Effects of mercury on visible/near-infrared reflectance spectra of mustard spinach plants (*Brassica rapa* P.)

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Some spectral characteristics of leaves of *Brassica rapa* P. may be associated with foliar mercury content.

Abstract

Mustard spinach plants were grown in mercury-spiked and contaminated soils collected in the field under controlled laboratory conditions over a full growth cycle to test if vegetation grown in these soils has discernible characteristics in visible/near-infrared (VNIR) spectra. Foliar Hg concentrations (0.174 - 3.993 ppm) of the Mustard spinach plants were positively correlated with Hg concentration of soils and varied throughout the growing season. Equations relating foliar Hg concentration to spectral reflectance, its first derivative, and selected vegetation indices were generated using stepwise multiple linear regression. Significant correlations are found for limited wavelengths for specific treatments and dates. Ratio Vegetation Index (RVI) and Red Edge Position (REP) values of plants in Hg-spiked and field-contaminated soils are significantly lower relative to control plants during the early and middle portions of the growth cycle which may be related to lower chlorophyll abundance or functioning in Hg-contaminated plants.

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1. Introduction

Global atmospheric mercury burdens may have increased up to five-fold since the Industrial Revolution (EPRI, 1994) as a result of fossil fuel combustion, ore smelting, and waste-incineration (EPA, 1997; DEFRA, 2002). Natural and anthropogenic point-sources of Hg including sulfide ores, the chlor-alkali industry, dental amalgams, battery production and hat making (Alloway, 1995; Irwin, 2002; Varekamp et al., 2003) have also contributed significantly to the global Hg burden. An important component of any remediation strategy is the assessment of the spatial distribution of Hg contamination in a local environment, particularly as there may still remain many areas contaminated with Hg that are poorly documented.

The spatial extent of mercury contamination in soils is typically established through field sampling and laboratory analysis. Remote sensing is a fast, non-destructive measurement that offers a synoptic view of a region. Soil Hg may be detected directly using reflectance spectroscopy (Kemper and Sommer, 2002), but many contaminated regions are covered by vegetation. Despite a substantial amount of literature exploring the effects of Hg on vegetation (Siegel et al., 1984; Panda et al., 1992; Cho and Park, 2000; Kabata-Pendias and Pendias, 2001) and the effects of other metals on vegetation spectra (Horler et al., 1983; Davids and Tyler, 2003; Schuerger et al., 2003), there are no studies of the effects of Hg on the visible/near-infrared (VNIR) spectra of vegetation.

We measured spectra of Mustard spinach (*Brassica rapa perviridis*) plants grown in Hg-contaminated soil in the laboratory over an entire growth cycle to establish baseline spectral characteristics from which changes due to Hg stress can be distinguished. Mustard spinach plants were selected for their short growth cycle (allowing a complete cycle to be observed) and because *Brassicaceae* family plants have been shown to accumulate metals including Zn, Pb, Se, S, Cr (Banuelos et al., 1997; Broadley et al., 2001) and Hg (Leonard et al., 1998;
Caille et al., 2005). We then used multiple stepwise linear regression to identify any relationships between foliar Hg content and VNIR spectral response.

2. Background

2.1. Effects of Hg on vegetation

Mercury may enter plant foliage through two primary pathways: 1) uptake of the oxidized from (Hg(II) or methyl mercury), adsorbed onto soil particles and/or dissolved in soil water through roots (Lindberg et al., 1979; Rea et al., 2002); and 2) absorption of Hg vapor through stomata, some of which may be derived from volatilization from the surface of Hg-rich soils (Ericksen et al., 2003; Caille et al., 2005). Plant root Hg concentrations are dominated by pathway #1, while foliar Hg concentration may be dominated by pathway #2 (Lindberg et al., 1979; Frescholtz et al., 2003). The contribution of each pathway to a particular plant is a function of the plant species, plant health and bioavailability of Hg in any particular environment (Adriano, 1986; Leonard et al., 1998). Significant correlations exist between foliar and soil Hg concentrations in a range of experiments and are observed to be both species and temporally dependent (Cocking et al., 1995; Gupta and Chandra, 1998).

The effects of Hg on plants have been well documented (Putra and Sharma, 2000; Kabata-Pendias and Pendias, 2001). Mercury harms plants through impairment of the synthesis and metabolism of chlorophyll (Clusters and Van Assche, 1985; Küpper et al., 1998; Cho and Park, 2000), chromosomal damage (Panda et al., 1992) and inhibition of root and shoot growth (Godbold and Hütterman, 1986). These effects may result in visible symptoms of stress including leaf chlorosis, necrotic leaves and leaf tips, and stunted growth (Siegel et al., 1984; Kabata-Pendias and Pendias, 2001). We therefore expect that Hg stress may result in decreased foliar chlorophyll content and/or damage to internal leaf structure.

