicity suggests that cytokinin transport to the leaves will be reduced. Skene (1975) suggested that a reduction in cytokinin transport from roots to leaves promotes leaf senescence. Leaves undergoing senescence will, by definition, lose chlorophylls, carotenoids, RNA, proteins, and lipids from the chloroplast membranes, and the leaves will become chlorotic. In fact, leaf chlorosis is often observed in laboratory treatment of plants with toxic quantities of heavy metals.

It has been proposed that abscisic acid (ABA) and xanthoxin are products which arise from the breakdown of carotenoids in plant leaves (Burden and Taylor, 1976). At present no studies have been reported which examine the changes in leaf ABA in response to heavy metal treatment. As mentioned above, however, carotenoid levels decrease in senescent leaves. Furthermore, the level of ABA in leaves is known to increase in response to water deficiency (Wright and Hiron, 1969; Livne and Vaadia 1972), and other conditions of physiological stress (Vaadia, 1976). Of the products linked to carotenoid breakdown, xanthoxin is a powerful growth inhibitor which has also been suggested as a precursor to the cellular production of ABA, and ABA is a powerful antitranspirant. Thus it is likely that the production of ABA is one plant response to heavy metal stress, and it has been found that plants treated with excess heavy metals have a lower transpiration rate (higher stomatal resistance) than the same species under control conditions (Bazzaz et al., 1974).

The observed leaf reflectance in the near infrared from about 1300 to 2500 nm is, to a large degree, controlled by the quantity of liquid water present in the leaf (Tucker, 1980). Simply stated, the more water in the leaf the lower the leaf reflectance, due principally to water absorption of radiation in these wavelengths. In the hydroponic studies by Horler et al. (1980) all plants were abundantly supplied with water. The increased stomatal resistance in the leaves of heavy metal treated plants suggests that more water will be present in these leaves than in the leaves of control plants. Consequently one would expect a decrease in the near infrared reflectance in the leaves of hydroponically grown metal treated plants. In the present investigation sugar maple seedlings were grown in clean silica sand. Although the plants were watered regularly, pure sand has an extremely low water holding capacity. Thus it is likely that the seedlings were subjected to some degree of water stress in the hot greenhouse environment. In a situation where water is limited, as in the present investigation, the stomates of all plants will close in response to water deficits, and the plant with the more well developed root system will be better able to absorb available water. As previously stated, the root systems of heavy metal treated plants are generally less well developed than the root systems of control plants. Thus, under conditions of water deficits one would expect the leaves of *control* plants to contain relatively more water, and consequently exhibit a lower near infrared reflectance than the leaves of metal treated plants.

To conclude, the results of the present and former investigations suggest that under the influence of heavy metal stress leaf reflectance generally increases in the visible wavelengths from 450 to 700 nm. In the present study a statistically significant increase in leaf reflectance was consistantly observed in the wavelengths dominated by chlorophyll absorption in the red wavelengths (660 and 700 nm). This region may be most sensitive for discriminating metal stressed from unstressed vegetation in geological exploration.

On the other hand, the changes in near infrared leaf reflectance in response to heavy metal stress may be considerably more variable than originally expected. Two counteractive mechanisms are proposed to explain the differences between this investigation and that reported by Horler et al. (1980) in observations of near infrared leaf reflectance: the increased stomatal resistance, and the reduced root development resulting from heavy metal treatment. Under the assumptions of the two proposed mechanisms the leaf reflectance observed in the wavelengths from about 1300 to 2500 nm will fluctuate in response to different moisture regimes. Furthermore, this model of plant response suggests that under different water regimes the near infrared leaf reflectance of metal stressed vegetation may sometimes be higher, and at other times lower than the reflectance of unstressed vegetation, depending on the water treatment. This variability could seriously reduce the utility of the near infrared spectral region from 1300 to 2500 nm as an indicator of heavy metal stress for geological exploration. Future research should be designed to validate or disprove this model, and establish the fundamental plant response to the interaction between water availability and heavy metal stress.

