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Spectral Detection of Lithium Uptake in Vegetation for Forensic Locating of Methamphetamine Lab Sites

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Abstract

Methamphetamine is a controlled substance having a great potential for abuse and addiction. Millions of Americans have tried this stimulant, so the need to stop its production is rapidly increasing. One approach that could assist in this regard is the use of spectral analysis of vegetation growing on land suspected of being a methamphetamine dump site. This could allow law enforcement to use multispectral data to examine the surrounding land under suspicion and obtain probable cause for a warrant for further forensic investigation of the site. As part of its production, lithium metal is introduced with anhydrousammonia to convert pseudoephedrine to methamphetamine. After this process, the remnant lithium is discarded nearby outdoors where weedy plants (such as *Arabidopsis thaliana*) can uptake the waste. The amount of lithium in Arabidopsis tissue can be ascertained through biochemical analysis and relatively high concentrations have the potential to affect reflected energy in particular spectral wavelengths. An experiment was conducted to determine if lithium uptake into the tissue of Arabidopsis could be discerned spectrally. T-test and linear regression evaluations found that the spectral responses of lithium treated plants were statistically different from those of controls in certain visible and near-infrared regions.

Keywords: Spectral, biochemical, vegetation, methamphetamine, *Arabidopsisthaliana*, forensic

1. Introduction

Methamphetamine (meth) is a schedule II controlled substance that poses considerable concern for its damaging effect on an individual's health and the threat of widespread abuse and addiction.

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First synthesized in 1887 and used for medicinal purposes during World War II, many soldiers returned home addicted and a market of buyers and sellers was established. Although banned for use in 1968, it has become a popular and increasingly abused drug because it is cheap and easy to manufacture using readily available materials and household chemicals (e.g., lithium metal obtained from batteries and ephedrine from over-the-counter decongestants). The introduction of lithium metal into clandestine methamphetamine laboratories was uncovered by Ely and McGrath (1990) in which lithium/ammonia/ammonium chloride is used to reduce ephedrine tomethamphetamine. In 2005, nearly 2,146 kg of meth was seized by the U.S. Drug Enforcement Agency and it was reported to be in widespread use in southeast Asia,Australia and the Czech Republic (Methamphetamine Center for Disease Control Drugscope, 2012). With approximately 5 million Americans having tried methamphetamine, the need to stop its production is rapidly increasing.

Since lithium is a primary ingredient in meth synthesis, biochemical evaluation of this metal's transport into the plant tissue of a commonly found weed (e.g., *Arabidopsis thaliana*) would enable the determination of its concentration with certain levels allowing spectral analysis to identify its presence. Discardment of lithium-laced waste after methamphetamine production onto nearby land areas having such a plant could also allow law enforcement equipped with spectral scanning devices the means to detect relevant levels of this metal as an indicator of an illegal drug site similar to that used today to locate cannabis growth (Gunther, 2005). To ascertain the feasibility of this forensic approach, a controlled lab experiment was conducted to find out if lithium metal was being transported into the leaf tissue of *Arabidopsis* and at what levels its concentration would induce a discernible spectral reflectance response in visible and near-infrared (NIR) wavelength regions.

2. Materials and Methods

2.1 Preliminary Background

Lithium is utilized in the Birch method of methamphetamine production, in which the principal components are pseudoephedrine, anhydrous ammonia, and sodium or lithium metal (U.S. Department of Toxic Substances, 2003). Pseudoephedrine hydrochloride (HCl) is dissolved in a solvent such as acetone/ethyl alcohol and filtered to remove any filler.

The pseudoephedrine HCl is mixed with liquid anhydrous ammonia and then lithium metal is introduced. An acidic solvent is then added to separate the methamphetamine HCl from the ammonia/lithium by acid/base extraction. As the layers separate, methamphetamine HCl will dissolve into the organic layer since salts, which are ionic, are water-soluble, while neutral molecules will not. This organic layer is kept while the bottom layer containing the lithium and ether is discarded. Hydrogen chloride gas is bubbled through the methamphetamine HCl forming a precipitate. This precipitate is filtered and the solid methamphetamine hydrochloride is dried and packaged (The Drug Identification Bible, 2012).