Background levels of Hg in soils range from 0.05–1.10 ppm (Davis et al., 1997; Kabata-Pendias and Pendias, 2001) and in vegetation (grasses, mosses, leaves and fruit) ranges from ~0.003 to 0.100 ppm (Rasmussen et al., 1991; Kabata-Pendias and Pendias, 2001). Documented critical levels of Hg toxicity in plant tissues range from 0.5 ppm (Kloke et al., 1984) to 3 ppm (Davis et al., 1978; Siegel et al., 1987), but depend on plant species and age. Tobacco plants grown in soils with 50 ppm Hg exhibited symptoms of Hg stress (Heaton et al., 1998). Che et al. (2003) reported toxicity in Cottonwood trees at between 8 and 40 ppm soil Hg and plant death at 400 ppm soil Hg. Barley plants showed visible symptoms of stress grown in 5 ppm soil Hg and genotoxicity above 22 ppm soil Hg (Panda et al., 1992).

2.2. Effects of stressors on plant spectra

The spectrum of a healthy plant is characterized by strong absorptions at ~450 nm and ~680 nm due to chlorophylls and strong reflectance in the NIR (700–1200 nm) due to internal scattering of light at the cell wall-air interfaces (e.g., Wooley, 1971; Fig. 1). Spectral indices that utilize wavelengths around 700 nm (the red edge region) have proven sensitive to both variations in leaf chlorophyll and leaf cell structure as this region includes both the ~680 nm chlorophyll absorption and the rise of NIR reflectance (e.g., Horler, 1983; Curran et al., 1992; Buschmann and Nagel, 1993; Gitelson et al., 1996). Commonly reported effects of both stress and senescence on vegetation spectra are increased overall reflectance in the visible, due to loss of chlorophyll (e.g., by destruction or lack of synthesis), and decreased reflectance in the NIR, due to damage to leaf cell walls and mesophyll tissue (Horler et al., 1980; Boyer et al., 1988; Carter, 1994; Carter and Knapp, 2001; Schwallier et al., 1983; Buschmann and Nagel, 1993; Smith et al., 2004).

To evaluate vegetation stress in reflectance spectra, we examine the entire 400–2300 nm spectral interval and focus on the following spectral wavelengths specific to chlorophyll and several carotenoids, which are also involved in photosynthesis (e.g., Mimuro and Katoh, 1991): 430 nm (Chl a), 448 nm (Chl b, carotenoids), 471 nm (carotenoids), 642 nm (carotenoids), 642 nm (Chl b), 662 & 680 nm (Chl a), and the green peak (550 nm); we also consider NIR reflectance at 800 nm (Fig. 1a). In addition we employ two simple reflectance (R) indices, a Normalized Difference Vegetation Index (NDVI) and Ratio Vegetation Index (RVI) (Gitelson and Merzlyak, 1994, 1996; Gitelson et al., 1996):

\[
\text{NDVI} = \frac{(R_{800} - R_{679})}{(R_{800} + R_{679})}
\]

\[
\text{RVI} = \frac{R_{750}/R_{700}}{}
\]

NDVI is a widely used index that has been shown to be strongly correlated with biomass of vegetation and leaf area index (e.g., Tucker, 1979; Turner et al., 1999; Hansen and Schjoerring, 2003). Narrow band NDVI values derived from hyperspectral data have been correlated with nutrient deficiencies that alter foliar intercellular airspace (Estep and Carter, 2005). RVI has been shown to correlate directly to chlorophyll a concentration (Gitelson and Merzlyak, 1996; Gitelson et al., 1996). Thus, we would expect a decrease in both NDVI and RVI with increasing plant stress.

The red edge position (REP) is the wavelength of the maximum value of the first derivative of the spectra in the RE region and is positively related to chlorophyll concentration and biomass of leaves and canopies (Horler et al., 1983; Demetriades-Shah et al., 1990; Filela and Peñuelas, 1994; Curran et al., 1995; Gitelson et al., 1996; Sims and Gamon, 2002; Imamishi et al., 2004). This parameter can be estimated by mathematically fitting the derivative curve (e.g., Miller et al., 1990; Dawson and Curran, 1998); however, our data are of sufficient spectral resolution to determine the REP by direct inspection of the maximum value of the first derivative curve in the ~700 nm region (Fig. 1c). Reduction of chlorophyll and leaf structure damage that may accompany metal stress leads to the narrowing and reduction of the major chlorophyll absorption at ~680 nm and decreased NIR reflectance, flattening of the slope between the far red and the shoulder of the NIR and causing the REP to shift to shorter wavelengths (Horler et al.,...
1980, 1983; Miller et al., 1985; Buschmann and Nagel, 1993; Filella and Peñuelas, 1994; Mariotti et al., 1996; Davids and Tyler, 2003; Kooistra et al., 2004).

