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Spectral reflectance as an indicator of foliar concentrations of arsenic in common sunflower (Helianthus annuus)

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SPECTRAL REFLECTANCE AS AN INDICATOR OF FOLIAR CONCENTRATIONS OF ARSENIC IN COMMON SUNFLOWER (*Helianthus annuus*)

A Thesis

by

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ABSTRACT

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Studies were conducted to investigate the use of spectral reflectance by foliage of common sunflower as a potential indicator of arsenic contamination of soil. Germination method was developed for sunflower seeds, and cohorts of sunflower seedlings in hydroponic tanks were established. The cohorts were exposed to 0 ppm, 5 ppm, 7.5 ppm, and 10 ppm treatments of As (V) and reflectance measurements of foliage were collected using a spectroradiometer during two experiments.Results demonstrated the feasibility of using spectral reflectance by foliage of common sunflower as a potential indicator of arsenic contamination. In both experiments, arsenic concentrations in leaf tissues were directly proportional to arsenic concentrations in hydroponic solutions in which such plants were grown. Although the effect(s) of arsenic accumulation had minimal impact on reflectance of visible wavelengths, the effects on NIR reflectance were substantial and resulted in a progressive decrease in reflectance as arsenicconcentrations in foliage increased.

DEDICATION

I dedicate this work to my family; their continuous support and encouragement were decisive to accomplish this goal. They are my strength and inspiration. I thank my husband, Robert for his understanding every time that I had to spend the weekends and afternoons working at the laboratory, to my children, Yuri and Gerardo, who motivated me to continue my academic studies, Rob and Page, and to my parents, Oscar and Yuridia.

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CHAPTER I

INTRODUCTION

Environmental issues are paramount in many scientific, activist, industrial, educational, governmental, political, and community groups. A clearer understanding of the environmental impact and economic implications of using a multitude of products and practices has been the driving force behind recent control and legislation on countless substances, including arsenic. Air, water and soil need to meet stringent safety standards to allow populations of humans and other organisms to thrive without harmful consequences. There is a continuous quest in knowledge and technology to facilitate the monitoring of substances that determine the air, water and soil quality.

Arsenic is listed by the Environmental Protection Agency (EPA) and the World Health Organization (WHO) as a known carcinogen; it is associated with animal and human skin, lung and bladder cancers (Ng et al., 2003). High doses of arsenic can be deadly and it has been reported as the possible cause of death of Napoleon and the American president Zachary Taylor (Feldmann, 2001). However, even lower doses can pose a hazard as scientific research suggests that the health of infants and small children may be at risk by exposure to lower levels of arsenic as result of soil ingestion (Calabrese et al., 1997)

In humans, high levels of arsenic in drinking water can cause hyperkeratinization (thickening) and hyperpigmentation of the skin on the feet, hand and torso (Nahar et al., 2007). As reported by World Health Organization, acute and chronic oral exposure to arsenic has resulted in arsenicosis, with consequent serious damage to the vascular system, including Blackfoot disease (a progressive loss of circulation in the fingers and toes that may lead to gangrene), and cyanosis of fingers and toes. Increased frequency of spontaneous abortions and congenital malformations has been linked to arsenic exposure, and has caused anemia in humans exposed by the oral route. In addition, direct irritations of the gastrointestinal mucosa can occur. In 2007, researchers at the Dartmouth Medical School reported that arsenic is also a potent endocrine disrupter (Davey et al., 2007).

Global attention was recently directed to Bangladesh which appears to be facing the largest mass poisoning of a population in history as result of arsenic contamination in its drinking water supplies. In the 1970's, efforts by the government and international agencies to provide the rural population with adequate water supplies was resolved by obtaining it from groundwater sources. However, in 1993 it was discovered that the water wells were contaminated with naturally occurring inorganic arsenic (Nahar et al., 2007; Ng et al., 2003).

High concentrations of arsenic in groundwater have also been reported in many other countries such as Argentina, Mexico, India, Romania, Lao PRD, China, and Vietnam (Nahar et al., 2007). Arsenic contamination of groundwater is also known to be a problem in United States (Twarakavi and Kaluarachi, 2006).

Arsenic contamination of groundwater not only poses a threat to supplies of drinking water, but also opens the possibility of irrigating agricultural crops with water contaminated with

arsenic. Long term irrigation with arsenic contaminated water is likely to increase arsenic concentrations in crops (ImamulHuq et al., 2003).

As a result of the Bangladesh catastrophe, governmental entities around the world introduced new environmental guidelines to control exposure to arsenic. In March 2001, the EPA Office of Drinking Water set a maximum contaminant level (MCL) for arsenic in drinking water to 10 ppb (0.01 mg/L). On the same note, the Agency for Toxic Substances and Disease Registry (ATSDR), reports that arsenic is listed by EPA as a hazardous air pollutant (HAP), and they have established an emissions limit of inorganic arsenic from copper smelters, glass-manufacturing, and arsenic plants.

The U.S. Food and Drug Administration (FDA) established tolerance levels for arsenic in by-products of animals treated with veterinary drugs that range from 0.5 ppm to 2 ppm. EPA began to phase out household ant poisons containing sodium arsenate in 1989, canceled all registered uses of inorganic arsenic for non-wood preservative purposes, and pressure treated wood with chromium-copper-arsenic (CCA) has been phased out for residential use. Arsenic is second only to lead as the main inorganic contaminant in the original National Priority List (NPL) of Superfund sites (Davis et al. 2001). In addition, arsenic is one of the toxic materials regulated under the Resource Conservation and Recovery Act (RCRA).

Arsenic in the Environment

Elemental arsenic (As) is a steel grey color metalloid that is commonly found in the environment in combination with oxygen, chlorine, or sulfur forming organic and inorganic compounds. The toxicity or arsenic varies between forms, but in general it has been established that the inorganic forms of arsenic are more toxic than the organic ones. Although intensive

research has been conducted in this area, insufficient data are available regarding to the stability of organic arsenicals in soil, whether or not they remain in organic form or are converted to inorganic forms as result of microbes, geochemical condition changes, and other environmental processes (Cullen and Reimer, 1989: Meharg and Hartley-Whitaker 2002).

Arsenic occurs naturally in the environment by release into the air by volcanoes, through weathering of arsenic-containing minerals and ores, and various geothermal processes. It can also be found as a result of industrial uses that include leather and wood treatments (Stilwell, 2002), herbicides and pesticides (Kristen, 2000), some livestock managing practices, manufacturing metals and alloys, transpose to petroleum refining, and burning fossil fuels and wastes (Melamed, 2004).

In the past, the global input of arsenic to soils by human activities was estimated to be between 52,000 -112,000 ton per year (Nriagu, 1994). As result of these activities the arsenic pollution becomes a topic of concern in many geographical areas of the world, including United States. Recent studies in Texas showed that arsenic and associated trace metals were clustered at three physiographic hotspots: the Southern High Plains, the Gulf Costal Plains, and West Texas. According to the investigation conducted by Lee and Herbert (2003), arsenic concentrations in the latter two areas are likely the result of natural sources, whereas high arsenic concentration in the Southern High Plains region probably reflects the extensive use of arsenic agrochemicals (herbicides, pesticides, desiccants) in production of cotton and other agricultural crops.

The Lower Rio Grande Valley (LRGV) encompasses Hidalgo, Starr, Cameron, and Willacy counties in Southern Texas where cotton production has long represented one of the mainstays of the local economy. The extensive use of arsenical compounds as cotton defoliants and desiccants contributed to a legacy of arsenic pollution in the area. The fact that arsenic does

not degrade readily and that arsenical compounds were used extensively for many years suggests that a major arsenic contamination problem may presently exist in the LRGV region although the extent is unknown. As agricultural land in the LRGV is increasingly converted to uses other than agriculture, the potential for human exposure to arsenic has amplified. Effective monitoring and measurement of arsenic in soil and groundwater is critical due to the considerably toxicity and ubiquitous nature of As. As urbanization of the LRGV region continues, the need for regular monitoring at sites where arsenic containing waste has been disposed of and sites where it occurs naturally at elevated levels is expected to become increasingly important.

Current Laboratory and Field Assays to Measure Arsenic

Graphite Furnace Atomic Absorption Spectrophotometry (GF-AAS) has become a routine method for the determination of many trace elements in a variety of sample matrices. Melamed (2004) identifies GF-AAS as an accepted analytical method for measuring arsenic in the environment. The laboratory assay of As based on this method involves pretreatment, either with acidic extraction or acidic oxidation digestion of the sample. During the pretreatment stage all the arsenic in the sample is transferred into an arsenic acid solution, which is subsequently measured as it is deposited in the instrument.

Other current analytical methods to measure arsenic also involve pretreatment, such as Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), Atomic Fluorescence Spectroscopy (AFS), and Hydride Generation Atomic Absorption Spectroscopy (HG-AAS).

Some of the limitations for the use of GF-AAS and other current laboratory assays to quantify arsenic are the requirement of bulky instruments which are expensive to operate and

maintain, and the need of fully equipped laboratories to preserve and operate them. Traditional methods for detection of As soil contamination are based on tedious, expensive and time consuming soil sampling techniques. The effectiveness of the critical long- term need to monitor and measure arsenic in soil and water will be dependent not only of traditional chemical analysis, but on the application of reliable and cost-effective methodologies for its surveillance and detection. These facts have triggered the interest to develop field assays in which lower sensitivities may be acceptable for purposes of site surveys or sample screening.

In a review of technologies and field measurements for arsenic prepared by Melamed (2004), the currently available field assays to measure arsenic include colorimetric test kits, Portable X-ray fluorescence (XRF) equipment, and Anodic Stripping Voltammetry (ASV). One of the advantages of XRF is the possibility to measure arsenic in dry solid samples, such as soil and dry sludge, without the need of previous acidic extraction or acidic oxidation digestion of the sample. These field assays present significant differences in safety, accuracy and reproducibility, and all they require a fixed laboratory analysis of duplicates to verify the performance of the field technology; the main advantage is that field assays can produce inexpensive and large number of screening results in a short time. Moreover, Melamed (2004) lists the biological assays (the use of bacteria and plants) as one of the potential promising technologies for arsenic detection in field investigations.

Unlike other pollutants, arsenic cannot be transformed into a non-toxic material; it can only be transformed into a form that is less toxic when exposed to living organisms in the environment. The metabolic transformations capable of converting arsenic into mobile forms can affect plants that are growing on high arsenic level conditions; therefore, vegetation changes can be a possible indication of arsenic contaminated soils.

Wild Sunflower as an Indicator Plant

Wild sunflower (*Helianthus sp.)* belongs to the family Asteraceae. Is a native annual and one of the common plant species found growing in areas that were previously used for agricultural purposes in the region of the LRGV. Wild sunflower has tolerance to salinity, has low nutrient requirements, and plants are able to adapt to different soil textures. Furthermore, Raab et al., (2005) reported that arsenite and arsenate are taken up readily by sunflower. These characteristics make wild sunflower a good candidate as a biological indicator of arsenic contamination of soil.

Wild sunflower (*Helianthus annuus*) grows up to 9 feet in height, and produces yellow blooms during most of the summer period. It is also one of the most diverse of the sunflower species in terms of geography and habitat (Everitt et al. 1999) and has been used in several investigations to improve the cultivated sunflower *(Helianthus annuus* L.) also referred to as the common sunflower. It is also reported that germination of this species is inhibited by strong dormancy systems (Chandler and Jan, 1985; Maiti, 2006).

Seed dormancy is a survival mechanism acquired during evolution that allows seeds to postpone germination until favorable growth conditions occur. Some species can produce seeds with different degrees of dormancy in the same year or produce seeds that can have long term dormancy in order to create a seed bank that will allow the species to survive long periods under adverse conditions (Baskin and Baskin, 1985). Factors that influence dormancy can be classified as exogenous and endogenous. Exogenous factors are associated with the seed coat permeability to water, and sometimes are also referred as seed coat dormancy (Maiti, 2006); endogenous factors are those to relate to the germination ability of the embryo.

Seed dormancy in *H. annuus* appears to be primarily related to exogenous factors (Hernandez and Orioli, 1985). The seed coat imposed dormancy can be overcome by methods that include soaking the seed in water, scarification and embryo excision. Thus, in the preliminary stages of this study, we developed a practical method to facilitate and standardize the germination of field-collected sunflower seeds (discussion to follow).

Biochemical Dynamics of Arsenic Uptake by Plants

A high arsenic level in the soils represents a reason for concern in relation to plant uptake and consequent access way into wildlife, livestock and human food chains. The mechanism in which arsenic is taken up from the soil and can be incorporated into the plant is a key concept in the evaluation of risk caused by arsenic contaminated soils.

During the past several decades extensive research has been conducted in both, soil and hydroponic conditions, to understand the physiological and biochemical mechanisms that govern the arsenic uptake by plants (Marin et al., 1993; Thornton, 1994; Onker and Hosser, 1995; Carbonel-Barrachina et al., 1995; Stilwell, 2002; Tu et al., 2003; Wang et al., 2002; Raab et al., 2005; Duester et al., 2010).

Much progress has been made to understand the toxicity of arsenic, and the dynamics of how arsenic is taken up and metabolized within the plant. Existing information in this area suggests that the major parameters affecting the level or arsenic in plant tissue are dependent of the type of plant (Onker and Hosser, 1995; Carbonell-Barrachina et al., 1995; Stilwell, 2002; Raab et al., 2005; Meharg and Hartley-Whitaker, 2002), the part of the plant root vs. shoot (Tu et al., 2002; Ma et al., 2001; Raab et al.,2005), the concentration and form of arsenic in the solution (Raab et al.,2005; Meharg and Harley-Witaker, 2002), the amount of organic matter present in

soil (Stilwell, 2002), and the amount of iron oxides and phosphorus added to solution or to soil (Meharg and Macnair, 1992).

The plant uptake of arsenic growing in contaminated soils was researched on vegetables by Thornton (1994), who reported differences between plant species and amounts of iron and phosphorus present in soil. Other reports also confirm the relation between the levels of arsenic in plant tissue and different plant species (Onken and Hossner, 1995; Meharg and Hartley-Whitaker, 2002; Stilwell, 2002). In relation to the present study, sunflower plants have been reported to uptake arsenic in both arsenate and arsenite species (Raab et al., 2005).

At the present time, it is well established that plants vary in their sensitivity or resistance to arsenic. A wide range of plant species have been identified growing on arsenic contaminated land, and it appears that the adaptive resistance usually involves alteration of arsenic transport mechanism (Bleeker et al., 2003; Meharg and Hartley-Whitaker, 2002). Despite these considerations, the recent discovery of the As-hyperaccumulating Brake fern *(Pteris vittata)* is exceptional, since *P. vittata* can metabolize, transport and store high levels of arsenic mainly in the fronds (Ma et al., 2001). This exceptional ability of some plants to hyperaccumulate arsenic in their fronds is opening the possibility of using certain fern species in phytoremediation, a multidisciplinary approach where plants are used to extract metal contaminants during the process of environmental restoration (Ma et al., 2001; Pickering et al., 2000; Tu et al., 2002).

Arsenic localization within the plants has been the subject of numerous studies. For example, Monni (2002) reported that old stems collected from trees (*Emperum nigurm)* growing in polluted areas contained arsenic in the primary cell walls of vascular tissues, especially in the living ray cells of phloem where arsenic concentration was higher than tissues of dead cells (xylem and sclereids). In the same context, Meharg and Macnair (1992) noted that when a toxic

element like arsenic is absorbed by plants, the plant will most likely limit translocation, resulting in accumulation primarily in the roots. Similar trends are reported by Burlo et al. (1999), in a study on the effect and accumulation of arsenic by tomato plants, which resulted in higher accumulation in roots and less translocation to shoots and fruits. Comparable results also have been reported in an experiment using sunflower plants, (*H. annuus)* plants took As quite readily, whether it was presented as arsenite or arsenate, resulting on higher accumulations detected in roots, followed by stems, and then leaves (Raab et al.,2005).