While only small amounts of lithium are required for the production of methamphetamine, nearly 95% of the lithium is consumed (U.S. Geological Survey, Mineral Commodity Summaries, 2012). The waste is often dumped on surrounding land and into streams. Previous research on the dumpsites of leftover lithium has mostly been performed on soil (Nelson et al., 1999). Lithium is the lightest of the alkali metals and occurs naturally in soil ranging from 7 to 200 ppm. The uptake of lithium into plant roots occurs quickly through Na^+ or K^+ ion channels. These channels regulate the flow of ions across the membrane in a plant's cells.

Lithium can either block these salt channels or pass through freely resulting in uptake into plant tissue by mass flow. Once in the leaf tissue, Li^+ is immobile and at high concentrations can cause chlorosis and even death, possibly due to channel blockage or competitive binding against other essential salts (Schrauzer, 2002).

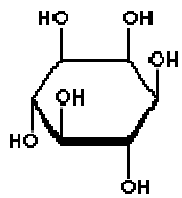
Arabidopsis thaliana can be used as a representative, weedy plant to detect lithium at methamphetamine dump sites because it is commonly found there. It was ideal for this study because of its size, life cycle, growth period, and established physiological requirement. In *Arabidopsis*, lithium can be taken up across cell membranes through nonselective cation channels (NSCCs) or through specific potassium channels. These nonselective cation channels transport sodium and potassium, and lithium is moved passively along a diffusion gradient as the growing plant rapidly uptakes solutes (Demidchik and Tester, 2002). Selective ion channels allow only certain ions to pass through into cells. These channels often utilize active transport. Through active transport solutes are pumped across membranes against their electrochemical gradient. For example, in the membranes of most plant cells, selective channels will allow potassium ions to pass but not sodium (Campbell and Reece, 2002).

Kent (1941) found that these channels have a selectivity of 0.73 for Li^+, nearly two to three-folds lower than K^+, suggesting lithium's permeability flow is much slower than K^+/Na^+. Yet, these channels are not specific for lithium. Instead, lithium is transported into the channels through mass flow and is found in the fast growing tissues due to the channels taking up water and ions for growth (Kent, 1941).

Lithium presents the same salinity stresses on these channels much like potassium or sodium (Demidchik and Tester, 2002). This high salinity stress threatens plants in two ways.

First lithium, like any ion, can create negative water potential in the soil, and thus a water deficit even though there is plenty of water present (Campbell and Reece, 2002). Second, a negative water potential can cause an excess of sodium and other ions which are toxic at high levels. Plants can counteract this effect in several ways including: the increase in stress hormones and myoinositol, the production and increase in concentration of solutes, the subsequent change to a more negative water potential than the surrounding soil, the sequestration of toxic ions in vacuoles, and the increase of root pressure and water transport. One of the major stress response molecules is the carbohydrate myoinositol (Figure 1). Myoinositol is stored in the phloem and transported to the tissue experiencing salt stress. The carbohydrate facilitates the solute accumulation and long-distance transport (Nelson et al., 1999). Another major anti-stress molecule is abscisic acid (ABA). ABA is a plant hormone that initiates signal transduction pathways that trigger the stress responses. For example, ABA induces an increase in anti-stress proteins and solutes. In addition, ABA triggers stomatal closure of gas pores on leaf surfaces through which water can evaporate and the action of myoinositol is reduced (The Arabidopsis Information Resource, 2012).

Figure 1 Structure of Myoinositol



Dicotyledens will also respond using molecular synthesis, enzyme induction and membrane transport as well as preventing toxic buildup by trapping the ions in the vacuole (The Arabidopsis Information Resource, 2012).

Arabidopsis and most plants respond to lithium stress in a manner similar to its response to Na^+ stress (Demidchik and Tester, 2002). Thus, *Arabidopsis* species can tolerate a broad range of water potentials in marginally arable, polluted or disturbed soil. This protection against salt stress allows for plants to uptake lithium from the soil, even when lithium is present in potentially toxic levels, and for the potential sequestration of lithium in shoot materials at concentrations high enough to detect lithium increases in plants at clandestine meth lab dump sites.

2.2 Sample Preparation and Spectral Evaluation

The focus of this study was on evaluating lithium bromide and *Arabidopsis* Wassilewskija (Ws-2) wild type seeds. A growth media for the Arabidopsis seeds consisted of calcium nitrate, magnesium sulfate, monopotassium phosphate, potassium nitrate, ferric ethylene-diaminetetraacetic acid, a micronutrient solution, and sucrose. A lithium 1000 ppm standard in 2% nitric acid solution was also prepared, and lithium chloride and lithium fluoride were used to study the effect of ions on spectral reflectance. Additionally, sodium chloride was used to study the effect of different salts on the spectral response.