3. Methods

3.1. Growth experiment design and sampling

We conducted growth experiments with Mustard spinach plants (*Brassica rapa perviridis*) at Wesleyan University from July—September of 2004. Control soils with background (<500 ppb) Hg levels were collected from the Wesleyan University campus. We used soils from two areas with severe Hg contamination from the historic hat-making industry in Connecticut (see Varekamp et al., 2005): the Dianna Knit factory in Norwalk, CT and a lot on Barnum Court in Danbury, CT, the latter of which is a designated Brownfield site. These field soils (sandy loam) were air dried for one week and then sieved in stainless steel screens to <2 mm.

A second set of soils was prepared by adding mercuric chloride (Mallinckrodt Chemical Works, NY, NY) to control soils. Spike solutions (100, 50, 25 and 1 ppm) were created by sequential dilutions of HgCl₂ dissolved in deionized water and then added to batches of control soil. The spiked soils were thoroughly mixed with a plastic spade and left in a hood to equilibrate for 1 week.

To improve aeration and nutrient content all Hg-spiked and control soils were mixed (50:50) with Promix potting soil (Premier Horticulture Inc., Red Hill, PA). Each soil treatment was distributed by weight (800 g per pot) into a 12.5 cm diameter plastic pot, with 3 pots assigned to each treatment level. Plastic petri dishes were placed underneath each pot. In sum, a total of seven treatment levels were used for the experiment: one control soil, four Hg-spiked soils and two Hg-contaminated field soils.

Pots were arranged in a hood in a randomized block design to control for position with respect to the hood opening. Six 48-inch, 40 watt, full-spectrum bulbs were placed above the pots. An automatic timer was used to maintain a 16 h light/8 h dark cycle. Temperature and humidity in the hood were monitored throughout the experiment. The temperature ranged from 26.2 °C to 21.8 °C at night. Plants were watered daily to field capacity.

Four Mustard spinach seeds were planted in each pot. Seedlings were thinned to one plant per pot one week after germination. The pots were rotated 180° daily to prevent seedlings from leaning over in response to air circulation, and pot positions within each block were rotated every three days during the experiment to minimize further effects of position in the hood on plant growth. Plants were grown for a total of 53 days, from 7/17/04 to 9/07/04, and were harvested after they began to flower.

After seedlings were two weeks old, leaf samples were collected weekly. One mature leaf was removed from each plant with clean stainless steel scissors. Leaves were washed with tap water, rinsed with deionized water and then air-dried for one week. Dry leaves were macerated and stored frozen until analysis. Soil samples were collected weekly to determine if soil concentration fluctuated over the course of the experiments. Soil samples were dried, homogenized, sieved to <2 mm and stored frozen until analysis.

3.2. Mercury analysis

Soil and plant samples were analyzed for total Hg content at Wesleyan University and the University of Connecticut with a Milestone Direct Mercury Analyzer 80 with an absolute detection limit of about 0.5 ng Hg. Soil samples were analyzed in nickel sample boats whereas leaf samples were analyzed in quartz sample boats. Samples were handled and analyzed according to EPA

Fig. 1. Average of all spectra collected for selected treatments on day 21 of the experiment. (a) Reflectance spectra. Individual wavelengths considered in one regression model are indicated; absorptions due to chlorophyll and carotenoids are specified. (b) 1st derivative of (a). (c) Portion of (b) focused on the red edge. Arrows indicate red edge position (REP).
method 7437 (USEPA, 1998) although leaf samples were not analyzed within the 28-day limit as directed by method 7437. Standards used include NIST SRM 1515 apple leaves (44 ppb ± 4 ppb), SRM 2709 San Joaquin soil (1.4 ppm ± 0.08 ppm), and the Canadian Research Council standards Mess 3 (0.091 ± 0.008 ppm) and Pacs 2 (3.4 ± 0.5 ppm). Precision for all standards was better than 5%. Duplicates of all soil and leaf samples (when possible) were analyzed to minimize variance.

3.3. Spectral data collection and analysis

Reflectance spectra were obtained at Wesleyan University using an ASD FieldSpec FR spectroradiometer (Analytical Spectral Devices, Boulder, CO) with a wavelength range of 350–2500 nm, a sampling interval of 1.4 nm between 350–1000 nm and 2 nm between 1000–2500 nm, and a spectral resolution of 3 nm between 350–1000 nm and 10 nm between 1000–2500 nm. The spectrometer is equipped with a 1 m long fiber optic sensor with a 25° field of view. Individual plants were placed on a target beneath two tungsten quartz halogen lights 180° apart with an incidence angle of 26°; each light was 50 cm from the target. Spectra were collected by positioning the fiber optic sensor within ~5 cm of each leaf by hand. Individual spectral measurements were an average of 5 scans and each leaf was sampled 10 times and subsequently averaged for further analysis; two to seven leaves were sampled per plant. Reflectance spectra were normalized with a white Spectralon® (sintered Halon) panel.