Since the toxicity of arsenic is dependent of its chemical form, it is critical to understand the interactions between the physiological plant processes and the toxic dose response. Arsenic is a non-essential element for plants, which interferes with metabolic processes in most plants at high concentrations and thus inhibits plant growth and development (Marin et al., 1993); decrease in plant height (Marin el al., 1993), causes a decrease in tillering (Rahman et al., 2004); reductions in yield (Carbonell-Barrachina et al., 1995; Rahman et al., 2007); reduction in root growth (Aberdin and Meharg, 2002), and even death (Marin et al., 1992) as obvious manifestation of arsenic induced phytotoxicity.

Plants deal with arsenic in various ways. One of the mechanisms used by many plants in response to high levels of arsenic is by reducing its uptake (Bleeker et al., 2003; Meharg and Hartley-Whitaker, 2002). However, the Chinese fern (*P. vittata)* resists arsenic by hyperaccumulating arsenic in its tissues and exhibits outstanding capabilities to extract low levels of arsenate from soil (Ma et al., 2001). Arsenic resistant plants that utilize the pathway to decrease arsenic uptake can still accumulate considerable amounts of arsenic in their tissues. Meharg, (1994) suggests that the arsenic resistant plants either compartmentalize and/or transform arsenic to less toxic species in order to withstand high cellular arsenic loads.

Inorganic arsenic species are generally more toxic than organic ones. The two phytoavailable forms of arsenic are arsenate (AsV) and arsenite (AsIII), which is the most toxic form. Less abundant methylated arsenic species, like dimethylarsinic acid (DMA) and monomethyl acid (MMA), are also found in soils and can also be taken up by plants (Marin et al., 1993; Abedin et al., 2002). In a short term study to investigate how arsenite, arsenate, dimethylarsinic acid, and momomethylarsonic acid were accumulated by eight rice (*Oryza sativa)* varieties in two growing seasons (wet and dry). Abedin et al, (2002) reported that all arsenic species were uptaken, although differences were significant between plant and arsenic species. Similar relations between the arsenate, arsenite and dimethylarsinc acid are reported by Mohamed and Meharg (2008) in relation to species uptake and toxicity in maize roots (*Zea mays L.),* the latter study suggested lower influx and toxicity of DMA compared to arsenate and arsenite. In both investigations, arsenate uptake decreased in the presence of non-phosphate starved plants, there was little or no increase of arsenite in the presence of phosphorus, and in the case of maize plants, DMA influx decreased 90 % in the presence of phosphorus. The affinity between arsenate and phosphate for the same transport mechanism is accountable for the As/P relation, which be discussed in a separate section. In the case of DMA, the uptake mechanism has not been confirmed in the literature at the present time.

The fact that arsenic can be found in the environment as organic and inorganic compounds, and that it can convert between different forms represents a complicated situation to determine the specific chemical forms present, a procedure known as arsenic speciation, and consequent evaluation of toxicity level (Tu et al., 2003; Wang, et al., 2002).

Arsenic speciation in determination of toxicity levels is evident as organic arsenic species are generally considered to be less toxic than inorganic species to a wide range of organisms,

including terrestrial and aquatic plants, animals and humans (Meharg and Harley-Witaker, 2002). However, another investigation on comparative phytotoxicity levels of three arsenic species (monomethylarsonousdiodide –MMAsIII, sodium arsenite, and sodium arsenate) on two aquatic plants and one algae reports the highest overall toxicity shown by MMAsIII, an organic form of arsenic, followed by the inorganic AsIII form (Duester et al.,2010).

As new studies have been conducted to understand how arsenic is metabolized in plants, it is important to note that some existing investigations do not consider chelation as the arsenic is characterized from extraction of plants tissue (Meharg and Hartley-Whitaker, 2002). Plants produce phytochelatins (PC's) in response to exposure to high metals concentrations, which suggests the possibility that under certain pH extraction conditions, the arsenic form characterized is different from the one that originated from the plant. The time dependent formation of arsenic-phytochelatin (As-PC) complexes on common sunflower (*H. annuus)* has been investigated by Raab et al.,(2005) who found that plants took up As quite readily, whether it was presented as arsenite or arsenate. However, different As species had an impact on the formation of As-PC complex in the leaf, but not in the root or stem. As a consequence, the formation of arsenic- phytochelatin (As-PC) complex seems to be critical parameter to be studied when As toxicity values are to be estimated.

The predominant form of arsenic in aerobic soils is arsenate, and due to its analogy to phosphate competes for the same transport mechanism in plants (Wang et al., 2002; Meharg and Hartley-Whitaker, 2002). Interactions of arsenate and phosphate at different concentrations have reported that increased phosphate supply decreased arsenic uptake markedly. Non resistant plants can be made more resistant to arsenate at higher phosphorus levels (Wang et al., 2002; Thornton, 1994; Mehar and Macnair, 1992). A well known mechanism to resist high levels of arsenate

employed by many plant species is achieved by reducing the uptake of arsenate through a suppression of the high-affinity phosphate/arsenate uptake system in the resistant plants. Furthermore, unlike arsenate uptake, neither phosphorus starvation nor the presence of phosphate affected arsenite uptake, presumable to be processed by the enzyme systems in plants as an analogue of nitrogen (Wang et al., 2002; Meharg and Hartley-Whitaker, 2002).

Merharg and Harley-Witaker (2002), also addressed the importance of considering the role of mycorrhizal association when evaluating the phosphate/arsenate interaction. One of the principal roles of mycorrhizal fungi in association is obtaining phosphorus for their plant hosts, which may result in enhanced arsenate acquisition from contaminated soils. Soil conditions play a critical role in the process of plant absorption of inorganic components, including arsenic. In order for the plant to uptake the components from the soil these need to be in solution, not bound to the soil (Meharg and Hartley-Whitaker, 2002). Moreover, if the solubility of these components is too high they could rapidly percolate away from the root area. Tu and Ma (2002) report that the low solubility of $FeAsO_4$ and $AlAsO_4$ was the rationale for the arsenic uptake deficit found on ladder brake (*Pteris vittata)* as different concentrations of organic and inorganic forms of arsenic added to soils were evaluated.

Arsenic forms inorganic and organic complexes in the environment, and considering that plants can access inorganic arsenic in form or arsenate (As V) or arsenite (As III) when in soil solution, is necessary to recognize that microbial transformation can methylate and demethylate arsenic species present in soils, transforming inorganic forms of arsenic into organic forms and vice versa (Turpeinen et. al., 1999). Additional factors to consider in relation to soil parameters are pH and redox conditions, since in soil-based studies, pH and redox conditions suggest an effect on the availability and consequent phytotoxicity of inorganic and organic arsenic species

(Marin et al., 1993). Arsenate species are predominant at moderate and high redox potentials, while arsenite species occur under more reducing conditions (Meharg and Hartley-Whitaker, 2002). This would suggest that soil parameters influence the toxicity of arsenic species due to altered availability.

Hydroponics is a technology for growing plants in nutrient solutions with or without the use of an artificial medium to provide mechanical support. It has been used in numerous laboratory investigations and commercial agricultural operations with the objective to control the aerial and root environment of the plants. The selection of hydroponic systems for the purpose of the present study offers the possibility to control adverse climate, air and root temperature, light, water, and plant nutrition, as the arsenic accumulation on foliage of sunflower is investigated and minimize the effects due to other factors.

Foliar structural changes, such as breakdown of chloroplast and decline in chlorophyll production appear as result of increased metal accumulation (Barcelo et al., 1988); metal accumulation also has an effect on the reduction in size of mesophyll cells (Zhao et al., 2000). Furthermore, Rahman et al., (2007) in a recent study focus on the effect of arsenic on photosynthesis and the effects on photosynthetic pigments, chlorophyll-a and chlorophyll-b, and their correlation with yield and growth, suggest that the reduction of growth and yield is the result of reduced chlorophyll content in rice leaf due to arsenic toxicity.

The main photosynthetic pigment in green plants is chlorophyll-a. It absorbs blue and red light to make it enter an excited state, and therefore appears as green as green wavelengths are reflected. The difference in the composition of chlorophyll-a and chlorophyll-b is a side chain radical (in *a* it is $-CH_3$, in *b* it is CHO) and both are effective photoreceptors. The absorption maxima of chlorophyll-a are wavelengths 430 nm and 662 nm that of chlorophyll-b are lambda

of 453 nm and 642 nm, which correspond to the blue and red areas; the common green peak appears at 550 nm (Campbell, 2007).

Photosynthetic pigments, water content of plants and leaf internal structure govern plant reflectance (Penuelas and Filella, 1998; Campbell, 2007). Spectral reflectance measurements have been used to identify and assess the status of plant species, many of which exhibit detectable changes in reflectance of electromagnetic energy in response to various stress factors; such as diseases (Summy et al., 2005 and Campbell, 2007), moisture shortage or maturity changes (Campbell, 2007), and metal pollution (Maruthi et al., 2007).

Potential Use of Remote Sensing Techniques

Remote sensing has been defined as " the practice of deriving information about the Earth's land and water surfaces using images acquired from an overhead perspective, by employing electromagnetic radiation in one or more regions of the electromagnetic spectrum, reflected or emitted for the Earth's surface" (Campbell, 2007). Remote sensing techniques are commonly used to monitor the physiological condition of native, exotic and agricultural crops (Campbell, 2007; Jensen, 2007; Lillesand et al., 2004; Summy, 2005).

Spectroscopy, the field of science devoted to the detailed examination of spectral data, is a fundamental component of remote sensing research. Instruments used in spectroscopy are designed to collect radiation and to divide it into spectral regions that are then measured electronically (Campbell, 2007). The field spectroradiometer, one of the instruments used in spectroscopy, consists of a measuring unit with a probe connected to it by a fiber optic cable. This measuring unit is made of an array of photosensitive detectors, with filters or diffraction gratings to separate radiation into several spectral regions, and subsequently projected onto

detectors. Spectral measurements are often reported as reflectance measured in terms of radiance but more recent software is designed to convert radiance measurements to present reflectance in real time to facilitate comparison with other data (Campbell, 2007). Typical spectroradiometers are sensitive to electromagnetic radiation (EMR) in the ultraviolet (300 - 350nm) visible (400-700nm) and near-infrared (700 or 800 nm -1,100 nm) and may also measure reflectance in the mid-infrared region (1,300-3,000 nm).

The amount of leaf reflectance in different areas of the spectrum is affected at distinctive regions of the spectra depending of photosynthetic pigments, leaf internal structure and water content. Changes in visible reflectance are indication of changes in leaf pigmentation and /or changes in photosynthesis. Stress factors that reduce photosynthetic efficiency are commonly correlated with increases in reflectance (or conversely, of reduction in absorption) of blue and red wavelengths. Thus an increase in red to levels of green gives perception of yellow and consequential chlorosis. The near infrared (NIR) region between 700 nm and 1300 nm is particularly useful to assess the status of vegetation as spectral reflectance is controlled by internal structure of the leaves, more specifically by the structure of the spongy mesopyll tissue (Campbell, 2007), and involves wavelengths which are not detectable by the human visual system. Barcelo et al., (1988) reports foliar structural changes, such as breakdown of chloroplast and decline in chlorophyll production as result of increased metal accumulation. Moreover, metal accumulation also has an effect on the reduction in size of mesophyll cells (Zhao et al., 2000). In the case in which the stress factor has not yet disrupted photosynthesis, but has altered the structure or configuration of the air spaces in the spongy mesopyll tissue, reflectance changes in the NIR area may be present and visible symptoms could be minimal and difficult to distinguish or entirely absent. In the former situation, spectroradiometer measurements or other types of

imagery acquired by remote sensing may be particularly valuable, as they provide the means to detect stress factors before visible symptoms become evident.

Vegetation canopies are composed of many separate leaves that may vary in their size, orientation, shape, and coverage of the ground surface. Shadowing tends to decrease canopy reflectance below the values normally observed in the laboratory for individual leaves; this effect is the result of available energy for reflection from soil or lower leaf layers (Campbell, 2007).

Spectral measurements obtained under laboratory conditions may involve plant handling. Although the acquisition of spectral measurements of foliage attached to plants tends to minimize physiological stress caused by handling, other serious problems become apparent. Under given lighting conditions, slight changes in leaf orientation may result in significant changes in both visible and NIR wavelengths. Moreover, superimposing of foliage of plants may result in a substantial increase of NIR reflectance relative to what will be obtained with the spectral readings of single leaves of plants.

In order to facilitate spectral measurements of foliage in the laboratory, variations on foliar spectral properties had been investigated in relation to handling techniques after leaf clipping (Foley et al., 2006). Maintaining water content within the samples is known to be an important factor to consider, since the changes in water levels can affect absorption in the visible and NIR areas as leaves are drying. After assessing the time constraints between leaf collection and spectral measurements of leaves wrapped with moist gauze around their petiole, Foley et al. (2006), suggests that no common time limit could be observed for leaf clipping and reflectance measurements hence measurement time constraints are dependent on the properties of the leaf or species. Therefore the purpose of the present study on sunflower, determination of the optimal time to obtain spectral measurements was determined (discussion to follow).

The possibility to minimize or correct the effects on leaf spectral measurements accounted for other factors rather than the arsenic will increase our ability to compare reflectance signatures of foliage subjected to different treatments in a controlled experimental environment.

The efficient application of remote sensing techniques supported by traditional laboratory analysis to evaluate the potential use of spectral signatures to monitor the effect of arsenic accumulation in foliage of wild sunflower (*H. annuus)* may well provide an effective adjunct to conventional chemical analysis for detection of arsenic contaminated soil in south Texas and other regions.

Objectives

The overall objective of this investigation was to evaluate the use of wild sunflower (*Helianthus annuus)* as potential indicator plant for arsenic contamination, and the use of remote sensing techniques as adjunct to conventional chemical bioassays.

Specific objectives include:

- Investigate and develop a practical method to facilitate germination of field-collected sunflower seeds, and establish cohorts of sunflower seedlings in hydroponic tanks in the laboratory.
- Evaluate the effects of leaf excision and various background materials on spectral reflectance of sunflower leaves under artificial lighting.
- Develop spectral profiles for foliage of common sunflower plants exposed to selected levels of arsenic in hydroponic solution.
- Evaluate the concentration of arsenic content on foliage with graphite furnace atomic absorption analysis (GF-AAS).

CHAPTER II

MATERIALS AND METHODS

Germination of Sunflower Seeds

The seeds of common sunflowers (*Helianthus annuus*) were field collected from several areas in Hidalgo County, TX. and mixed to avoid differences due to collection location. Then, a practical method to break the dormancy of the seeds was investigated. At first a standardized germination test in Petri dishes was conducted by placing approximately 80 seeds per Petri dish in four replications. This group of seeds was used as a control. The remaining seeds were soaked overnight for a period of approximately 15 hrs as recommended by Maiti et al. (2006) in unsterilized distilled water, designating this procedure as soaking treatment. Independently of other treatments, and in order to avoid disease infestation, in a laminar flow hood all seeds were treated with a solution of 70% Ethyl alcohol and distilled water for 5 minutes followed by immersion in a solution of a commercial hypochlorite solution diluted at 50% (v/v) with deionized distilled water for 10 minutes. Alternation of three rinses with sterilized distilled water followed the application of each solution. This was established as the disinfecting treatment.

The efficacy of a solution of Hydrochloric acid (HCl) and distilled water as a dormancy breaking agent was also evaluated. Two different concentrations (1.0 M HCl and 0.1 M HCl) were applied to the seeds for a period of 25 minutes and 55 minutes followed by three rinses with distilled water prior to the time disinfecting treatment were tested. These groups of sunflower seeds were designated as chemical treatments.