The *Arabidopsis* growth media, a pre-plant fertilizer used to boost micronutrients during plant growth, consisted of: 0.47 g $Ca(NO_3)_2$, 0.50 g KNO_3, 0.50 g $MgSO_4$, 2.5 mL 20 mM FeEDTA, 1.0 mL micronutrient, 5.0 mL 0.5M KH_2PO_4, and 10.0 g sucrose. To test the spectral reflectance of lithium in *Arabidopsis,* nine different concentrations of solid LiBr were added with the growth media (50mL) to produce treatment concentrations of: 700 ppm, 200 ppm, 70 ppm, 60 ppm, 50 ppm, 40 ppm, 30 ppm, 20 ppm, and 7 ppm (Table 1) (Campbell and Reece, 2002).

Table 1 Amount of lithium bromide added to *Arabidopsis* growth media to achieve desired treatment concentrations

	7ppm	20ppm	30pm	40ppm	50ppm	60ppm	70ppm	200ppm	700ppm
LiBr (g)	0.003	0.006	0.009	0.012	0.015	0.018	0.023	0.059	0.215

To determine whether the lithium spectral response observed was due to the lithium metal and not the bromine anion in the salt, a growth media was prepared that contained enough lithium chloride and lithium fluoride to obtain 7 ppm lithium which was then compared to a 7 ppm lithium bromide growth media. To ascertain whether the lithium salt was distinguishable from other salts spectrally, a growth media was prepared that included enough sodium bromide to obtain 200 ppm which was then compared to a 200 ppm lithium growth media.

Spectral scanning analysis was performed on the lithium treated leaves of *Arabidopsis* to detect differences in the visible and near-infrared (NIR) wavelength regions. The analysis was conducted in a controlled room setting using a Spectron Engineering SE-590 Spectroradiometer having a wavelength range from 368.4 to 1113.7 nanometers. During scanning, the leaf samples were laid as a flattened, circular mound under the spectroradiometer and illuminated with quartz halogen lights. Initially, two scans per sample were made of a Spectralon reflectance panel which was used to determine the average background reflectance or standard. Then after two scans of each sample, a spectral curve was generated as percent reflectance vs. wavelength. This was a ratio of the two sample scans with the standard. To ensure there was no statistical variation in the scanning process, the leaves were rearranged between scans. Deviations in the spectral curve peaks in particular wavelength regions from plant leaves having varying lithium levels were noted.

After spectral analysis was performed, the plant leaves were prepared for measurement of their lithium tissue concentration. The instrument used to detect lithium was a Perkin Elmer 1100 Atomic Absorption Spectrometer.

Emission was determined for each lithium bromide sample, and a calibration curve for an emission intensity signal of lithium versus concentration was generated with five standards ranging from 50 ppb to 5 ppm Li^+ in diluted HNO_3 (Manton, 1950). This same method was employed on treatments containing LiCl and LiF. The emission intensities of each of the unknowns were compared to the emission intensities of the known standard in a calibration curve. The calibration curve method measures the instrument signal versus concentration of standard solutions. With this information, concentrations of lithium in *Arabidopsis* tissue were calculated using Equation 1 to determine the amount of lithium in the tissue and not the digested solutions where X is the concentration of the digested solution (mg/L) and mass is the dried weight (kg) of the tissue prior to digestion.

Equation 1: $y = ((X(0.25L)) / mass)$

To determine the statistical relationship between the varying lithium concentrations in *Arabidopsis* tissue and percent reflectance, linear regression was performed. T-test analysis was carried out to ascertain if any significant differences existed in the spectral curves. A treatment group mean was compared to a control group mean, with the level of significance set at 0.95. Both two-tailed distribution and two-sample of equal variance tests were used.

Finally, the first derivative of the lithium spectral response curves was plotted to identify any unique changes in them resulting from different lithium concentrations. This analysis acts to reduce any background noise in the original scanned data and enables further evaluation of curve variations (Liew, 2001).