To reduce noise, the first derivative calculations were performed on spectra that were smoothed with a 5 point moving average. First derivative values of the smoothed spectra were calculated as the change in reflectance divided by the change in wavelength between those values (Smith et al., 2004). Correlation coefficients (r) were calculated between R or its first derivative (ΔR) and foliar Hg concentration for each date and each treatment.

3.4. Statistical analysis

Statistical analyses were performed in SAS 9.1 (SAS Inc., Cary, NC, USA). Regression analysis was conducted to evaluate the relationship between soil and leaf Hg concentrations (Sokal and Rohlf, 1995). Analysis of variance (ANOVA) was used to determine if there were statistically significant differences in leaf Hg among different treatment groups (Sokal and Rohlf, 1995). Normality and homogeneity of variance were evaluated prior to ANOVA. Post-hoc power calculations were performed manually (Sokal and Rohlf, 1995) and with the use of G*power (Erdfelder et al., 1996).

The relationship between foliar Hg and spectral properties was modeled using stepwise multiple linear regression is SPSS 11 (SPSS, Inc. Chicago). The models are of the form:

Foliar Hg concentration = \( b_0 + b_1(S_{l1}) + b_2(S_{l2}) + \cdots + b_n(S_{ln}) \),

where \( S_l \) is the reflectance (R) or its first derivative (ΔR) at wavelength, \( \lambda \), and \( b_n \) are best-fit coefficients that maximize the equation’s coefficient of determination \( r^2 \). Independent variables are included in the model when the significance of the model (as measured by the significance of the F-test) is <0.10, and are excluded if the significance is >0.15. The high number of independent variables results in high intercorrelation between them, introducing redundancy into the equation. The magnitude of multicollinearity was assessed using a variance inflation factor (VIF) = \( 1 - r^2 \) for the regression of a particular independent variable against all other independent variables. We limited the model to variables with a VIF <10 (Yoder and Pettigrew-Crosby, 1995).

Three separate stepwise regression models were created for reflectance data of each treatment and the spiked experiments on each sample date for: 1) the entire wavelength interval except for the following regions that were noisy due to instrument and/or atmospheric noise: 350–400 nm, 1350–1500 nm, 1850–1975 nm, and 2300–2500 nm; 2) the 400–900 nm region, where plants have been observed to respond to stress due to metals (Horler et al., 1980; Schwaller et al., 1983; Davids and Tyler, 2003; Schuerger et al., 2003; Koostra et al., 2004); and 3) the wavelengths and vegetation indices specific to plant chlorophyll and pigments: 430 nm, 448 nm, 471 nm, 550 nm, 642 nm, 662 nm, 680 nm, 800 nm, REP, RVI, and NDVI. Stepwise regression models were also run for the ΔR for each treatment and the spiked experiments on each sample date over the region 500–900 nm (wavelengths <500 nm were noisy); the first derivative data over the 500–2300 nm interval were excessively noisy and not included in a regression model as a whole.

4. Results

4.1. Mercury in soils and leaves

Mercury contents of the spiked and contaminated field soils are one to two orders of magnitude higher than those of control soils (Table 1). Soil Hg concentrations did not change significantly over the course of the experiment, and the reported values are averages over time for each treatment level.

Foliar Hg concentrations range from 0.17 ± 0.03 ppm to 4 ± 1.6 ppm (Table 2). Foliar Hg concentrations are considerably higher for all experiments on day 28 than for other days (Fig. 2). A possible explanation for these outlying data is that the hood failed temporarily during the period of growth prior to sampling day 28, allowing Hg vapor from the soils to accumulate in the chamber. The magnitude of the foliar Hg on day 28 correlates strongly with measured soil Hg (r = 0.99), which suggests that volatilization of Hg from the soil beneath each plant dominated the foliar Hg signal for that period.

Significant positive correlations (p < 0.01) between soil and leaf Hg are observed for the first two sampling dates but not during the rest of the experiment (Fig. 3). Soil and leaf Hg correlations are improved (r > 0.90) for the first 4 sampling dates if the data from the Spike 4 experiment are removed from the correlation. Due to the small sample size, there is not enough power to test individual sample dates for statistical differences between treatments with an ANOVA and thus the data are pooled. Measurements from days 28 and 53 are found to be outliers and are excluded from the pooled data set. Significant differences are observed for control versus spiked leaves (p < 0.01) and spiked versus field leaves (p < 0.01) but not for control versus field leaves.