To evaluate the germination activity of the sunflower seeds, the control group and treatments were placed in Petri dishes with a sterilized filter paper located at the bottom of each dish. Approximately 80 seeds were paced within each Petri dish and sterilized distilled water was used to dampen the filter paper. The Petri dishes were covered with the lids and sealed with parafilm. All treatments were replicated four times.

Petri dishes were placed in randomized order under a 14 hr light/ dark cycle at a temperature of 20° C in the laboratory. Seeds germinating by the 7^{th}day and total number of seeds germinating prior to 15^{th} day were counted and recorded. The seedling emergence data was analyzed using one-way analysis of variance (ANOVA) and means were separated using Tukey pairwise means comparison test (Sokal and Rohlf, 2000). Statistical tests were summarized by reporting the F-value, degrees of freedom for treatments and error, and the *P-value*. The F-value in the ANOVA indicates the occurrence (or lack of occurrence) of one or more differences among treatment means, and the range separation test (for example, Tukey pairwise comparison test) indicates where actual differences occur. Both are based on degrees of freedom (number of values that are free to vary) and provide a *P-value* that indicates the evidence that we have against the null hypothesis (no change).

Plant Culture and Arsenic Treatments

After seedlings reached the 2- 3 leaf stage, the plants were transferred to a hydroponic system and randomly separated in four groups. The hydroponics systems consisted of 8 liter black plastic tubs placed in racks with a continuous aeration pump unit connected to them, and a Styrofoam® floater cover with equidistant holes to support the plants. Light source continued to

be provided in a 14 hr light/ 10 dark cycle with artificial florescent bulbs, which are adequate for plant growth, hanging from the racks.

The chemicals reagent grade used during the experiment were purchased from (Sigma Chemical Co., St Louis, MO.). The seedlings were supplemented with 8 liters of Hoagland's nutrient solution (Hoagland and Arnon, 1950) modified with addition of iron (Misra and Sharma, 2006) and placed in the hydroponic tubs. Aluminum foil was placed around the floater edges to keep light out of the hydroponic solution to prevent algae growth. The temperature in the laboratory was 20ºC.

About two weeks after transplantation, when seedlings reached the 5 to 6 leaves stage, the plants were thinned out before the application of treatments and a minimum of five plants of similar growth stage were placed in a uniform distribution pattern on the floaters and kept in the tubs. One control group of 0 ppm, and three arsenic treatment groups corresponding to 5 ppm and 7.5 ppm and 10 ppm in form of K_2HAsO_4 solution were supplied to the plants in 8 liters of the nutrient solution. The potassium salt of As (V) was selected for solubility and to prevent possible sodium harm to plants. All treatments were carried out using five plants and arranged in randomized order in the racks. The arsenic treatments were supplemented with nutrient solution to avoid any nutrient deficiencies. The experiment was repeated in a second trial.

Spectral Reflectance Measurements

Spectral reflectance measurements were collected using a Fieldspec Pro® VNIR spectroradiometer from Analytical Spectral Devices Inc. (Boulder, CO, USA) with a sensitivity range to EMR extending from the ultraviolet (350nm) through the near-infrared region (110nm). Measurements were made using 1º and 18º field-of- view (FOW) target probes and a remote

cosine receptor (RCR) to measure incident radiation. Prior to each measurement, a "white reference" measurement was collected from a Spectralon® panel (ASD, Boulder CO, USA) to facilitate conversion of radiance measurements to real time measurements of percent reflectance.

Reflectance data and spectral profiles for wavelength regions (Blue, Green, Red and NIR) values were recorded for selected wavelengths in the blue (460nm), green (550nm), red (680nm) and NIR (850nm). Data were analyzed using ANOVA and means were separated using Tukey pairwise comparison test.

A previous evaluation of different materials to select a suitable background to be used during the collections of spectral measurements of leaves was investigated. The intensity and effects of background reflectance measurements by three candidate materials (two different black cloths and the black standard laboratory tabletop) and of sunflower foliage placed on each of these substrates were obtained. The same equipment and experimental set-up described previously was using during the investigation.

In order to optimize experimental comparison and facilitate the collection of spectral measurements from excised (detached) sunflower leaves, the effects of excision were also evaluated. Before the arsenic supplementation, spectral curves for individual sunflower leaves attached to plants were obtained, immediately after excision (0 min), 1 min, 2 min, and up to 5 minutes following excision.

Once that the leaf handling techniques were justified, leaves from three plants of each treatment and control were collected for spectral measurements and placed in 25 ml. beaker containing enough distilled water to cover the petriole. Then, the leaves were immediately transported to the area that was set to collect spectral information in the same laboratory. Each leaf was gently placed flat on top of the non-reflective background as a precautionary measure to

minimize changes in spectral amplitudes due to curvature of the leaf surface. Measurement lasted a few seconds, as the spectroradiometer reported a reading derived from the average of a set of 10 measurements. This procedure was followed weekly throughout the duration of the experiment1 and experiment 2.

Chemical Analysis

As we are interested in direct relationships between arsenic content and leaf spectral reflectance, the same excised leaves used to collect spectra measurements were air-dried and preserved for chemical analysis. The dried leaves were ground and accurately weighted in triplicate. Plant samples were digested using concentrated HNO_3 (Fisher Scientific, Houston, TX, USA). Digestion was performed on a hot plate until a clear digest was obtained. The digested solution was analyzed for As using graphite furnace atomic absorption spectrometry (GF-AAS). Standards for calibration of the instrument were prepared by diluting a 1000 mg/L As stock solution to make 10, 50, and 100 ppb As standard. All the digestions and GF-AAS measurements were carried out in triplicate. The parameters used for GF-AAS analysis are presented in Table 1. In addition, before analysis the sensitivity of the instrument was tested and found to be within +/- 20% of 40.0 pg/0.0044 As. The sensitivity check was performed by injecting 20µl of a 50µg/L solution of As and obtaining a signal of 0.11 As +/- 20%. The GF-AAS data analysis was performed using the Standard Error for the three replicate samples. Arsenic concentrations in foliage were analyzed using Analysis of Variance (ANOVA) and means were separated using Tukey pairwise comparisons test (Sokal and Rohlf, 2000). In several cases, a combination of spectral reflectance data and data relating to arsenic concentrations in foliage were analyzed using the nonparametric Kruskal-Wallis test (Sokal and Rohlf, 2000).

Table 1. Graphite Furnace Atomic Absorption Spectophotometry (GF-AAS) operating parameters used for the determination of As in sunflower digestions.

Parameter	Predry	Avg.	Ash	Atomization	Clean out
Temp (ºC)	110	130	1200	2000	2450
Ramp time (s)	1	5	10	0	1
Hold time (s)	20	30	20	5	2
Argon flow (ml/min)	250	250	250	0	250

Note: Other parameters were as follows: slit 0.7 low, matrix analysis $Pd(NO_3)_2$ 0.005mg + 0.003 mg $Mg(NO_3)_2$, λ=193.7 nm, and Zeeman background correction.

CHAPTER III

RESULTS

Seed Germination

During the preliminary stages of the project, a method for the germination of the field-collected sunflower seeds was developed following procedures described in Materials and Methods. Germination success was lowest in the untreated control and the group subjected to the soaking (15h) + disinfecting treatment (1.2% and 2.6% respectively) (Table 2). Germination activity improved when seeds were treated with HCl for 20 min, although no differences were detected among groups treated with 0.1 M and 1M solutions of HCl (13.7% and 13.5%). However, extending the treatment time to 55 min resulted in a remarkable increase in germination success of seeds treated with both concentrations of HCl (78.2% and 65.9% at concentrations of 0.1M and 1M, respectively). Based on the results of this study; the latter treatment was selected as the germination method for the sunflower plants propagation.

Table 2. Germination activity of wild sunflower seeds *(Helianthus annuus)* in response to different treatments.

Treatment	**Avg. Number of seeds**	**Avg. No. of emerged seedlings on 7th day***	**Avg. No. of emerged seedlings on 14th day***	**Avg. Total germination (%)***
Control	85	0a	1a	1.2 a
Soaking (15h) + Disinfecting	86	1a	3a	2.6 a
Soaking (15h) + Disinfecting + 0.1 M HCl (20min)	89	6b	14b	13.7b
Soaking (15h) + Disinfecting + 1 M HCl (20min)	89	7b	12b	13.5b
Soaking (15h) + Disinfecting + 0.1 M HCl (55min)	87	51d	75d	87.2d
Soaking (15h) + Disinfecting + 1 M HCl (55min)	82	37c	56c	65.9c

*Means within columns followed by the same letter are not significantly different at 5% probability level (analyzed by ANOVA; means separated by Tukey pairwise comparison test).

Effects of Background

Spectral reflectance measurements for three candidate background materials (two different types of black cloth and the black standard laboratory tabletop) are shown in Figs. 1 and 2. The standard laboratory table top and one type of black cloth were strong absorbers of both visible and NIR wavelengths, that is, they reflected essentially no EMR in either of these waveband regions, whereas the second type of black cloth absorbed visible wavelengths (400 – 800 nm) but exhibited an exponential increase in NIR reflectance (700 – 900 nm). As result of this effect, spectral reflectance measurements for foliage placed on the standard laboratory tabletop and on the NIR- absorbing black cloth were accurate in both the visible and NIR regions of the spectrum (Fig. 2). In contrast, spectral measurements for foliage placed on the second type of black cloth (NIR-reflecting) were accurate in the visible region, but were greatly magnified (and erroneous) in the NIR region (Fig. 3). Thus, the acquisition of accurate spectral measurements for foliage placed on a background substrate is dependent on the selection of a NIR-absorbing (rather than a NIR-reflecting) substrate.

Effects of Leaf Excision

Another important consideration in the acquisition of spectral measurements is the effect(s) of leaf excision on reflectance of visible and NIR wavelengths during a given period following detachment from plants. Spectral reflectance of plants used in this study were similar in all wavebands prior to excision, and showed no major changes in reflectance from the time leaves were excised (0 min) to 5 min following detachment as long as petioles were kept moist (Figs. 4 and 5). This time interval was deemed sufficient to collect spectroradiometer measurements and was used during the remainder of the study.

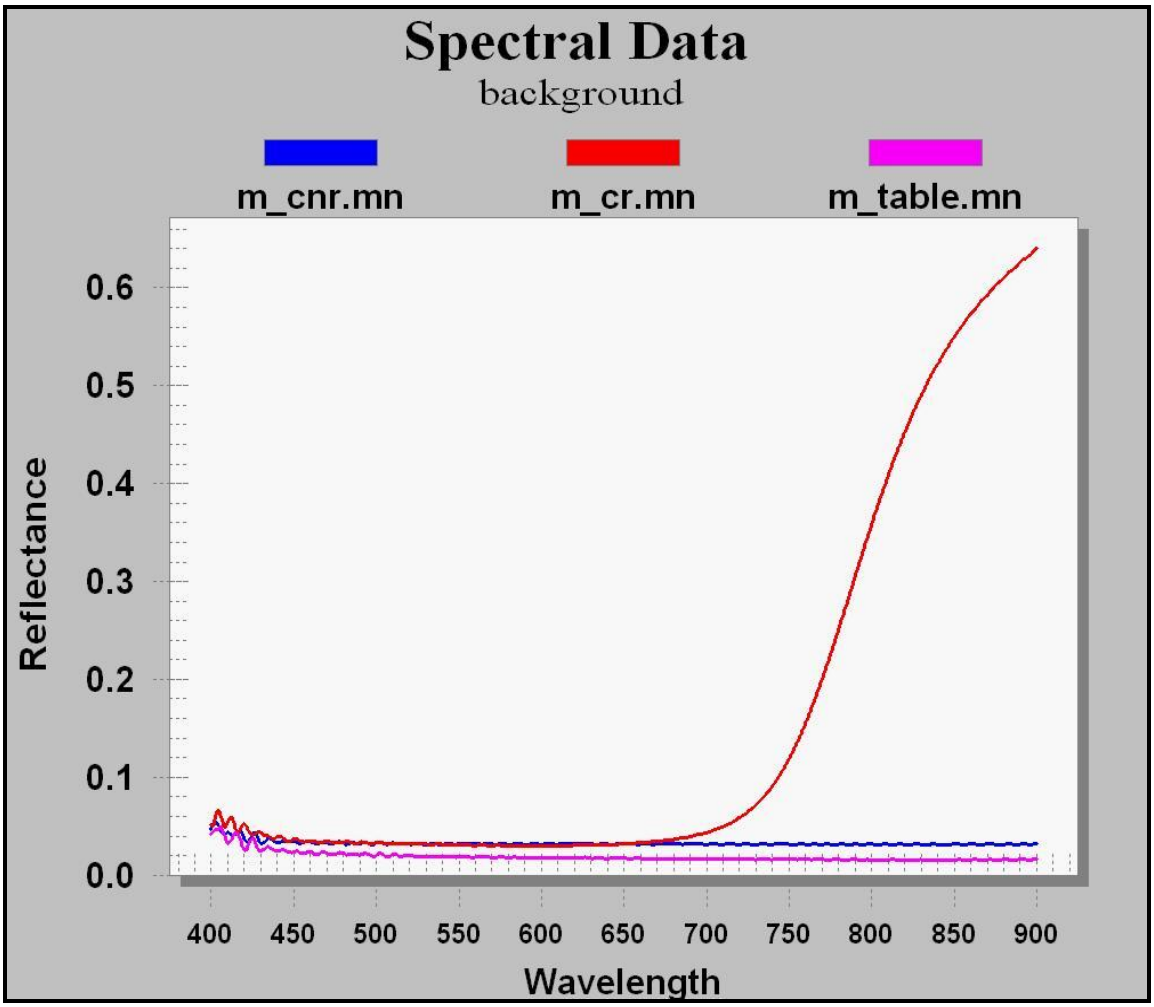

Figure 1. Mean spectra reflectance curves by three different black materials. The curve represent the mean of standard laboratory table top (magenta, m_table), NIR absorbing cloth (blue, m_cnr), and NIR reflecting cloth (red, m_cr).