3. Results and Discussion

In this experiment, the concentration of lithium in plant tissue (ppm) increased as the concentration of lithium in the growth media increased until a threshold was reached above 70 ppm (Table 2). The greatest standard deviation is seen in the higher treatments of 200 ppm and 700 ppm, the point at which the plant implemented defense mechanisms

Table 2 Concentrations of Lithium Treatments in *Arabidopsis* Tissue and Their Relative Standard Error Compared to the Lithium Concentration in the Growth Media

(Error reported is for the standard deviation from the mean of three experimental trials. PPM refers to the mg of Li/kg plant material.)

Concentration in media (ppm)	**Concentration in plant tissue (ppm)**	**RSD**
Control	13 ± 1	12
7	22 ± 2	11
20	33 ± 3	9
30	52 ± 5	9
40	39 ± 7	17
50	80 ± 5	6
60	91 ± 7	8
70	59 ± 5	9
200	142 ± 20	14
700	190 ± 16	8

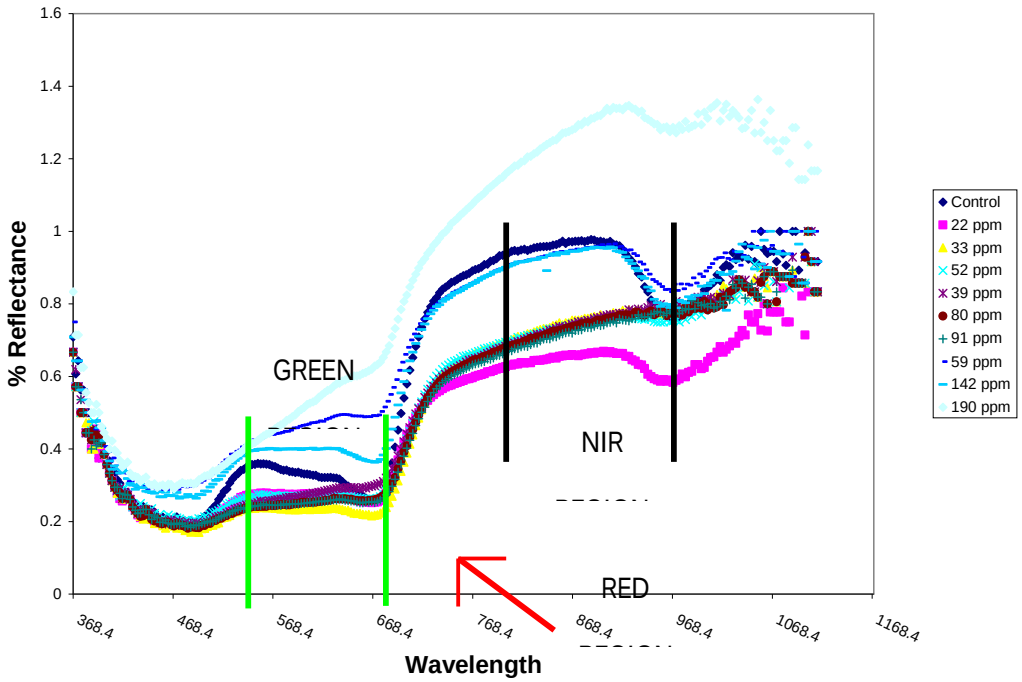
against salinity stress. A plant's response to salinity stress can vary among individuals, as seen by the data and chlorosis of the plant at 700 ppm which followed after nearly 8-10 days (Figure 2).

Figure 2: *Arabidopsis* Chlorosis (Right) of Plant Tissue Lithium Concentrations at 700 ppm Compared to the Control (Left)

With regard to the relationship between spectral reflectance and lithium concentration in *Arabidopsis* tissue, the spectral curves showed considerable variation in the percent reflectance of lithium treated plants when compared to the control plants not containing lithium (Figure 3).