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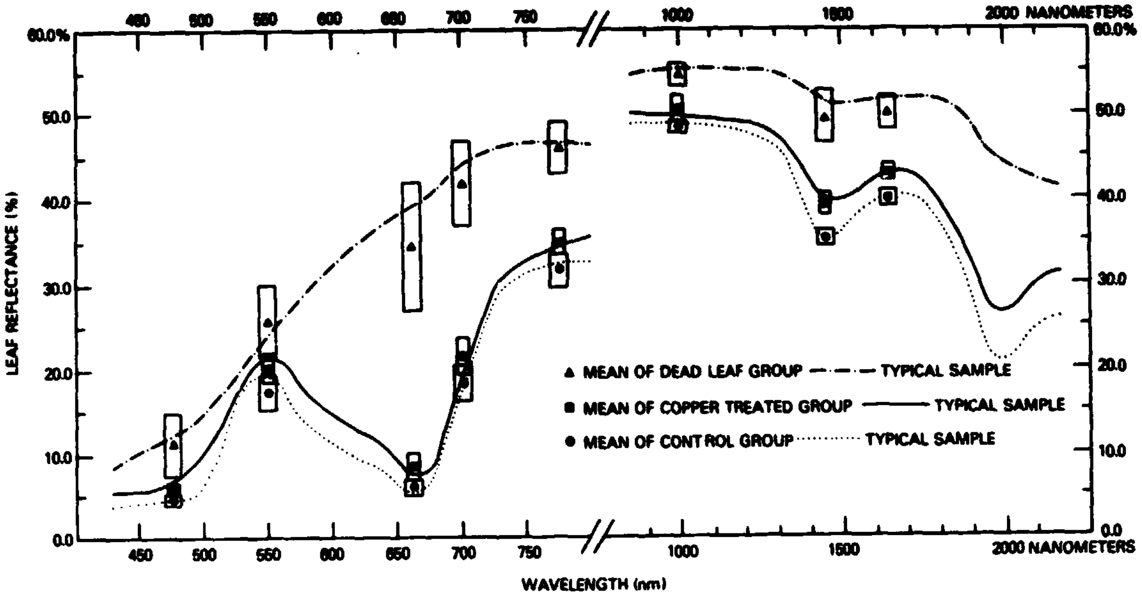

Figure 1. Hemispherical leaf reflectance for typical samples from the control, copper treated, and dead leaf groups. Mean spectral reflectance values for each group are identified at 475, 550, 660, 700, 775, 1000, 1450, and 1650nm, and the 95% confidence intervals are illustrated by boxes. Note the change in scale on the abcissa. The data from 425 to 800nm were collected with a photomultiplier detector, and a lead sulfide detector collected the data from 950 to 2100nm.

Table 1

Raw Reflectance, in Percent Relative to $BaSO_4$, for all Samples from Control, Copper Treated, Manganese Treated, and Dead Leaf Groups.