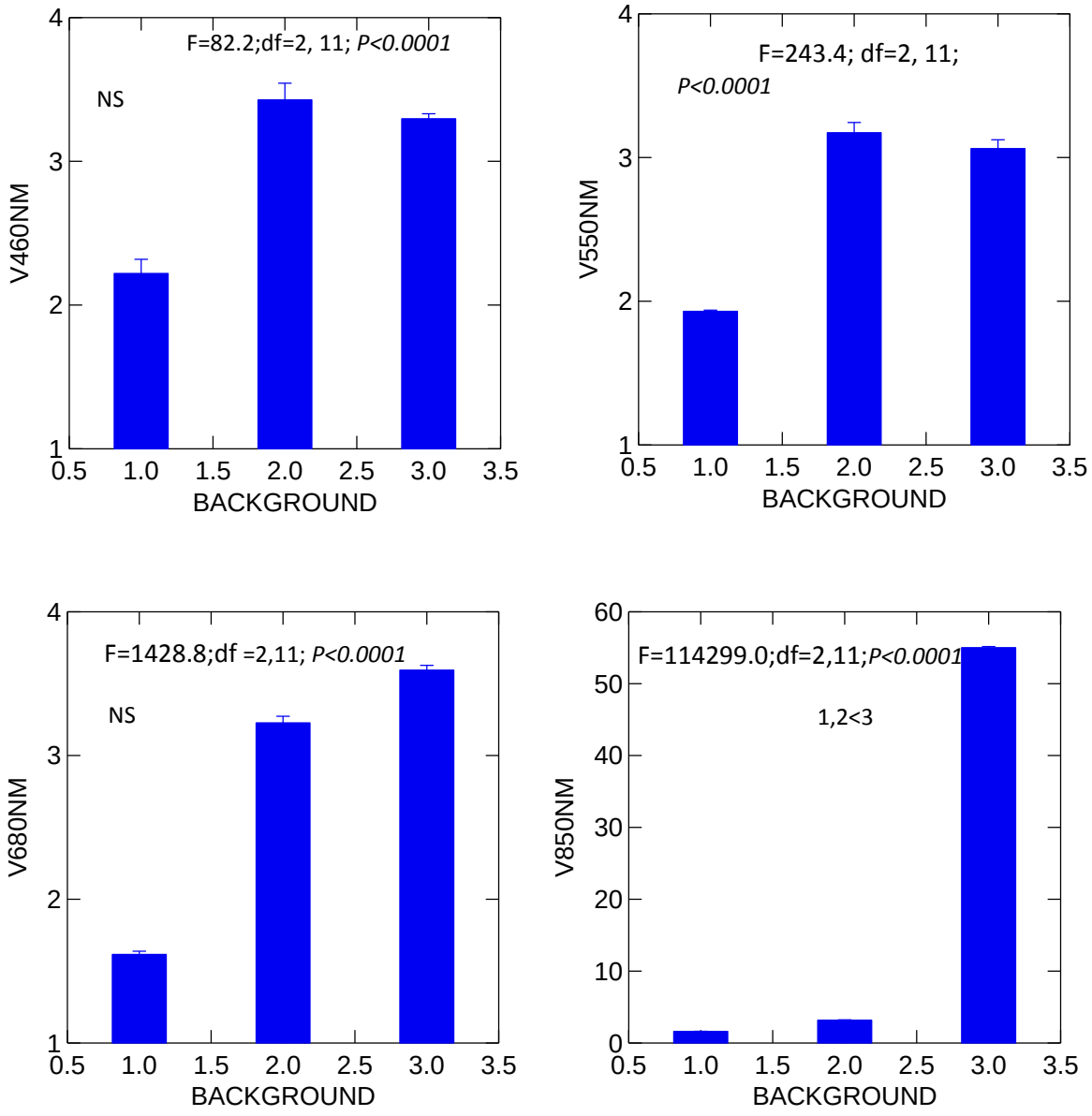

Figure 2. Spectral reflectance measurements for three different backgrounds. 1.0 laboratory table, 2.0 black non-reflecting cloth and, 3.0 black reflecting cloth at (460 nm), green (550nm), red (680 nm) and near-infrared (850 nm) regions of the spectrum; 2009.

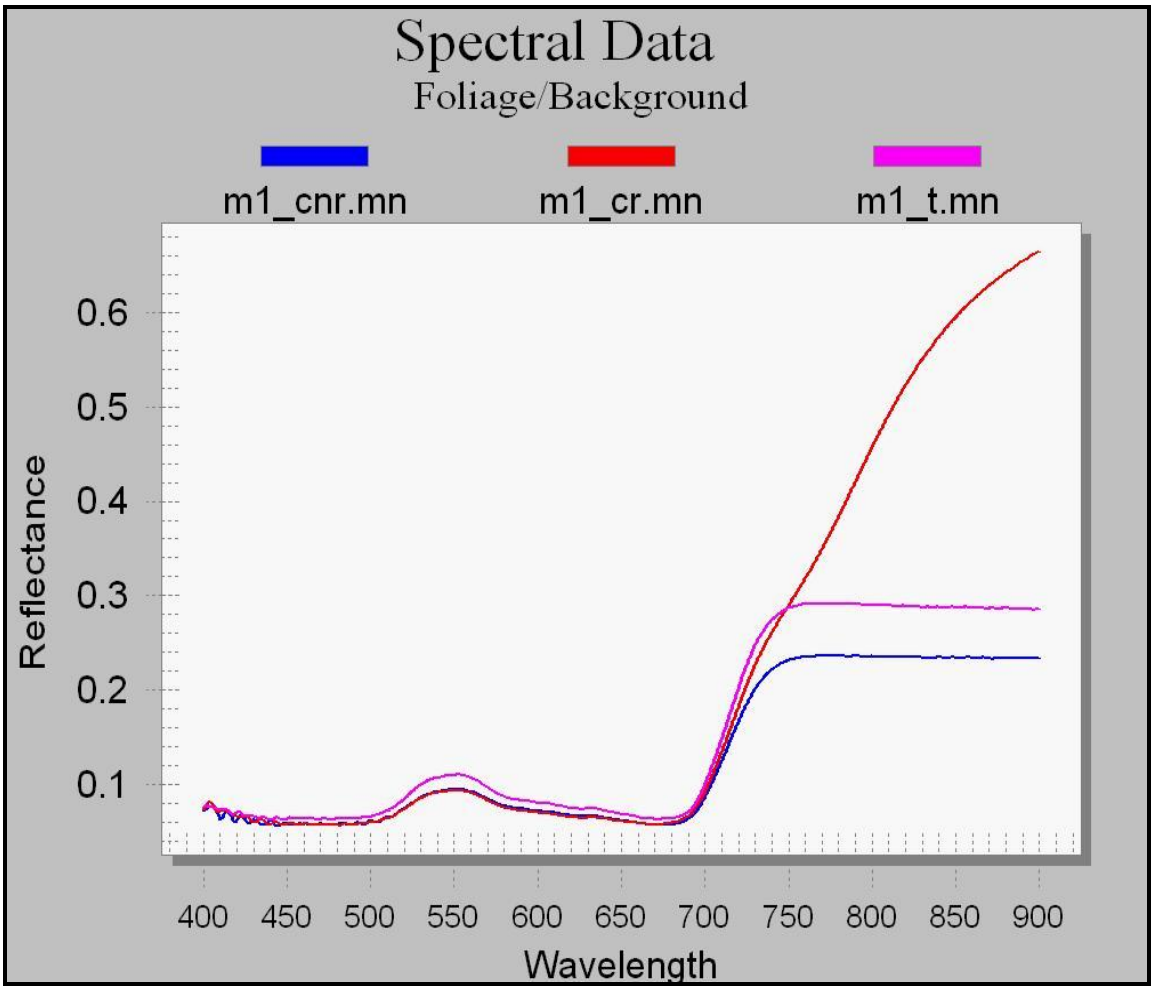

Figure 3. Effects of background on spectral reflectance of sunflower foliage placed on three black materials. NIR- absorbing cloth (blue, m1_cnr), standard laboratory table top (magenta, m1_t), and NIR-reflecting cloth (red, m1_cr.).

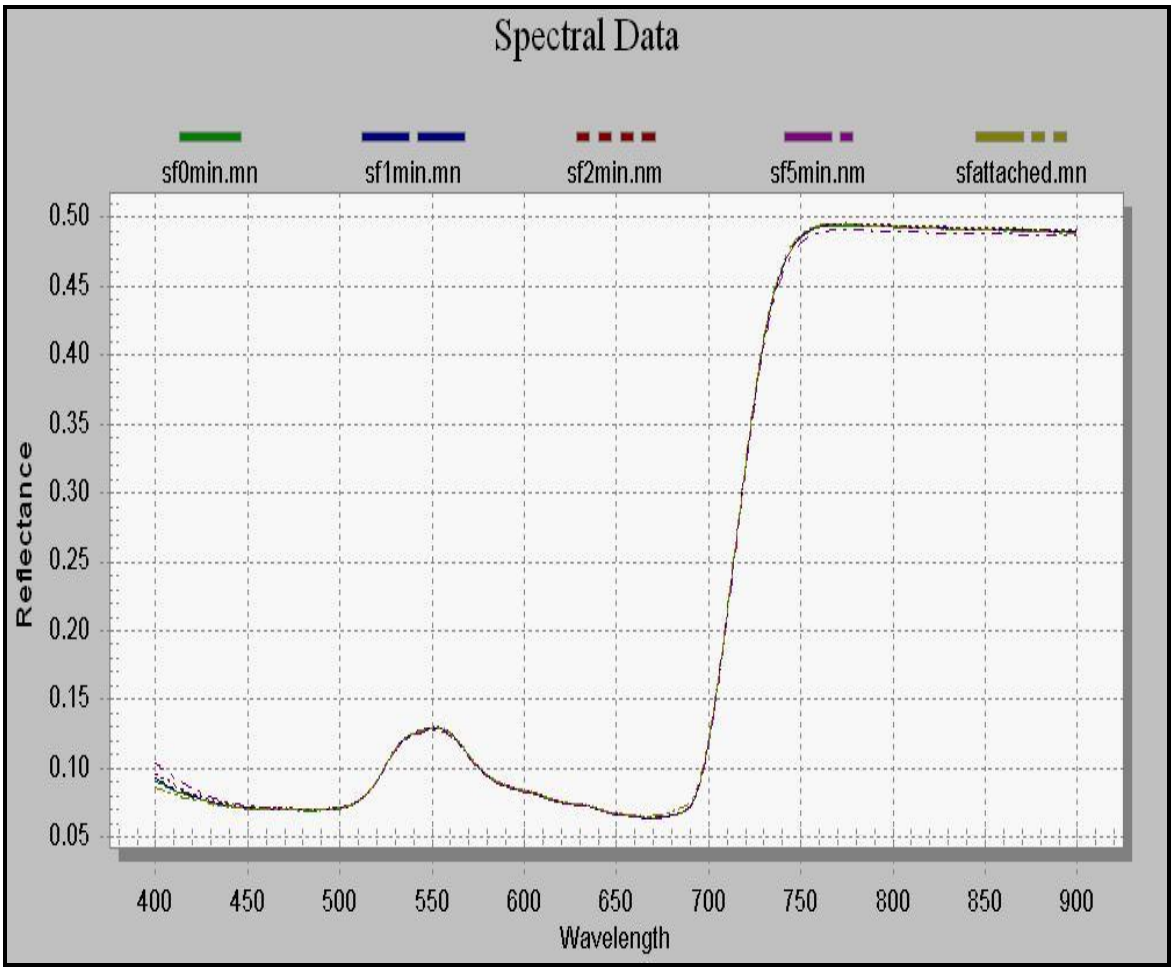

Figure 4. Effects of leaf excision at time series. Mean spectral reflectance curves for individual sunflower leaves attached to plants (sfattached), immediately after excision 0min (sf0min), and at intervals of 1 min (sf1min), 2 min (sf2min), and 5 minutes following excision (sf5min).

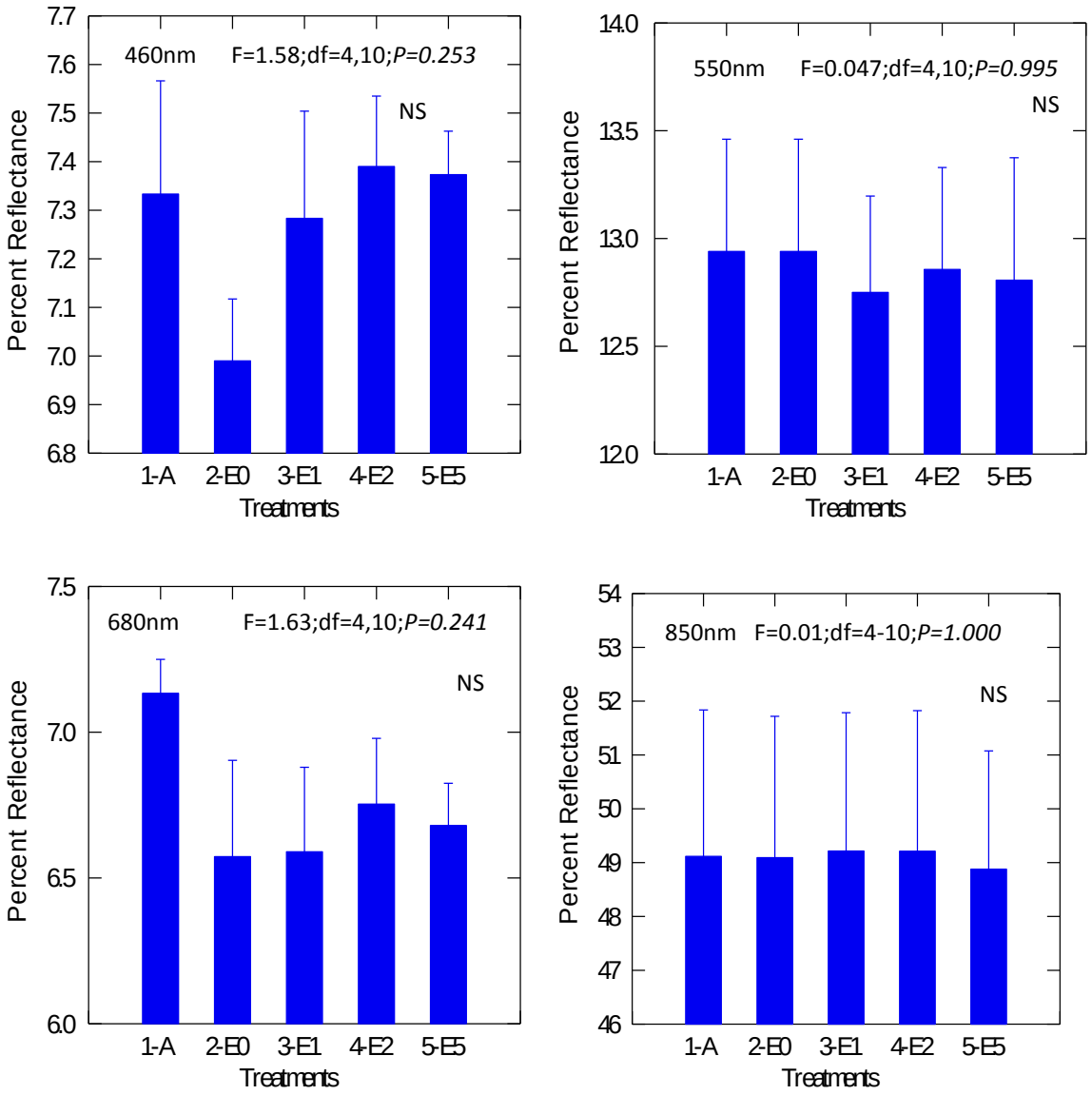

Figure 5. Spectral reflectance measurements for sunflower foliage as effect of leaf excision on time series in the blue (460 nm), green (550nm), red (680 nm), and near-infrared (850 nm) regions of the spectrum; 2009. The bars represent the mean of three measurements from leaves attached (1-A), immediately after excision (2-E0), at 1 min (3-E1), 2 min (4-E2), and up to 5 min (5-E5) after excision. Spectral reflectance of the sunflower leaves was measured under laboratory conditions before arsenic treatment applications.

Experiment 1

Spectral Reflectance Measurements

Reflectance measurements of excised leaves were collected at weekly intervals under artificial (quartz halogen) lighting conditions using a spectroradiometer with sensitivity to visible and NIR wavelengths.

By week 1 post-treatment, relatively small but significant differences among controls and treatment plants were evident in the blue, green and red regions of the visible spectrum (Fig 6). However, the relatively low reflectance of blue (460nm) and red (680nm) wavelengths in the two highest treatment levels (indicative of relatively high absorbance by photosynthetic pigments) suggested that no significant disruption of photosynthesis had occurred at this point (Fig.7). In contrast, the progressive and significant reduction of NIR reflectance from 26 – 33% in the control and low-level treatment group ($P>0.05$) to 12% and 8% in the mid- and high-level treatment groups ($P<0.001$) provided strong evidence that significant changes in the structure of the spongy mesophyll cells (the principal determinant of NIR scattering) had occurred. The progressive reduction of NIR reflectance continued during week 2 (Fig.8 and 9) and week 3 post treatment (Fig.10 and 11), when NIR reflectance was greatest in the untreated control (38%) and least in the group subjected to the highest arsenic treatment level (11%) ($P=0.26$). Thus, increases in arsenic exposure levels were accompanied by relatively small changes in reflectance of visible wavelengths, and relatively large and progressive decreases in NIR reflectance through time. The latter is a well-known symptom of plant stress (Campbell, 2007) and suggests that a major effect of As accumulation had been one or more structural changes in the spongy mesophyll layer of leaves which are the principal determinants of NIR scattering.