4.2. Relationship between spectral properties and foliar Hg concentrations

Correlograms of foliar Hg content and R across the entire spectrum for all treatment levels show the highest degree of overall correlation for the Control and Barnum

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Average experiment soil Hg concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Soil Hg (ppm)</td>
</tr>
<tr>
<td>Control</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>Spike 1</td>
<td>1.12 ± 0.10</td>
</tr>
<tr>
<td>Spike 2</td>
<td>14.2 ± 1.35</td>
</tr>
<tr>
<td>Spike 3</td>
<td>21.5 ± 2.87</td>
</tr>
<tr>
<td>Spike 4</td>
<td>39.4 ± 4.90</td>
</tr>
<tr>
<td>Dianna Knit</td>
<td>120 ± 11.3</td>
</tr>
<tr>
<td>Barnum Court</td>
<td>111 ± 25.7</td>
</tr>
</tbody>
</table>

n = 5 (±SD); precision is ±0.17 ppm; detection limit is 0.2 ppb.
Court treatments (Fig. 4). Two wavelengths are selected for inclusion in the regression model in the middle infrared region (>1000 nm) of the spectrum (Table 3), where spectral response is largely a function of water content of the leaves. Examination of the VNIR portion of the spectrum (Fig. 4b) shows that all spiked and the Dianna Knit treatments are negatively correlated to foliar Hg across the visible wavelengths. For this restricted wavelength interval, the regression model shows a significant \( (p = 0.06) \) negative correlation between green reflectance and foliar Hg content in the control plants (Table 4). The shape of the correlogram in the green region is broadly mimicked by the Spike 1, Spike 2 and Dianna Knit treatments (Fig. 4b). Regression of individual spectral bands and RVI, NDVI and REP values also indicate a significant \( (p < 0.1) \) negative correlation with green in the Control plants; Spike 2 and Spike 3 plants are modeled to have a positive correlation with REP (Table 4).

Correlation coefficients for the \( \delta R \) are generally greater than those for \( R \) (Fig. 4c), with the greatest values for the Control plants in the red portion of the spectrum. All treatments show similar behavior in the red edge region, displaying negative correlations to foliar Hg at \( \sim 700 \) nm and positive correlations at \( \sim 730 \) nm. Wavelengths selected for inclusion in the regression models are listed in Table 5.

### 4.3. Plant phenology and Hg

#### 4.3.1. External appearance of plants

During the first two weeks of the growth cycle plants from all treatment levels appeared similarly healthy. Plant leaves increased in size, number and green color from day 13 to day 34. By day 34 of the experiment, plants in the highest spiked soils (level 4) and the field soils exhibited chlorosis, leaf elongation and curling leaf edges. By day 53, leaves of plants in the spike levels 2 and 3 soils were chlorotic and elongated and plants in spike level 4 had necrotic leaf tips. Control plants and spike 1 soils had green, healthy-looking leaves throughout the growing season.

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**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hg concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 21</td>
</tr>
<tr>
<td>Control</td>
<td>0.43 ± 0.02</td>
</tr>
<tr>
<td>Spike 1</td>
<td>0.28 ± 0.00</td>
</tr>
<tr>
<td>Spike 2</td>
<td>0.54 ± 0.09</td>
</tr>
<tr>
<td>Spike 3</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td>Spike 4</td>
<td>0.90 ± 0.08</td>
</tr>
<tr>
<td>Dianna Knit</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>Barnum Court</td>
<td>0.19 ± 0.27</td>
</tr>
</tbody>
</table>

\( n = 3 \) (±SD) except for the control on days 21 and 34 where \( n = 1 \); precision is ±0.04 ppm; detection limit is 0.2 ppb.
experiment except one plant of each of these treatments had lighter green leaves by day 53.

The symptoms of plant stress observed in this study are common general responses of plants to stress including metal toxicity (Schuerger et al., 2003). Siegel et al. (1984) and Suszcynsky and Shann (1995) observed stress symptoms similar to those observed in our study, including curling leaves, for tobacco plants subjected to Hg vapor stress. The foliar concentration above which plants exhibited stress in our study (>0.5 ppm Hg) was similar to the concentration above which the tobacco plants showed symptoms of stress in their study (>490 ppb Hg).

4.3.2. Changes in spectral properties over the growth cycle

Correlograms of foliar Hg content of the spiked plants and R across the entire spectrum for all dates show the highest degree of overall correlation on days 34 and 46 (Fig. 5). Regression modeling shows a significant negative correlation between foliar Hg and green wavelengths on day 34 ($p < 0.1$) and a positive correlation with NIR wavelengths on day 46 ($p < 0.01$; Table 3). These wavelengths are also selected when the model input is restricted to the 400–900 nm interval (Fig. 4b; Table 5). Regression of individual

Table 3

<table>
<thead>
<tr>
<th>Sampling Date/Treatment</th>
<th>$\lambda$ (nm) in order of selection (sign of term)</th>
<th>$R^2$ (cumulative)</th>
<th>$p$ (cumulative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1525 (--)</td>
<td>0.79</td>
<td>0.04</td>
</tr>
<tr>
<td>Barnum Court</td>
<td>1842 (--)</td>
<td>0.85</td>
<td>0.08</td>
</tr>
<tr>
<td>Day 34</td>
<td>565 (--)</td>
<td>0.67</td>
<td>0.09</td>
</tr>
<tr>
<td>Day 46</td>
<td>723 (+)</td>
<td>0.99</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>1975 (--)</td>
<td>1.00</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Model takes the form: Foliar Hg concentration = $b_0 + b_1(R_{1a}) + b_2(R_{2a}) + \cdots + b_n(R_{na})$.