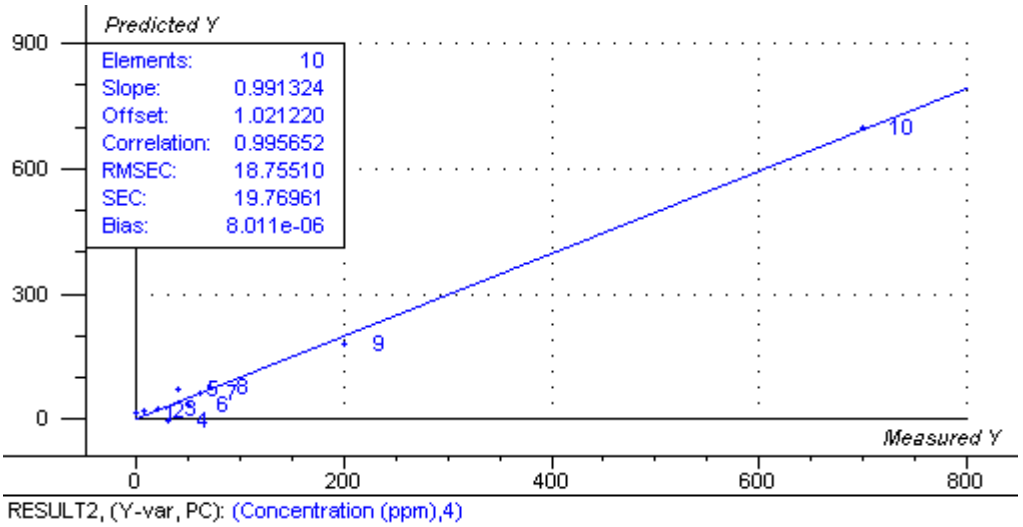
It appears that low amounts of Li^+ (22-52 ppm) lowered the percent reflectance at around 550 nm (green region), yet increased the percent reflectance in this region when treatment was equal to or greater than 59 ppm. At 670 nm (red region) low concentrations were indistinguishable from the control response, but percent reflectance increased above 70 ppm at this wavelength. This is not surprising since this region in where chlorophyll absorption is normally strong. However, as a plant responds to salinity stress and eventually approaches death, the chlorophyll is reduced as seen with the browning of the plant. Around 850 nm (NIR region) the percent reflectance dropped nearly 0.4% with small amounts of lithium (22-91 ppm) compared to the control, but it increased considerably with lithium concentrations of 190 ppm. The NIR region is also sensitive to changes in the cellular structure of a plant, so these variations in spectral reflectance can be attributed to mesophyll alterations produced by the different lithium concentrations.

Figure 3: Spectral Reflectance of *Arabidopsis* Tissue Grown In Growth Media Containing Varying Concentrations of Li^+



The statistical relationship between the varying lithium concentrations in *Arabidopsis* tissue versus the percent reflectance was determined by linear regression and showed a positive correlation between the predicted values and the measured values that proved to be significantly correlated at 99.5% (Figure 4). It also revealed that the root mean error of prediction (RMSEC) can predict within 19 ppm what the lithium concentration is in an unknown sample. Apparently, plants with 700 ppm (10) and 190 ppm (9) tissue concentrations are outliers.

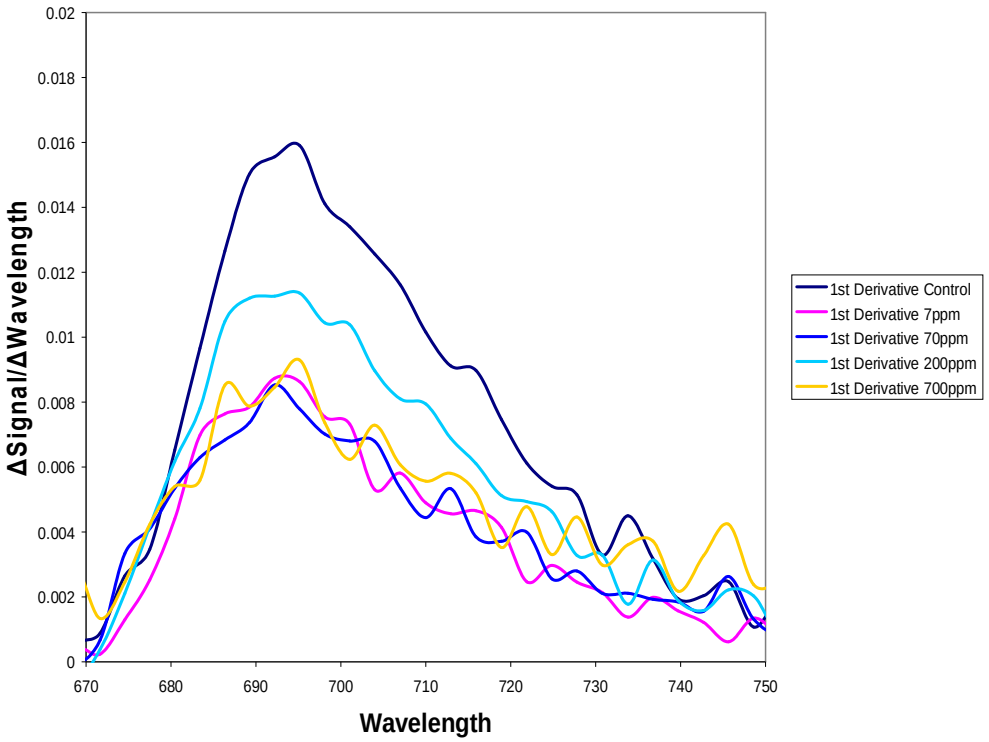
Figure 4: Linear Regression of Varying Lithium Concentration [ppm(Y-Axis)] in *Arabidopsis* Tissue Versus Percent Reflectance [nm(X-Axis)]