Chemical Analysis

As we are interested in the relationships between arsenic content and leaf spectral reflectance, the same excised leaves used to collect spectra measurements were air-dried and preserved for chemical analysis. Significant differences were detected in arsenic concentration of foliage between the control groups and the samples from the treated groups. Higher concentrations of arsenic were found in the group treated with highest concentration of arsenic (58mg/kg), followed by concentrations of arsenic detected in the medium and low arsenic treatments (44mg/kg and 26mg/kg, respectively), the lowest arsenic concentrations were observed in the untreated control group (<1mg/kg). Similar trends which became more pronounced were evident by week 2 and week 3 post treatment (Fig.12).

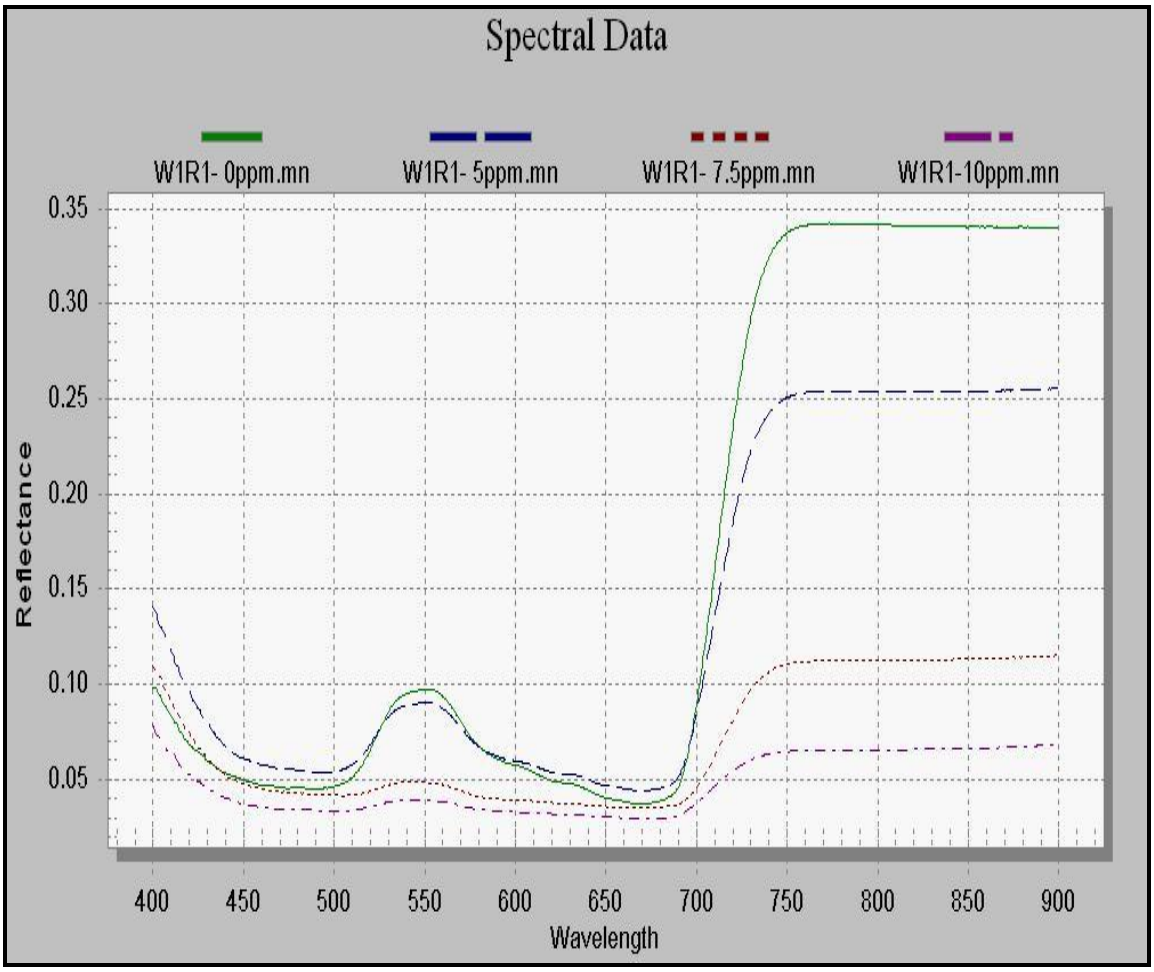

Figure 6. Mean spectral reflectance curves of sunflower leaves at one week of arsenic treatments; Experiment 1, 2009. The spectral curve represented in the graph is mean curve for each treatment.

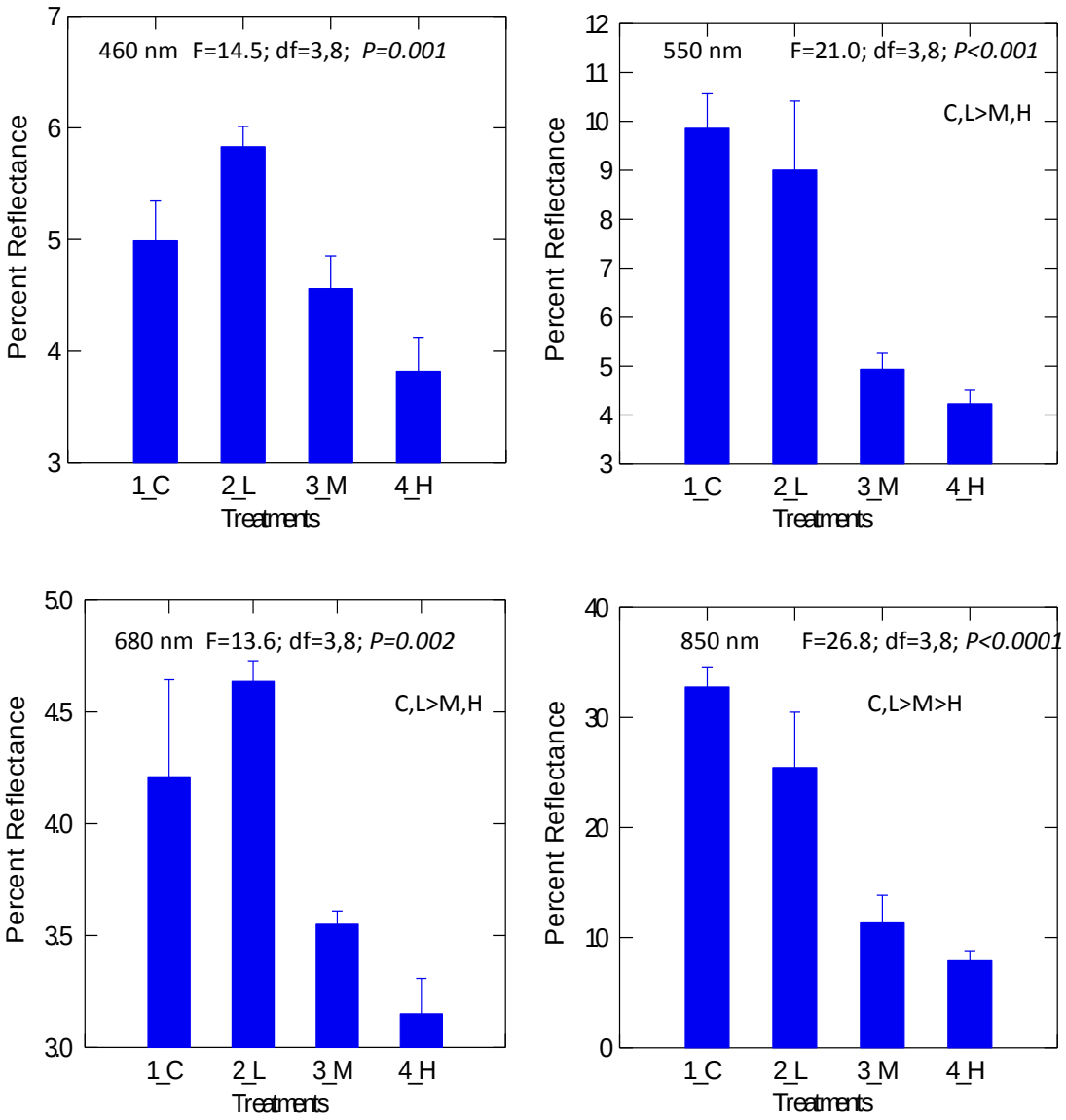

Figure 7. Spectral reflectance measurements for sunflower foliage in the blue (460 nm), green (550nm), red (680 nm) and near-infrared (850 nm) regions of the spectrum; Experiment 1, week 1 post treatment, 2009.Arsenic treatment groups: control group (1-C) 0ppm, low (2-L) 5ppm, medium (3-M) 7.5ppm, and high (4-H) 10ppm.

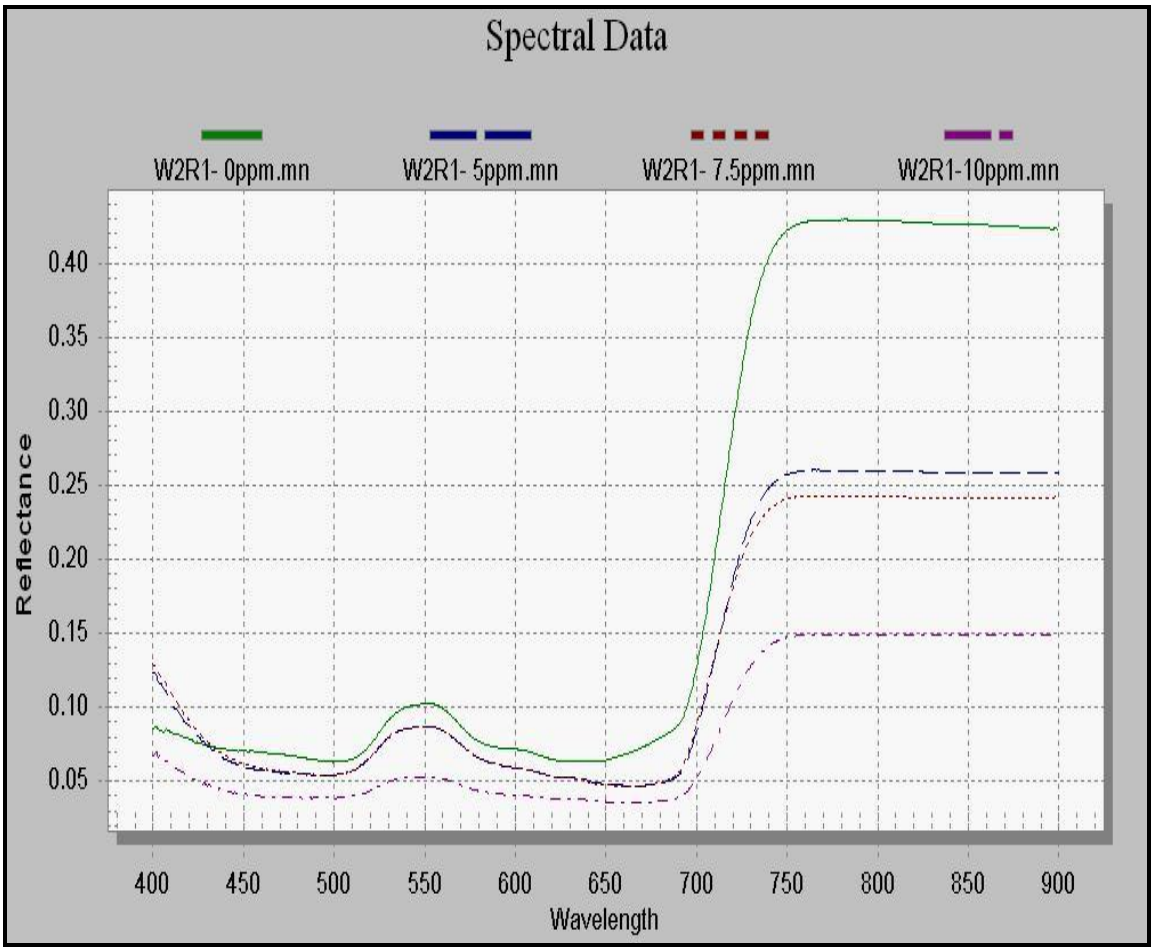

Figure 8. Mean spectral reflectance curves of sunflower leaves at two weeks of arsenic treatments; Experiment 1, 2009. The spectral curve represented in the graph is mean curve for each treatment.

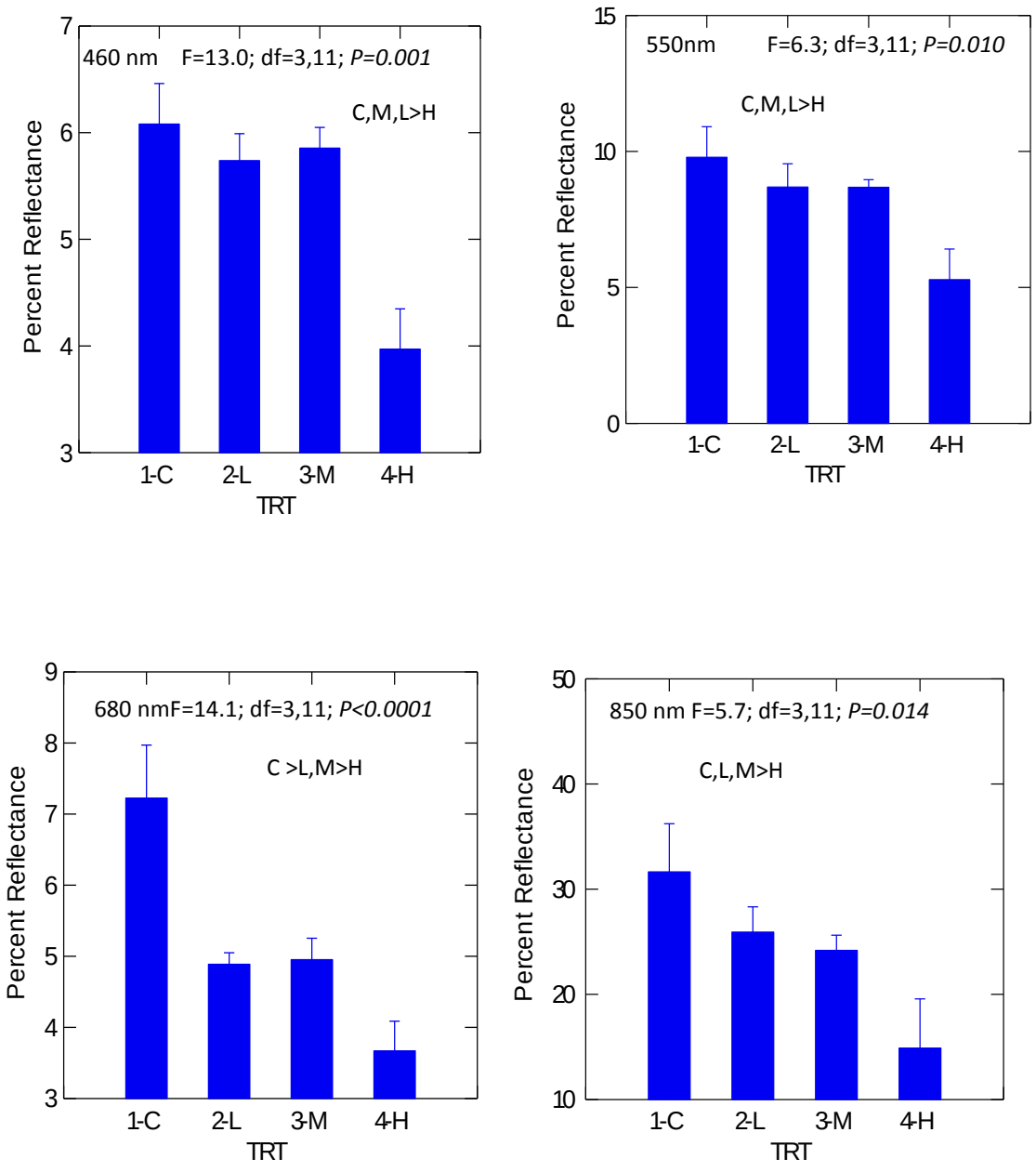

Figure 9. Spectral reflectance measurements for sunflower foliage in the blue (460 nm), green (550 nm), red (680 nm) and near-infrared (850 nm) regions of the spectrum; Experiment 1, week 2 post treatment, 2009. Arsenic treatment groups: control group (1-C) 0ppm, low (2-L) 5ppm, medium (3-M) 7.5ppm, and high (4-H) 10ppm.