4.3.3. Changes in spectral properties over specific dates

Correlograms of foliar Hg content and its 1st derivative for Hg treatment levels. Shaded areas correspond to $|r| < 0.6$, and are only for reference.

Table 4

<table>
<thead>
<tr>
<th>Spectral transformation</th>
<th>Sampling Date/Treatment</th>
<th>$\lambda$ (nm) in order of selection (sign of term)</th>
<th>$R^2$ (cumulative)</th>
<th>$p$ (cumulative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflectance</td>
<td>Control</td>
<td>550 (--)</td>
<td>0.75</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Spike 2</td>
<td>REP (++)</td>
<td>0.78</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Spike 3</td>
<td>REP (++)</td>
<td>0.71</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NDVI (--)</td>
<td>0.95</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Day 46</td>
<td>800 (+)</td>
<td>0.75</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>550 (+)</td>
<td>0.99</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>471 (+)</td>
<td>1.00</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

Model takes the form: Foliar Hg concentration = $b_0 + b_1(R_{1a}) + b_2(R_{2a}) + \cdots + b_n(R_{na})$. $S_\lambda$ is R or dR at wavelength, $\lambda$.

*a* Regressions were performed using the following bands: 430 nm, 448 nm, 471 nm, 550 nm, 642 nm, 662 nm, 680 nm, 800 nm, red-edge position (REP), ratio vegetation index (RV1), and normalized difference vegetation index (NDVI).
wavelengths and vegetation indices selects an additional NIR wavelength into the model on day 46 (Table 4).

Correlation coefficients for \( dR \) are greater than those for \( R \) (Fig. 4c), with the greatest values for plants on days 28 and 34 of the experiment. Significant \((p < 0.05)\) positive correlations between foliar Hg and \( dR \) are modeled by stepwise regression in the NIR region on days 21, 28 and 46; plants demonstrate a significant \((p < 0.01)\) negative correlation in the green region on day 34 (Table 5).

NDVI values of control, spiked and contaminated field plants have a small range \((0.80 - 0.85)\) among treatment levels and sample dates and do not differ significantly. In general, NDVI values are similar throughout the experiment then decrease between the last two sample dates.

RVI values generally increase between the first and second sample dates, remain relatively high through the third sample date and decline thereafter (Fig. 6a,b). Peak RVI values are reached at the second sample date for Control and Spike 3 plants, the third sample date for Spike 1, Spike 2, Barnum Court and Dianna Knit plants and the fourth sample date for Spike 4 plants. On average, RVI values

Table 5
Results of stepwise regression modeling of Hg in the spiked experiments and spectral properties over the 400—900 nm interval (reflectance, \( R \)) or 500—900 nm interval (1st derivative of reflectance, \( dR \)) \((\text{VIF} < 10)\)