The first derivatives of the lithium spectral curves were also plotted to see if there were any apparent variations in the curves with different lithium concentrations (Figure 5). These derivatives reduce any background noise in the original data and enable further discrimination of changes within a spectrum. They are computed using the equation: Δ signal / Δ wavelength (Tsai and Philpot, 1998). By comparing the first derivatives of the lithium treated *Arabidopsis* tissue to the control not containing lithium, variation was observed. Since the first derivative is the slope of the tangent line, a higher value indicates a steeper slope in the original spectral curve. In this analysis, higher levels of lithium appear to increase the percent reflectance in the spectral range of 680 nm to 750 nm, which was observed in the original curve. Lower concentrations of lithium appear to lower the percent reflectance from 680 nm to 750 nm but with no visible trends among treatments.

Figure 5: First Derivative Spectra of the Percent Reflectance of *Arabidopsis* Tissue Grown in Various Concentrations of LiBr

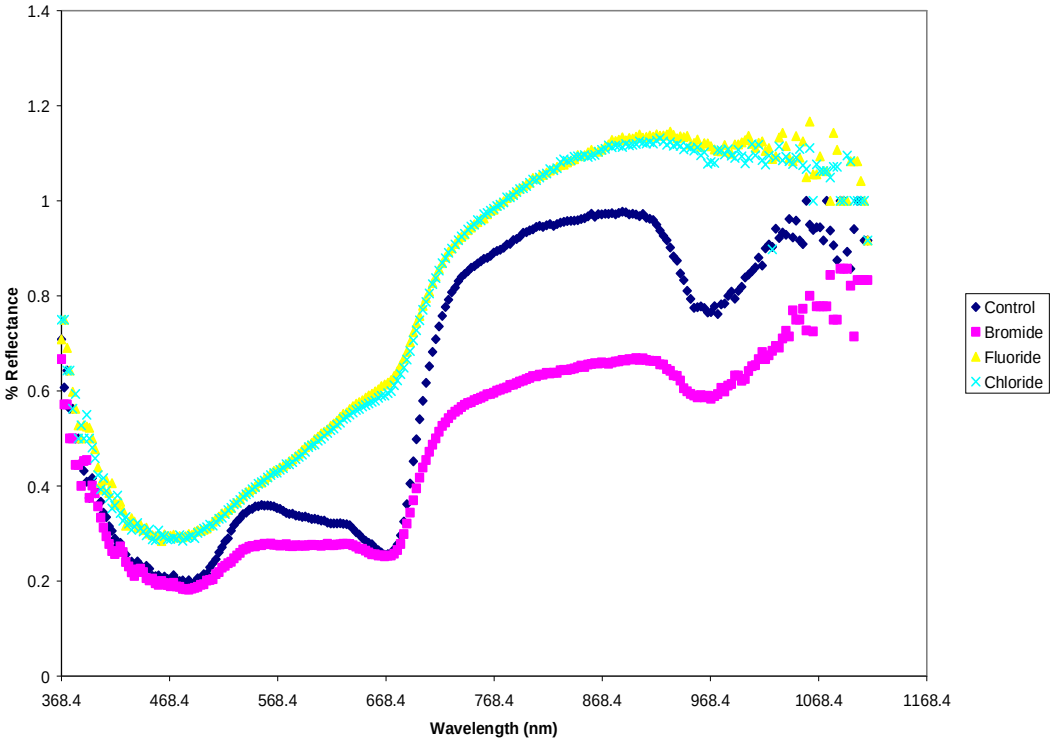
(The treatments from 20 ppm-60 ppm are not shown in this plot to better show variation among the higher treatments. Variation was smaller and less noticeable in the smaller lithium treatments.)