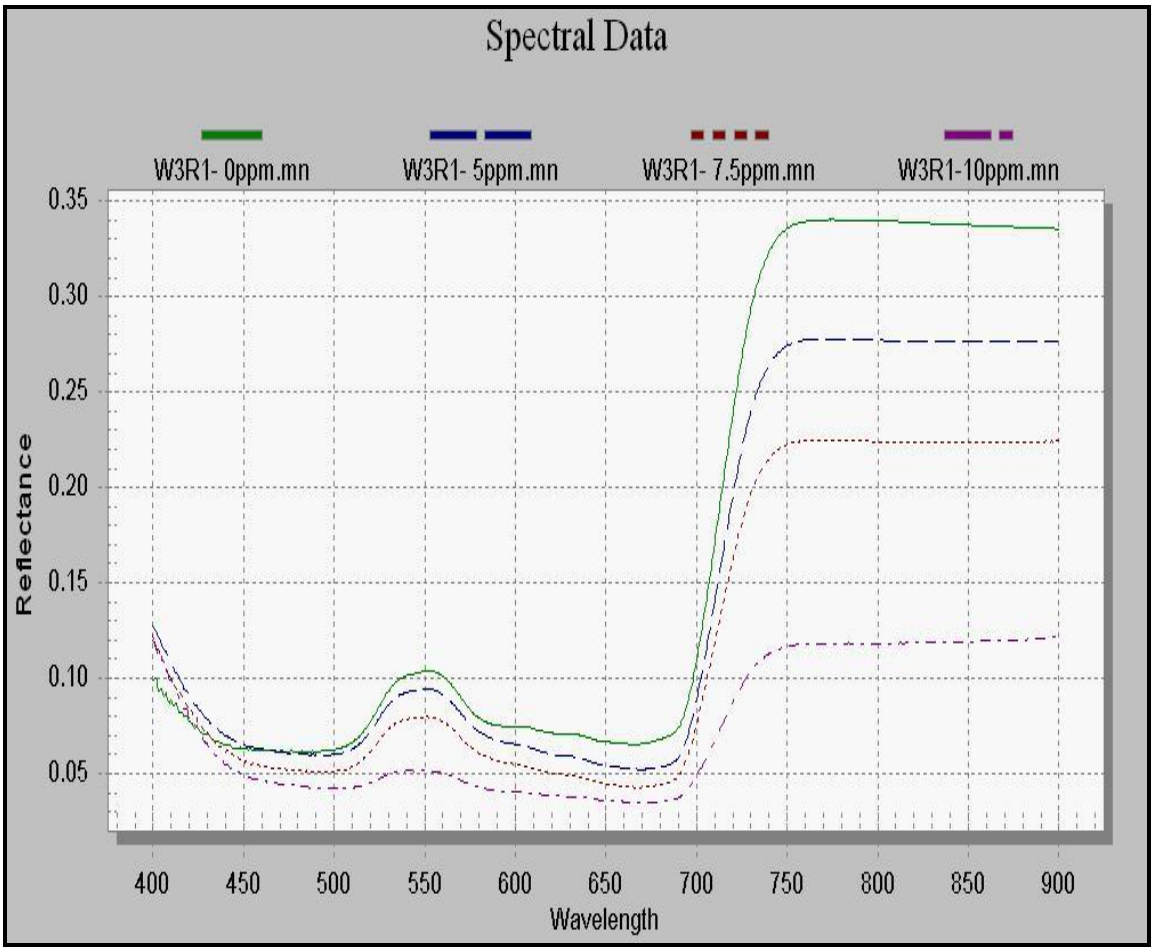

Figure10. Mean spectral reflectance curves of sunflower leaves at three weeks of arsenic treatments; Experiment 1, 2009. The spectral curve represented in the graph is mean curve for each treatment.

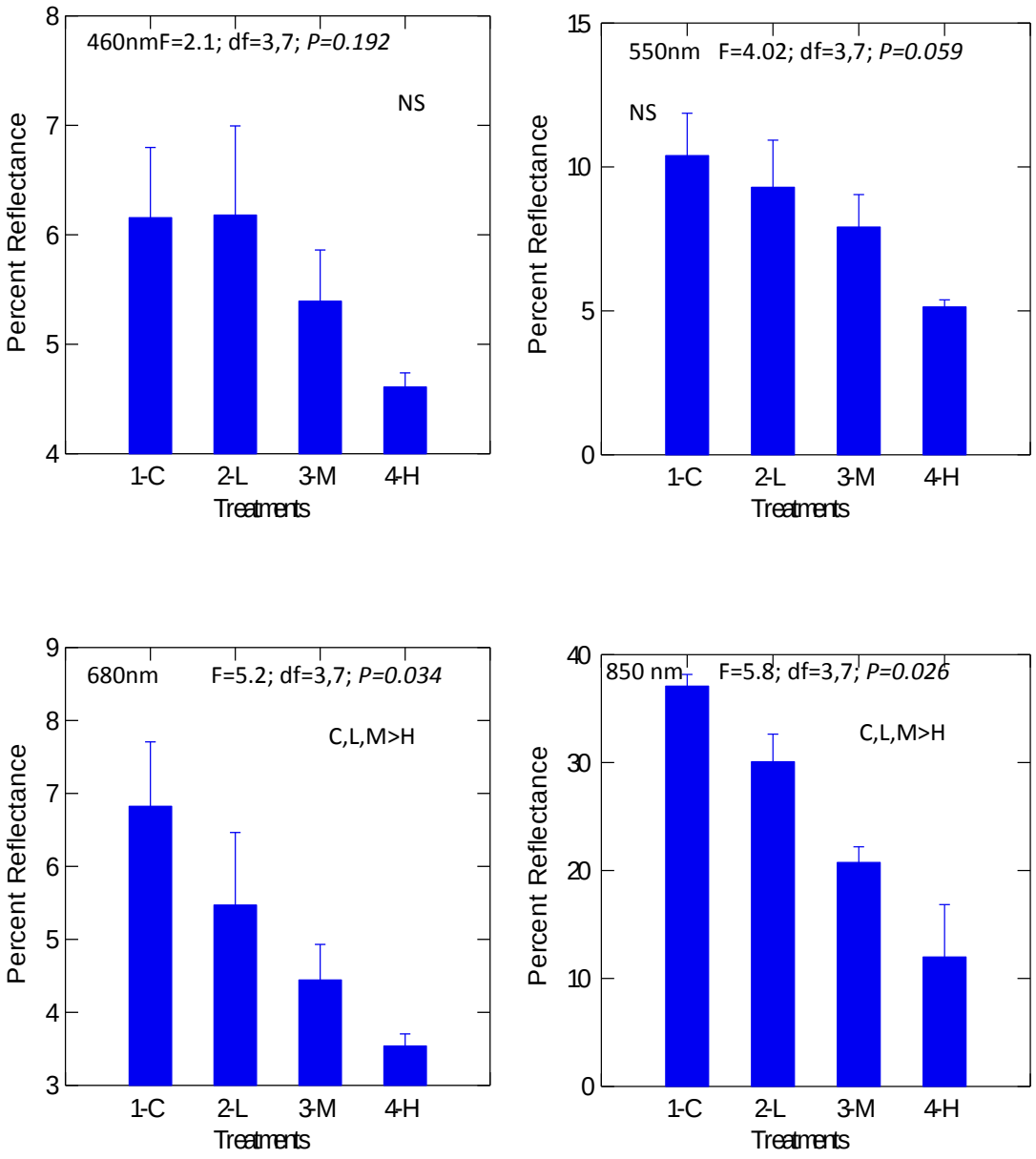

Figure 11. Spectral reflectance measurements for sunflower foliage in the blue (460 nm), green (550 nm), red (680 nm) and near-infrared (850 nm) regions of the spectrum; Experiment 1, week 3 post treatment, 2009. Arsenic treatment groups: control group (1-C) 0ppm, low (2-L) 5ppm, medium (3-M) 7.5ppm, and high (4-H) 10ppm.

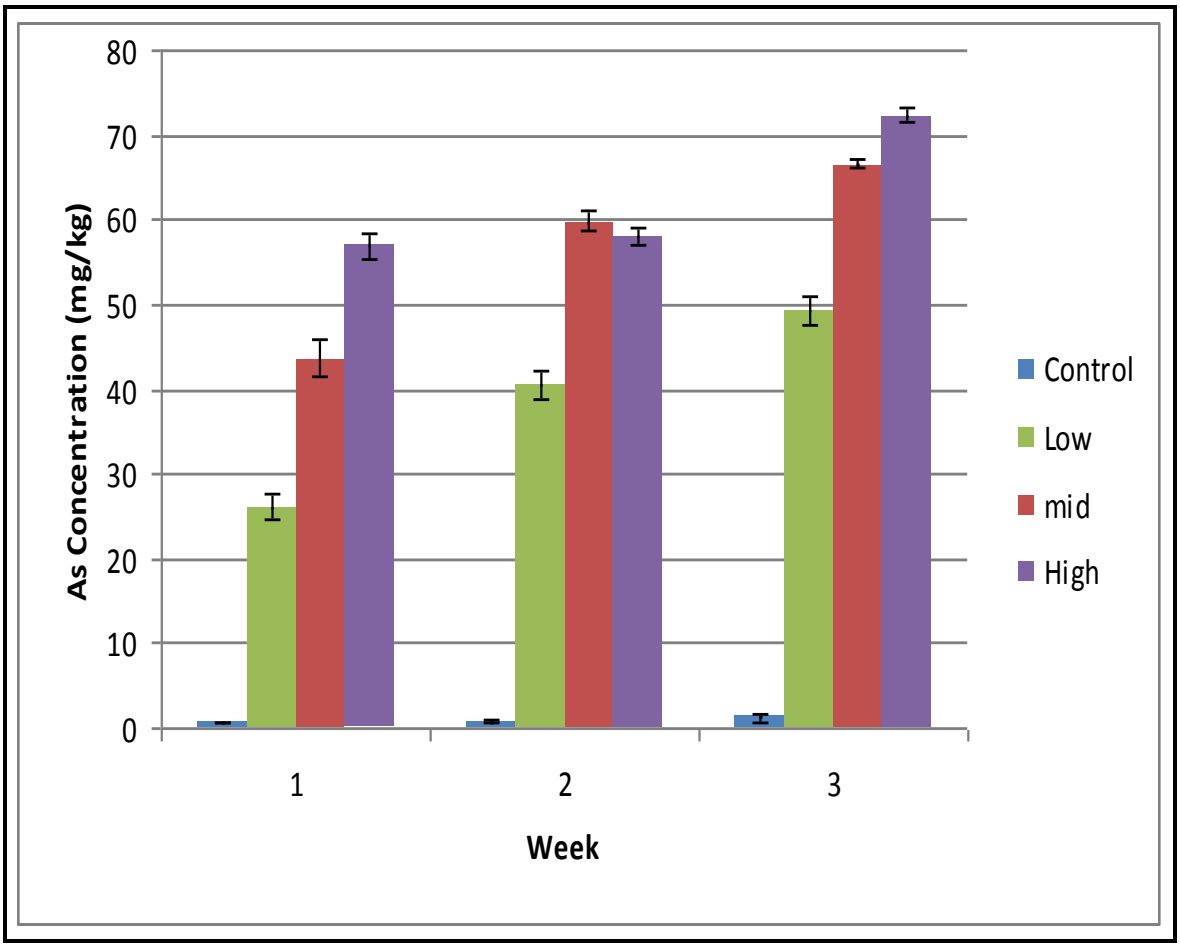

Figure 12. Arsenic concentration in the leaves of wild sunflower leaves. Bars represent mean of three replicates Experiment1, 2009.

Experiment 2

Spectral Reflectance Measurements

By the end of week 1 post treatment, slight differences in spectral reflectance were obtained in the blue and green regions of the spectra but no significant difference were evident on the red and NIR regions (Fig. 13 and 14). Nevertheless, by the end of week 2, similar trends to the ones obtained during experiment 1 became apparent. The magnitude of changes in blue and red (4-7% and 3.5-8%, respectively) suggest that the photosynthetic efficiency had not been disrupted during this time. However, the magnitude of difference in NIR (14-42%) suggested that significant changes in leaf structure (spongy mesophyll cells) had occurred during this period (Figs.15 and 16). The same trend continued during week 3 (Figs. 17 and 18). By week 3 post treatment, NIR reflectance was greatest in untreated controls (38%), intermediate among cohorts subjected to low and medium concentrations (27% and 24%, respectively), and least among cohorts subjected to the highest As concentration (12%). Conversely, by the end of week 4 the spectral measurements obtained indicate a considerable increased in the reflectance in the blue (5.5%) and red (7%) regions (Fig 19 and 20). The same trend was observed at week 5 as spectral measurements in the blue region were elevated (8.5%) suggesting that at this point the arsenic concentration in the foliage had additionally disrupted photosynthetic efficiency in the treated plants (Fig 21 and 22). Thus, by week 4 post treatment, stress caused by arsenic exposure had progressed to the point where photosynthesis was affected, and was most pronounced among treatments exposed to highest levels of arsenic.

Chemical Analysis

As in experiment 1, the same excised leaves used to collect spectra measurements were air-dried and preserved for chemical analysis. The results of this chemical analysis inducted trends similar to those noted in experiment 1. Significant differences were detected among untreated controls and treatment groups. Greater concentration of arsenic were found in the leaves of the higher arsenic treatments (30mg/kg), followed by the medium (23mg/kg) and low (15mg/kg) arsenic treatment groups and the lowest arsenic concentration was observed in the untreated control group. These trends became more pronounced by week 2, week 3, week 4 and week 5 post treatment (Fig. 23).

During both experiments, the GFAAS results inversely correlated to the changes in the NIR spectral reflectance by foliage of sunflower plants. The plants with the lowest NIR reflectance had the highest As concentrations, the plants with intermediate NIR reflectance had intermediate As concentration and the plants with the lowest NIR reflectance had little to no As content. At the end of week 5, changes in the size of the leaves were observed. Sunflower leaves from the untreated control groups were larger size than the treatment groups, and the size of leaves decreased as the arsenic concentration were increased (Fig. 24).

Later, both experiments were combined and analyzed using de Kruskal-Wallis statistical test. The results of the non-parametric analysis are shown in Figs. 25 and 26. As can be seen in the data, there is no significant difference detected in blue (KW=2.302;df=2;*P=0.316*), green (KW=5.809;df2;*P=0.055*), and red (KW=1.813;df=2;*P=0.404*) regions of the visible spectra (Fig. 25). However, there is a significant direct correlation between the NIR spectral reflectance and the arsenic concentration in the different treatments 5.0 ppm, 7.5 ppm, and 10 ppm (KW=9.750;df=2;*P=0.008)*)(Fig. 26 left). As the arsenic concentration in the hydroponic

solution is increased the spectral reflectance in the NIR spectral reflectance decreases over the five weeks of growth. In addition, Figure 26 right shows the non parametric analysis of the arsenic concentrations in the plant foliage vs level of arsenic in the hydroponic solution. As can be seen in Fig 26 right, as the arsenic concentration in the hydroponic solution is increased the arsenic concentration in the plant foliage also increases (KW=11.340;df=2;*P=0.003)*. A comparison of both these plots in Figs. 26 right and 26 left shows a correlation between decreasing spectral reflectance in the NIR and increasing arsenic concentration in the plant foliage.