Sample Number*	Collection Date	Wavelength (nm)							
		475	550	660	700	775	1000	1450	1650
Control Group									
0-109	9/5	4.5	15.7	6.0	21.3	36.6	49.9	36.1	41.8
0-103	9/5	4.5	19.2	5.7	19.0	32.3	49.0	35.6	40.5
0-106	9/12	5.1	12.9	4.9	17.5	31.0	48.2	32.8	38.5
0-103L	9/12	4.0	16.0	4.9	17.0	32.8	47.8	34.8	39.3
0-37	9/12	4.0	19.4	5.4	19.0	35.4	48.4	35.0	40.0
0-130L	9/5	6.0	17.0	5.3	16.5	30.0	48.7	35.0	39.8
0-4	9/5	5.7	16.5	7.0	16.8	27.8	48.1	36.3	40.4
0-34L	9/5	5.7	22.5	8.8	21.4	32.5	49.0	36.9	40.6
0-66	9/5	5.1	15.2	6.5	16.5	28.3	47.0	35.4	39.4
Copper Treated									
Cu-30-126	9/8	6.8	23.7	11.6	27.5	38.8	49.6	37.0	42.5
Cu-30-52	9/8	4.7	21.3	6.8	21.4	33.0	49.6	37.0	42.5
Cu-30-6	9/3	5.5	21.8	7.0	–	–	49.7	37.3	42.0
Cu-30-39	9/3	6.8	17.2	7.3	–	–	50.9	38.5	42.0
Cu-60-113	9/12	5.2	18.2	6.3	19.0	35.0	48.9	37.7	42.1
Cu-60-140L	9/5	4.3	15.0	9.4	20.0	31.0	59.7	40.8	43.8
Cu-60-47	9/5	5.0	16.5	6.1	19.6	33.0	52.0	43.9	46.0
Cu-60-35	9/5	7.3	21.5	14.0	25.8	36.0	49.1	41.3	43.9
Cu-60-41L	9/5	5.0	19.2	6.5	21.5	33.8	50.3	39.4	43.2
Cu-90-21L	9/3	7.5	23.9	11.0	24.0	38.2	49.0	39.4	42.2
Cu-90-20	9/3	5.7	21.0	7.2	19.4	35.5	50.0	40.0	43.0
Cu-90-60L	9/3	6.4	22.6	10.0	21.4	35.8	49.3	37.1	41.0
Cu-90-63L	9/3	5.7	18.9	6.6	16.6	33.8	49.5	39.4	42.2
Manganese Treated									
Mn-500-134L	9/8	4.2	16.5	6.0	20.4	31.9	47.1	34.5	39.5
Mn-500-39L	9/8	4.8	18.5	7.0	24.5	36.0	50.0	40.2	43.8
Mn-500-101L	9/8	4.5	18.5	5.9	22.7	35.2	47.8	35.0	40.4
Mn-500-58	9/8	5.2	18.5	7.6	24.5	35.7	49.3	34.5	40.3
Mn-1000-57L	9/12	4.8	19.3	7.2	23.0	36.8	47.0	34.9	40.0
Mn-1000-14L	9/3	6.0	14.8	7.0	19.5	34.5	49.4	40.9	43.4
Mn-1000-100L	9/3	5.0	15.0	6.0	17.7	32.0	46.7	34.2	38.2
Mn-1000-83	9/3	5.4	13.7	7.0	19.0	31.0	48.2	37.0	40.8
Mn-1000-119L	9/3	6.0	17.6	8.3	21.8	36.0	50.0	41.5	44.0
Mn-5000-32L	9/5	4.8	15.0	7.3	22.6	36.2	49.0	38.8	42.6
Mn-5000-19L	9/5	4.8	15.1	7.3	19.5	32.2	47.0	34.4	39.2
Mn-5000-78L	9/5	4.8	18.1	7.3	22.0	34.1	48.4	36.6	40.4
Mn-5000-95L	9/5	5.3	15.8	7.5	18.7	28.6	46.5	37.3	41.0
Dead Leaf Group									
0-57	9/12	17.0	32.1	32.2	43.1	48.6	55.6	52.0	52.0
Cu-30-154**	9/8	7.9	23.8	11.7	31.5	42.3	55.8	53.0	52.0
Cu-30-147	9/8	7.3	22.7	35.0	40.0	42.3	52.9	45.0	47.0
Cu-60-161L	9/12	9.0	19.0	39.7	43.8	45.7	53.8	50.8	50.2
Cu-60-85L	9/12	15.8	30.4	31.5	42.5	47.8	53.3	50.0	50.0
Cu-60-182L**	9/12	8.4	22.4	15.8	32.7	44.8	56.3	52.7	52.3
Mn-1000-144L	9/12	6.9	21.7	19.0	31.2	41.3	53.5	44.2	47.2
Mn-1000-119	9/12	12.0	24.0	39.6	44.1	46.5	55.5	51.6	51.8
Mn-1000-80	9/12	10.5	27.9	43.9	48.6	51.0	56.2	51.5	51.6

*Code: Cu = copper treated, Mn = manganese treated; 0, 30, 60, 90, 500, 1000, 5000 represents the level of treatment in parts per million. The letter, L, identifies samples taken from plants collected on forest sites which exhibited background levels of soil copper.

**Dead but green leaf, omitted from statistical treatment. See results section.

Table 2

Results of Analysis of Variance Tests to Compare the Control Group with Each of the Three Treatment Groups.

Wavelength	Manganese Treated		Copper Treated		Dead Leaf Group	
	$F_{1,21}$ (Experimental)	Attained Significance	$F_{1,21}$ (Experimental)	Attained Significance	$F_{1,14}$ (Experimental)	Attained Significance
475 nm	0.1114	0.7420	4.8281	0.0399	21.822	0.0004
550	0.2638	0.6132	5.6996	0.0270	18.398	0.0004
660	5.5489	0.0288	6.9048	0.0161	108.51	<0.0001
700	9.8011	0.0053	6.6566*	0.0189	150.17	<0.0001
775	2.9584	0.1009	6.7042*	0.0185	80.263	<0.0001
1000	0.3164	0.5800	4.6271	0.0001	121.73	<0.0001
1450	2.7978	0.1100	25.317	<0.0001	142.40	<0.0001
1650	2.2801	0.1467	31.679	<0.0001	161.01	<0.0001

*$F_{1,19}$

null hypothesis: $\mu(\text{control}, \lambda) = \mu(\text{treatment group}, \lambda)$
alternative hypothesis: $\mu(\text{control}, \lambda) \neq \mu(\text{treatment group}, \lambda)$

$F_{1,14}$ (critical, $\alpha = 0.05$) = 4.54
$F_{1,19}$ (critical, $\alpha = 0.05$) = 4.38
$F_{1,21}$ (critical, $\alpha = 0.05$) = 4.32