In order to determine if it was the lithium bromide that caused changes in the spectral curves and not the anion bromine, plants were grown in a media containing either lithium fluoride or lithium chloride.

Analysis was performed by comparing the percent reflectance of the treatment scan to the percent reflectance of the control scan at a specific wavelength. The spectral curves showed variation in percent reflectance of the LiCl and LiF compared to the LiBr treated plants (Figure 6).

Figure 6: Spectral Reflectance of *Arabidopsis* Tissue Grown in Growth Media Containing Either LiCl, LiF, or LiBr at 7 ppm



At low levels of Cl^- and F^-, percent reflectance increases steadily from 550 to 850 nm. At low levels of Br^-, percent reflectance also increases from 550 to 850 nm, but the spectrum yields a much lower overall reflectance response (0.2%) than the other two anions. The percent reflectance of the Br^- spectral response indicates that the anion does have a distinct percent reflectance and that lithium bromide can be spectrally distinguished from lithium chloride and lithium fluoride.

This further validates what was shown in Figure 5; that lithium, either by itself or in the bromide form, does produce a change in the cell wall structure of *Arabidopsis* enough to influence its spectral reflectance of NIR radiation, particularly at relatively high concentrations. It should be noted that methamphetamine waste also contains lithium hydroxide which is a corrosive alkali hydroxide that could change the pH of the media and produce changes in the spectral reflectance responses.

Finally t-test analysis, utilized to determine the significance of a treatment against the controls in each of these comparisons: various lithium concentrations versus no lithium; first derivatives of various lithium concentrations; different salts against a Li^+ control; and different anions against a Br^- control, proved these measured treatments to be significantly different from the control 95% of the time.

4. Conclusion and Application of this Research

The use of spectral analysis for the study of locational problems has been around for nearly three decades. Methamphetamine production and abuse has been around for even longer. By combining the evaluation of spectral reflectance with lithium content in vegetation growing on possible methamphetamine dump sites, it provides suggestive evidence of probable cause for a warrant to search for a nearby meth lab.

This investigation showed that certain levels of lithium produced a considerable reduction in reflectance in the near-infrared region compared to a control. While results only indicate a qualitative difference, lithium overall does produce a cellular change in *Arabidopsis* enough to influence its spectral reflectance of near-infrared radiation.

The findings support our hypothesis that lithium, either by itself or in the bromide form, can induce changes in a commonly found weed species that produce discernible differences in its spectral reflectance. This suggests that biochemical and spectral analyses of such a plant could be useful methods for detecting lithium contaminated methamphetamine waste using remote sensing techniques.

Despite the positive implications mentioned above, it is important to note that the spectral data analyzed in this study only provide qualitative indications of relatively high levels of salt being present in the soil, some possibly being in lithium form. Soil in general contains minerals, soluble salts, organic matter, gases and water that contribute ions responsible for salinization, like Na^+, K^+, Ca^{2+}, Mg^{2+}, Li^+ and Cl^-. The small amount of lithium normally found (~ 7- 200 ppm) can produce similar spectral responses as these other soil salt ions. Therefore with this in mind, a spectral response indicative of a high concentration of lithium could also be reflecting any number of other salts present in the plant.

Nevertheless, this methodological approach would allow law enforcement to use aerial/satellite multispectral imagery or field portable spectrometers to examine the surrounding land area of a suspected meth lab in order to obtain probable cause for a warrant. If law enforcement were to suspect that methamphetamine waste was dumped in an area, spectral analysis could verify that sufficiently high levels of a soil salt are present to affect a common weed plant, much in the same way the technique is used to find cannabis vegetation (Gunther, 2005).

Future examination should be done to derive spectral response data for other particular soil salts. It was found that spectral analysis is a useful qualitative method for identifying changes in lithium concentration, but not to detect differences among salts.

Finally, since it is LiOH that is the by-product found in methamphetamine waste, not LiCl, LiF, or LiBr which this study focused on, research should be done with LiOH or actual methamphetamine waste to yield more specific results.

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