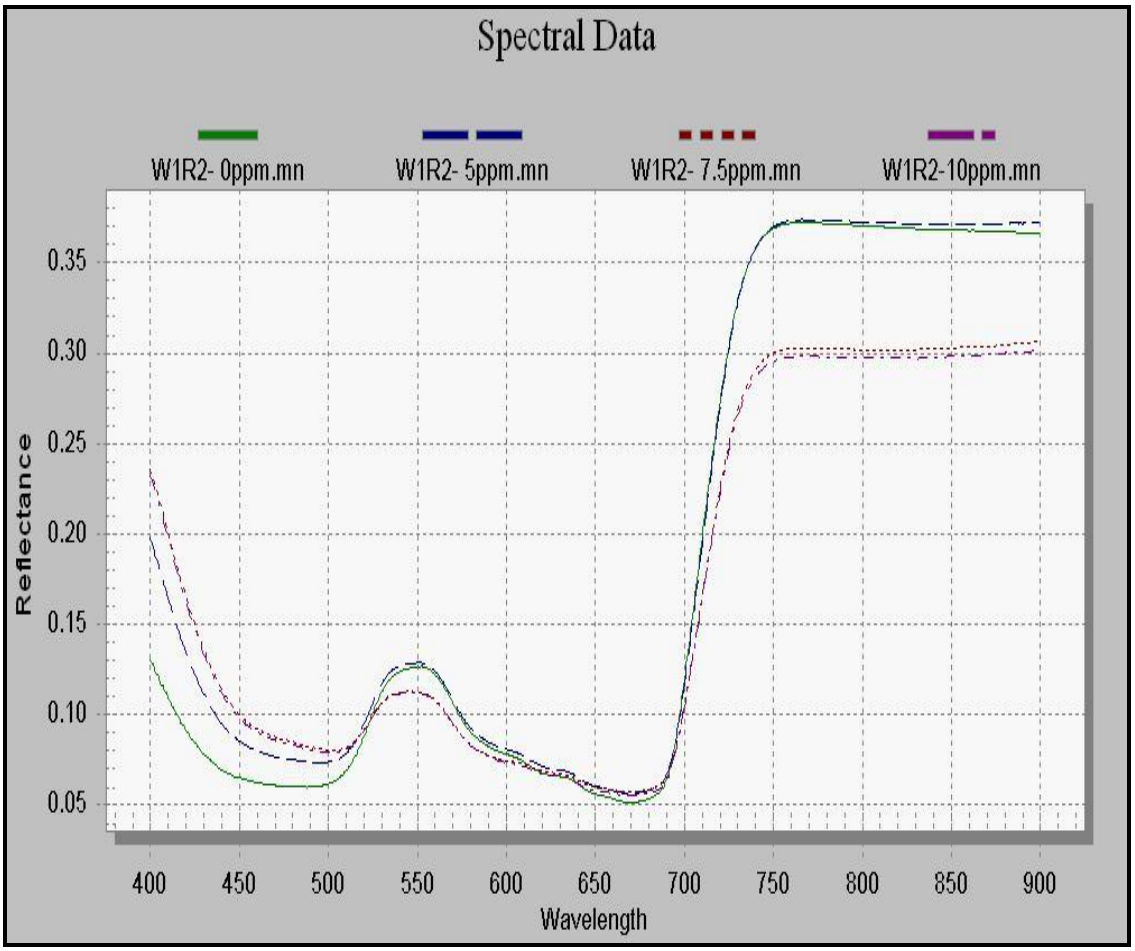

Figure 13. Mean spectral reflectance curves of sunflower leaves at one week of arsenic treatments; Experiment 2, week1, 2010. The spectral curve represented in the graph is mean curve for each treatment.

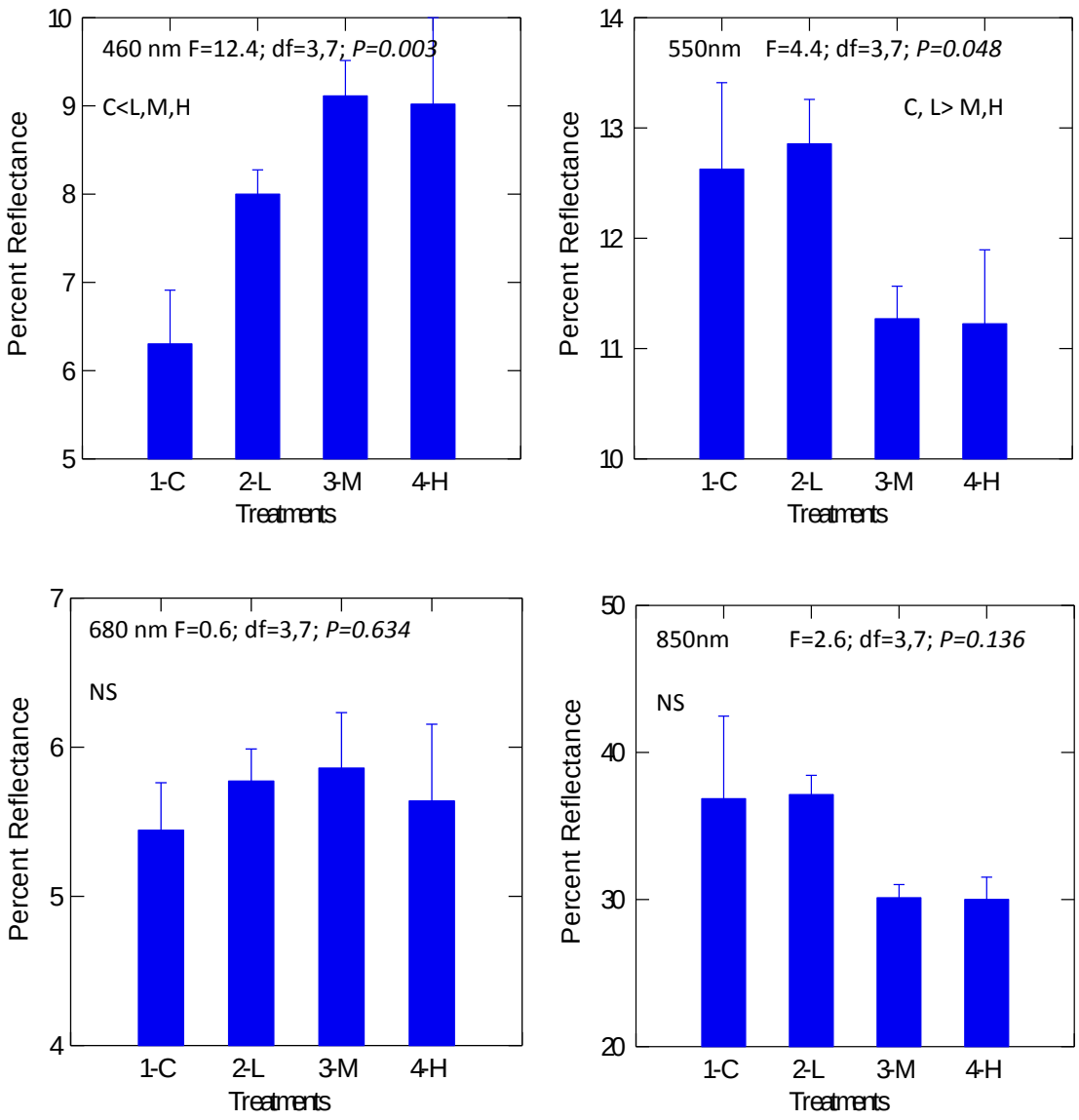

Figure 14. Spectral reflectance measurements for sunflower foliage in the blue (460 nm), green (550 nm), red (680 nm) and near-infrared (850 nm) regions of the spectrum; Experiment 2, week 1 post treatment, 2010.Arsenic treatment groups: control group (1-C) 0ppm, low (2-L) 5ppm, medium (3-M) 7.5ppm, and high (4-H) 10ppm.

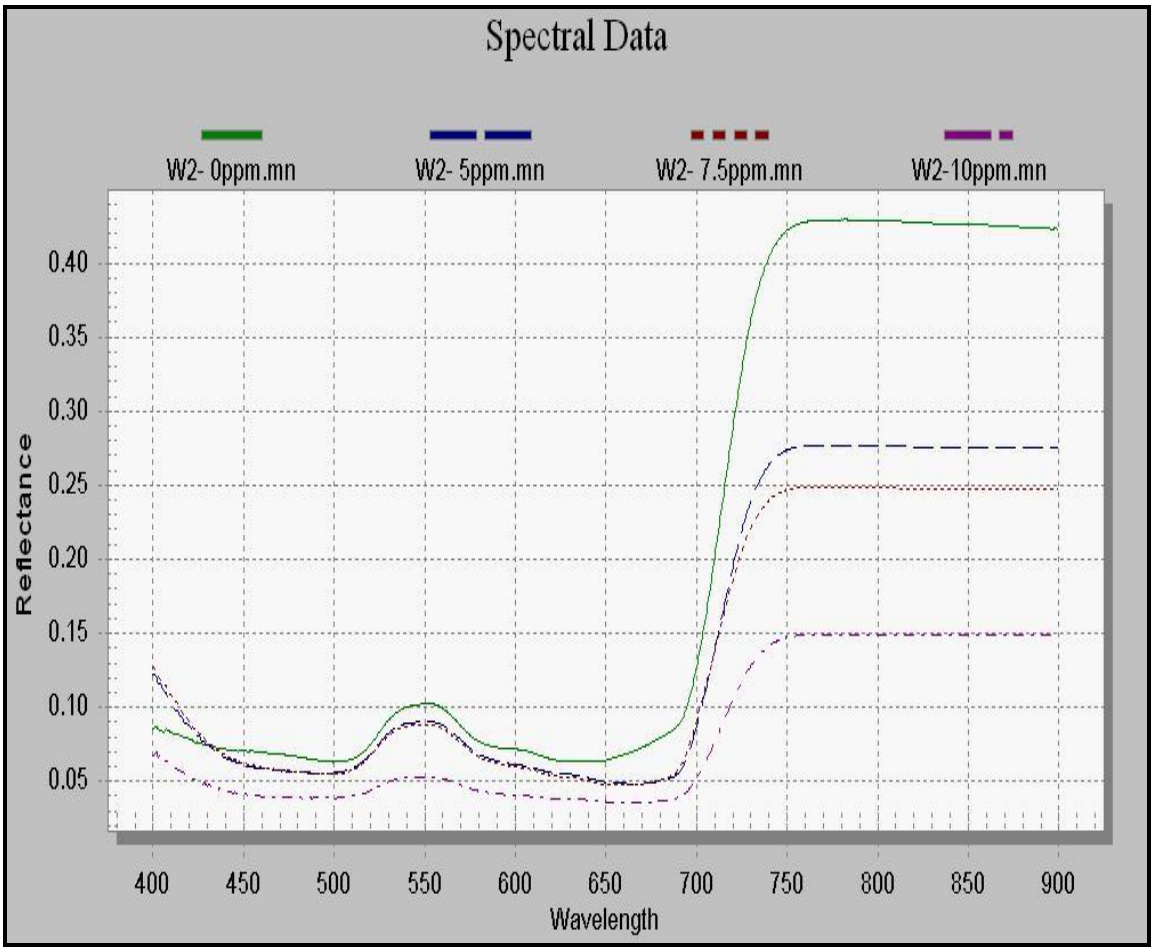

Figure 15. Mean spectral reflectance curves of sunflower leaves at two weeks of arsenic treatments. Experiment 2, week 2, 2010. The spectral curve represented in the graph is mean curve for each treatment.

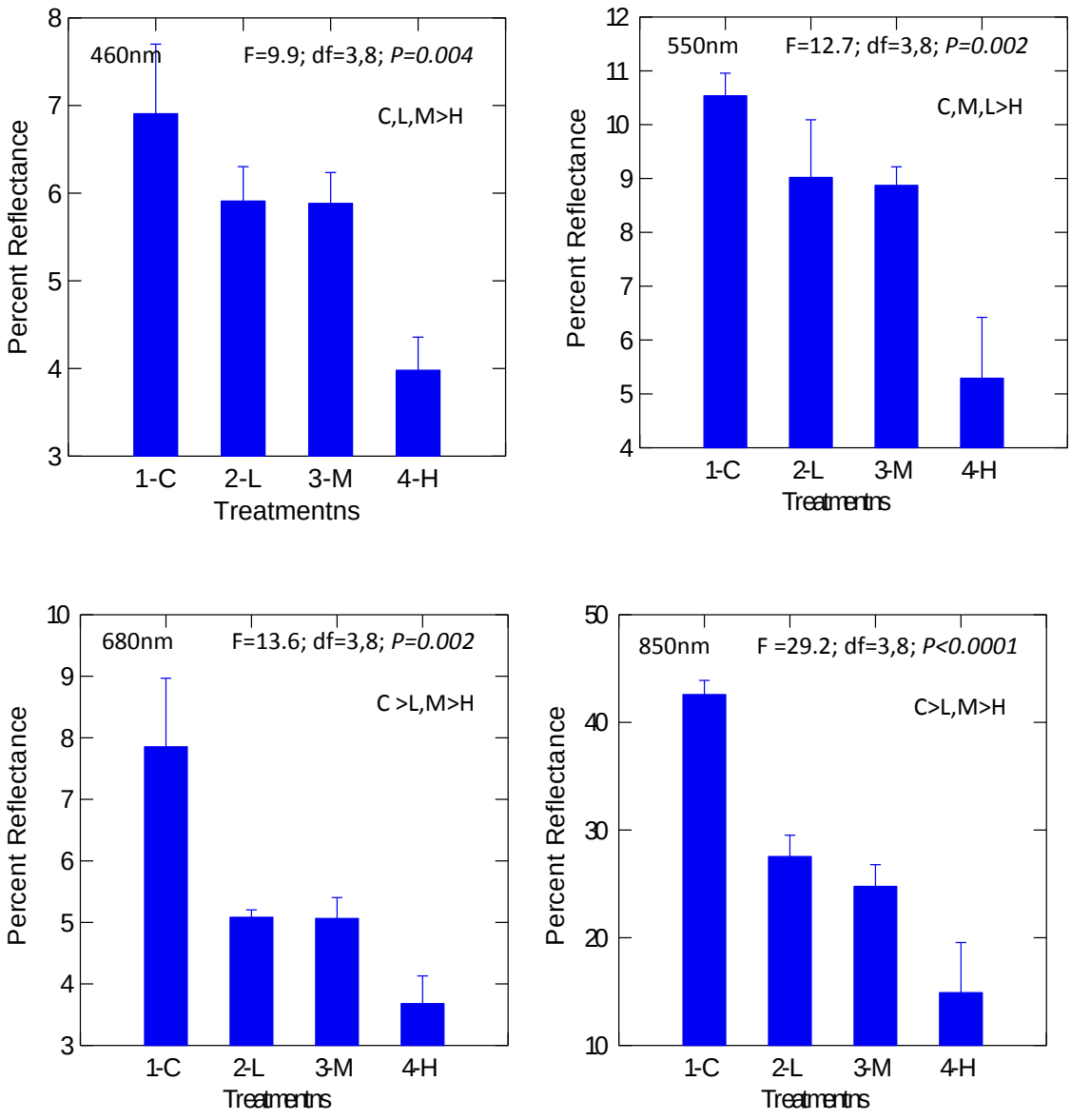

Figure 16. Spectral reflectance measurements for sunflower foliage in the blue (460nm), green (550nm), red (680nm) and near-infrared (850nm) regions of the spectrum; Experiment 2, week 2 post treatment, 2010. Arsenic treatment groups: control group (1-C) 0ppm, low (2-L) 5ppm, medium (3-M) 7.5ppm, and high (4-H) 10ppm.