<table>
<thead>
<tr>
<th>Spectral transformation</th>
<th>Sampling Date/Treatment</th>
<th>( \lambda ) (nm) in order of selection (sign of term)</th>
<th>( R^2 ) (cumulative)</th>
<th>( p ) (cumulative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflectance Control</td>
<td>560 (−)</td>
<td>0.76</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>1st Derivative of Reflectance Control</td>
<td>700 (−)</td>
<td>0.96</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>829 (+)</td>
<td>1.00</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>616 (+)</td>
<td>1.00</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>833 (−)</td>
<td>0.84</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>820 (+)</td>
<td>0.99</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>750 (−)</td>
<td>1.00</td>
<td>0.01</td>
<td></td>
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<tr>
<td>Spike 1</td>
<td>781 (−)</td>
<td>0.75</td>
<td>0.06</td>
<td></td>
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<tr>
<td></td>
<td>842 (+)</td>
<td>1.00</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>503 (−)</td>
<td>1.00</td>
<td>0.01</td>
<td></td>
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<tr>
<td>Spike 2</td>
<td>603 (+)</td>
<td>0.94</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>515 (−)</td>
<td>1.00</td>
<td>0.01</td>
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<tr>
<td></td>
<td>798 (+)</td>
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<tr>
<td>Spike 3</td>
<td>774 (−)</td>
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<td></td>
<td>712 (+)</td>
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<td>0.01</td>
<td></td>
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<tr>
<td></td>
<td>783 (−)</td>
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<tr>
<td>Barnum Court</td>
<td>895 (−)</td>
<td>0.89</td>
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<tr>
<td></td>
<td>650 (+)</td>
<td>1.00</td>
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<tr>
<td>Reflectance Day 34</td>
<td>565 (−)</td>
<td>0.67</td>
<td>0.09</td>
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<td></td>
<td>723 (+)</td>
<td>0.99</td>
<td>0.01</td>
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<tr>
<td>Day 46</td>
<td>841 (−)</td>
<td>0.98</td>
<td>0.01</td>
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<tr>
<td></td>
<td>529 (−)</td>
<td>0.98</td>
<td>0.01</td>
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<td>849 (+)</td>
<td>0.78</td>
<td>0.05</td>
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<td>676 (+)</td>
<td>0.99</td>
<td>0.01</td>
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<td></td>
<td>537 (+)</td>
<td>1.00</td>
<td>0.01</td>
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<tr>
<td>Day 53</td>
<td>785 (−)</td>
<td>0.79</td>
<td>0.04</td>
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<tr>
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<td>672 (+)</td>
<td>0.99</td>
<td>0.01</td>
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<tr>
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<td>829 (+)</td>
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Model takes the form: Foliar Hg concentration = \( b_0 + b_1(R_{l1}) + b_2(R_{l2}) + \cdots + b_n(R_{ln}) \). \( S_l \) is \( R \) or \( dR \) at wavelength, \( \lambda \).
of control plants are higher than those of spiked and contaminated plants on days 21–34, significantly so (p < 0.01) on days 21 and 28.

The averaged first derivative spectra show a single peak in the red edge region (Fig. 1c). Over the course of the growing cycle, all plants show a shift of REP to longer wavelengths as plants develop then shift back to shorter wavelengths with the onset of senescence (>day 34, Fig. 6c,d). The REP of plants in spike level 4 and Barnum Court soils reach peak values 6 days later than plants in the control, lower spike level and Dianna Knit soils (Fig. 6c,d). REP values of plants in Hg-spiked and contaminated field soils are generally shifted to shorter wavelengths relative to control plants on the second and third sample dates (Fig. 6d); these treatments are significantly different (p < 0.0001) on days 21 and 28.

5. Discussion

5.1. Relationship between soil and leaf Hg

The details of the Hg in the soil-plant-air system in this study are complex and depend on a number of factors including the mechanism of uptake (via leaf or root), and the ability of the plant to incorporate Hg which is a function of plant health (stress factors and senescence), and bioavailability of Hg (Ericksen et al., 2003; Frescholtz et al., 2003; Leonard et al., 1998). Significant differences are observed for control versus spiked leaves (p < 0.01), demonstrating an increase in foliar Hg for the plants grown in the spiked soils. These results agree with several other studies that found significant positive correlations between soil and foliar Hg concentrations (Barghigiani and Ristori, 1995; Cocking et al., 1995; Shaw and Panigrahi, 1986; Siegel et al., 1987; Gupta and Chandra, 1998). Soil and foliar Hg are significantly correlated (p < 0.01) on days 21 and 28 (Fig. 3a), and foliar Hg best represents soil Hg values during the green-up phase of plant development at the beginning of the growing season. Foliar Hg is lowest on day 53 for 6 of the 7 treatments indicating a loss of Hg by some mechanism during senescence.

The plants were grown inside a hood to minimize the contribution of Hg vapor uptake by the leaves, and some foliar Hg was probably derived from root uptake. However, atmospheric uptake of Hg is well demonstrated by the elevated levels of foliar Hg in the control plants. Additionally, the strong but temporary increase in foliar Hg levels on day 28 is consistent with the addition of Hg vapor.

The ANOVA of the pooled data show significant differences for spiked versus field leaves (p < 0.01), but not for control versus field leaves. This discrepancy may be due to a difference in bioavailability of Hg in the spiked versus
contaminated soils. Hg was added to the spiked soils in the relatively soluble form of HgCl₂ just before the experiment while Hg in the field soils has experienced a greater degree of Hg volatilization, and the weathering and removal of more mobile and soluble fractions over time. Thus it is likely that Hg in the spiked soils was more available for uptake through root and possibly also leaf pathways.

5.2. Relationship between spectral properties and leaf Hg

Several empirical studies of the relationship between R and chlorophyll content show strong negative correlations at 550 nm and 680 nm due to increased absorption at these wavelengths and a shift of the position of the red edge to shorter wavelengths (Carter, 1994; Buschmann and Nagel, 1993; Mariotti et al., 1996; Blackburn, 1999; Carter and Knapp, 2001). Plants grown in Hg-spiked soils have greater average R(550 nm) (not shown) and significantly lower average RVI and REP values (Fig. 6) than control plants at the beginning of the growth cycle. Similar spectral shifts have been reported in the spectra of vegetation subject to a variety of stressors under both laboratory and field conditions (Horler et al., 1980; Rock et al., 1988; Curran et al., 1995; Schuering et al., 2003; Li et al., 2005). As RVI and REP correlate strongly with chlorophyll content (Gitelson and Merzlyak, 1996; Horler et al., 1983; Demetriades-Shah et al., 1990; Fillela and Peñuelas 1994; Curran et al., 1995; Gitelson et al., 1996; Imanishi et al., 2004), these results are consistent with a reduction in chlorophyll content as a manifestation of toxicity by Hg. That the 550 nm, RVI and REP values of the contaminated plants show greater distinction from controls during the beginning of the growth cycle may indicate specifically that the Hg stress was most acute during greenup or that plants with lower foliar Hg concentrations may have greater sensitivity to the effects of Hg, in this case, <0.6 ppm excluding day 28. Although not significant, the general shape and magnitude of the correlograms of R and \( \delta R \) are most similar for the plants with the lowest amount of foliar Hg (Control, Spike 1, 2 and Dianna Knit; Fig. 4b,c), also consistent with an increased sensitivity at lower foliar Hg concentrations.

The correlation coefficients for foliar Hg content and \( \delta R \) are greater than those for R. All treatments exhibit similar behavior in the red edge portion of the spectrum, with negative values for \( R \) at \( \sim 700 \) nm and positive values for \( R \) at \( \sim 725 \) nm (Fig. 4c). Yoder and Pettigrew-Crosby (1995) recorded strong correlations between the chlorophyll content of leaves and the first difference of reflectance \((\log I/R)\), a good proxy for \( \delta R \) at 690 nm (positive values for \( R \) and 750 nm (negatives values for \( r \)). The correlations between foliar Hg and \( \delta R \) of each treatment in this experiment are qualitatively consistent with reduced foliar chlorophyll content due to Hg toxicity.

The correlograms yield both positive and negative relationships that vary as a function of treatment and sampling date, and the significant correlations of foliar Hg and \( R \) selected by stepwise regression appear generally inconsistent with what is expected if Hg is reducing the chlorophyll content of the leaf. The mixed results yielded by the experimental data may be due to the statistical limitations imposed by the small sample size. However, it is clear that phenological variability must be considered when examining foliar Hg concentration and leaf spectral characteristics. Our data show that RVI and REP values in the early to middle portions of the growth cycle are the most heavily influenced by Hg content. This portion of the growth cycle also facilitates the distinction of spectral variability due to Hg stress from that due to senescence.

Plants growing in field soils experienced early-onset senescence, and the average RVI and REP values of plants in field soils were generally lower and \( R(430, 448, 471, 550, 642, 662, 680 \) nm) generally higher than both control and spiked plants over the course of the experiment. Each of these spectral behaviors is consistent with increased plant stress. As the foliar Hg concentrations of the plants in these soils were not statistically different from those of the control plants, Hg is probably not the primary cause of the spectral stress indicators. Unpublished data show the Barnum Court and Dianna Knit soils to have elevated concentrations of Pb, As, Cu and Ni with respect to background levels; these elements are known phytotoxins (Kabata-Pendias and Pendias, 2001) that may have caused damage to chlorophyll and leaf structure of the plants in this study. The contaminated field soils from these former hat-making factory sites may also contain elevated levels of organic contaminants or deficient levels of micronutrients that could have contributed to plant stress.

6. Conclusions

This study is a first attempt to correlate foliar Hg concentration to high resolution VNIR spectral reflectance. Mustard spinach plants grown in Hg-spiked soils accumulated foliar Hg that varied in amount through the experiment. The soil:foliar Hg was significantly and positively correlated for most of the treatments at the beginning of the growth cycle. Foliar Hg was lowest at the end of the growth cycle. Thus care must be taken when attempting to measure foliar Hg from a single day or comparing Hg concentrations collected at different times of year.

Plants with the greatest amount of foliar Hg showed symptoms of stress earlier than plants of other treatments. Foliar Hg appears to influence spectral reflectance at several specific regions in the 400–2300 nm spectrum. The highest significant correlations are found for leaves in the Control treatment and for all treatments in the middle to end of the growth cycle. Equations generated by stepwise regression yield both positive and negative relationships between foliar Hg content and indicators of plant health.

A significant offset between average RVI and REP values of healthy and highly contaminated plants is greatest during the early portion of the growth cycle. This pattern is consistent with a reduction of chlorophyll concentration and/or impairment of metabolism in the young plants due to Hg stress. Further experiments are required to identify the potential causes of spectral variability shown here.
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