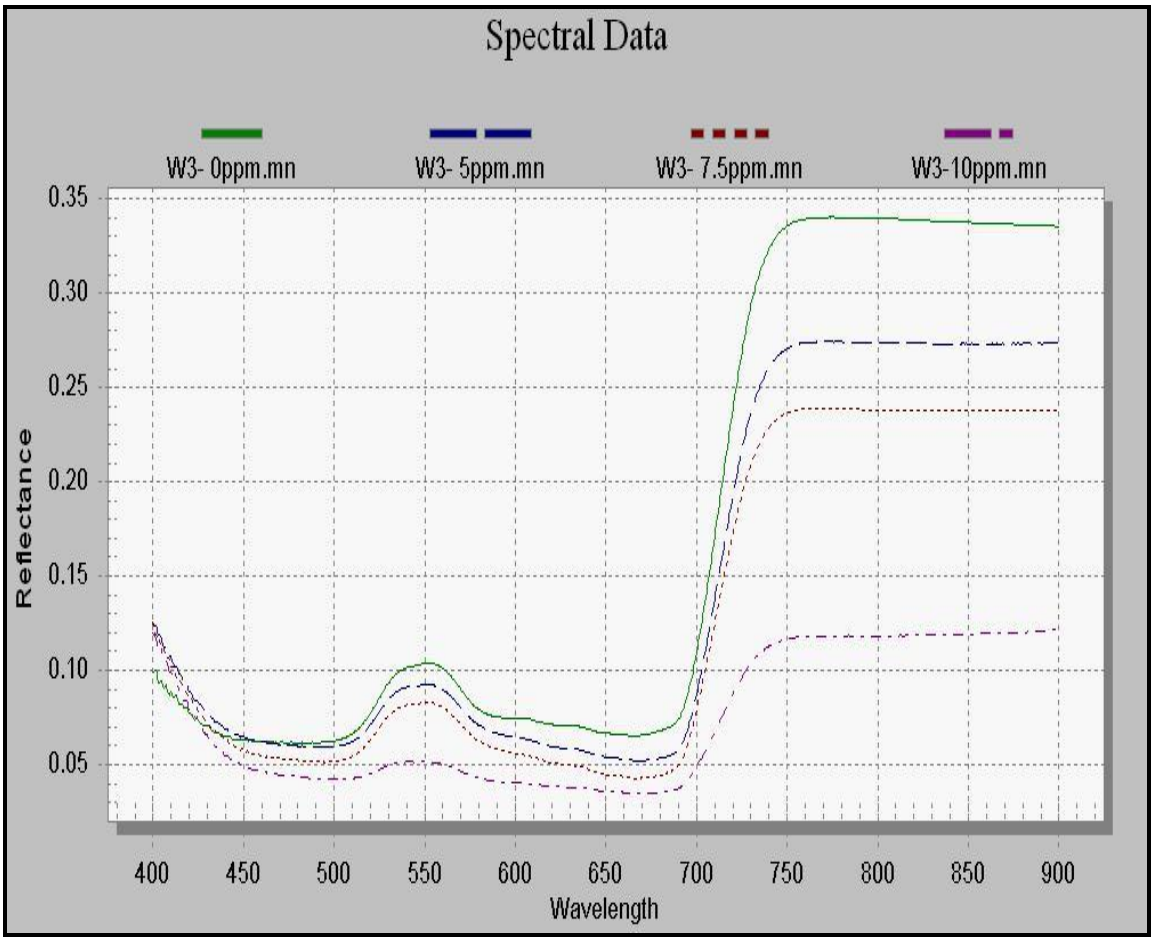

Figure 17. Mean spectral reflectance curves of sunflower leaves at three weeks of arsenic treatments. Experiment 2, week 3, 2010.

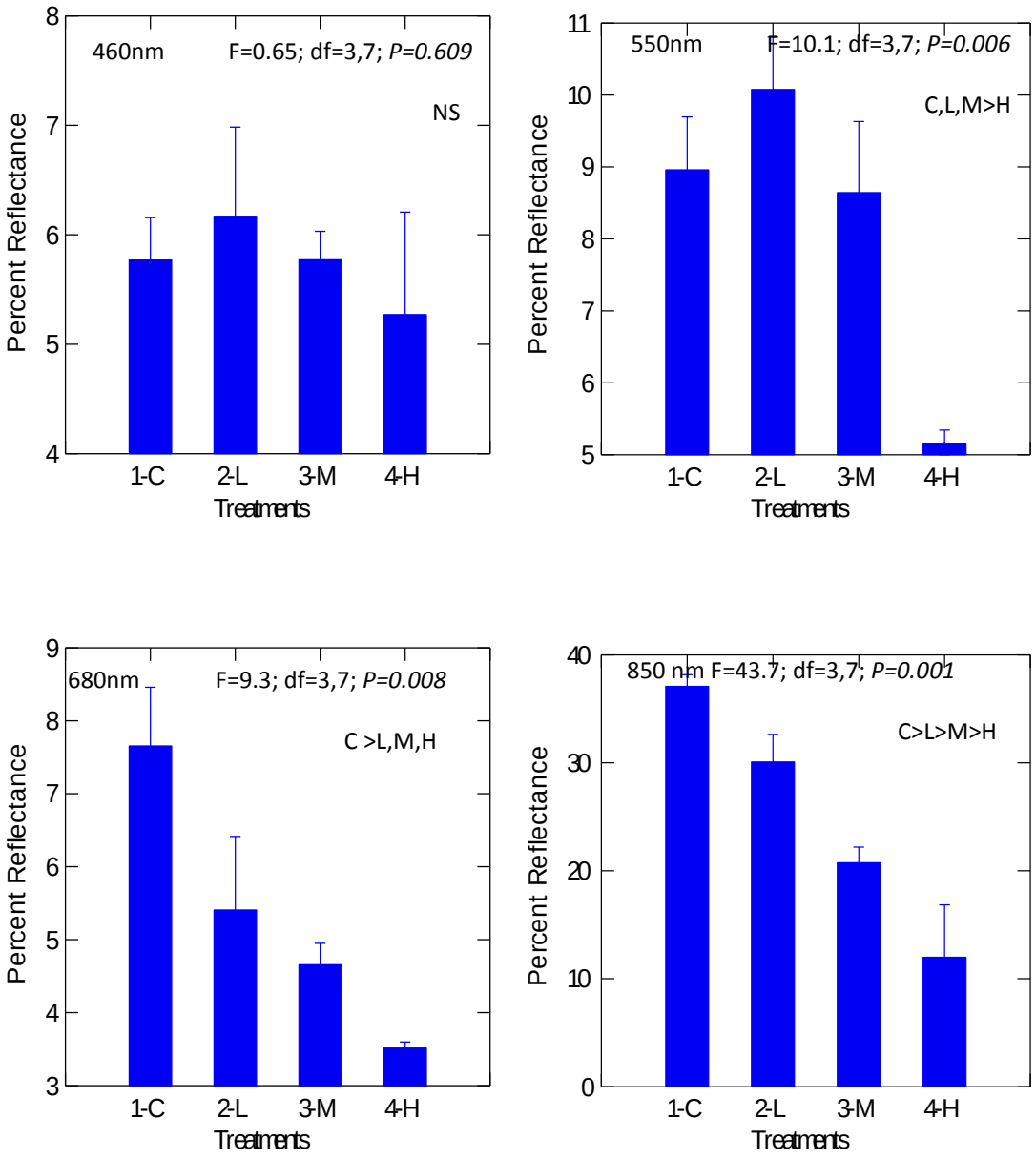

Figure 18. Spectral reflectance measurements for sunflower foliage in the blue (460nm), green (550nm), red (680nm) and near-infrared (850nm) regions of the spectrum; Experiment 2, week 3 post treatment, 2010. Arsenic treatment groups: control group (1-C) 0ppm, low (2-L) 5ppm, medium (3-M) 7.5ppm, and high (4-H) 10ppm.

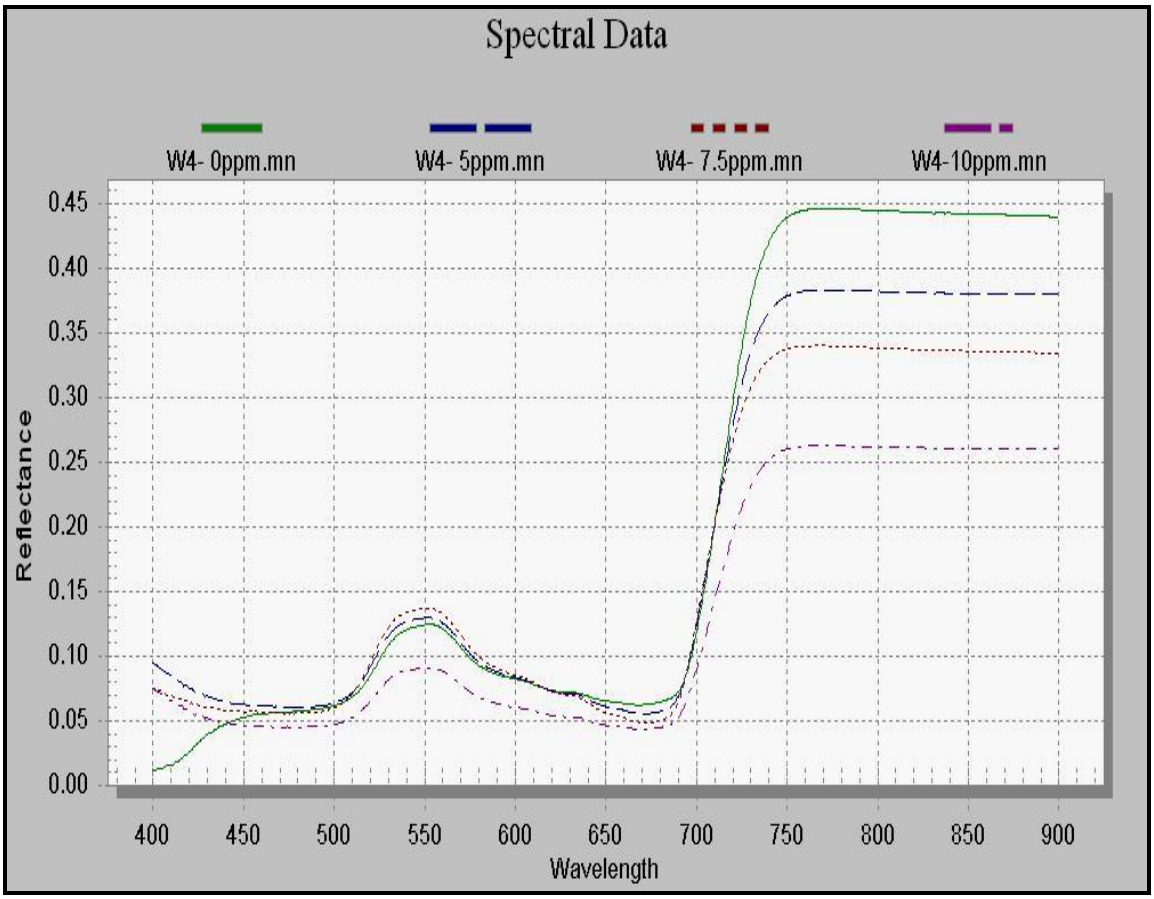

Figure 19. Mean spectral reflectance curves of sunflower leaves at four weeks of arsenic treatments. The spectral curve represented in the comparison graph is mean curve for each treatment during experiment 2, week 4, 2010.

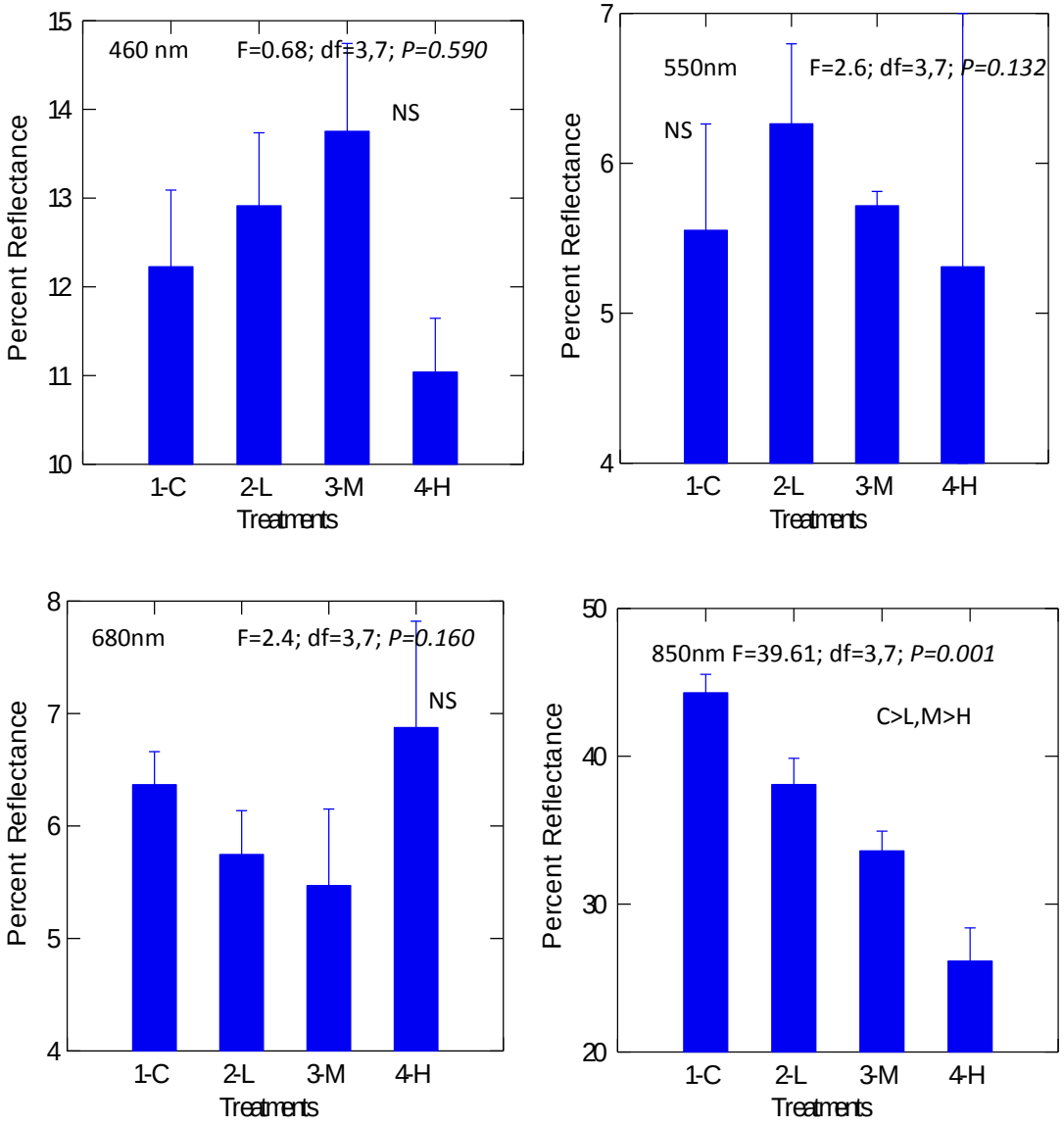

Figure 20. Spectral reflectance measurements for sunflower foliage in the blue (460nm), green (550nm), red (680nm) and near-infrared (850) regions of the spectrum; Experiment 2, week 4 post treatment, 2010. Arsenic treatment groups: control group (1-C) 0ppm, low (2-L) 5ppm, medium (3-M) 7.5ppm, and high (4-H) 10ppm.

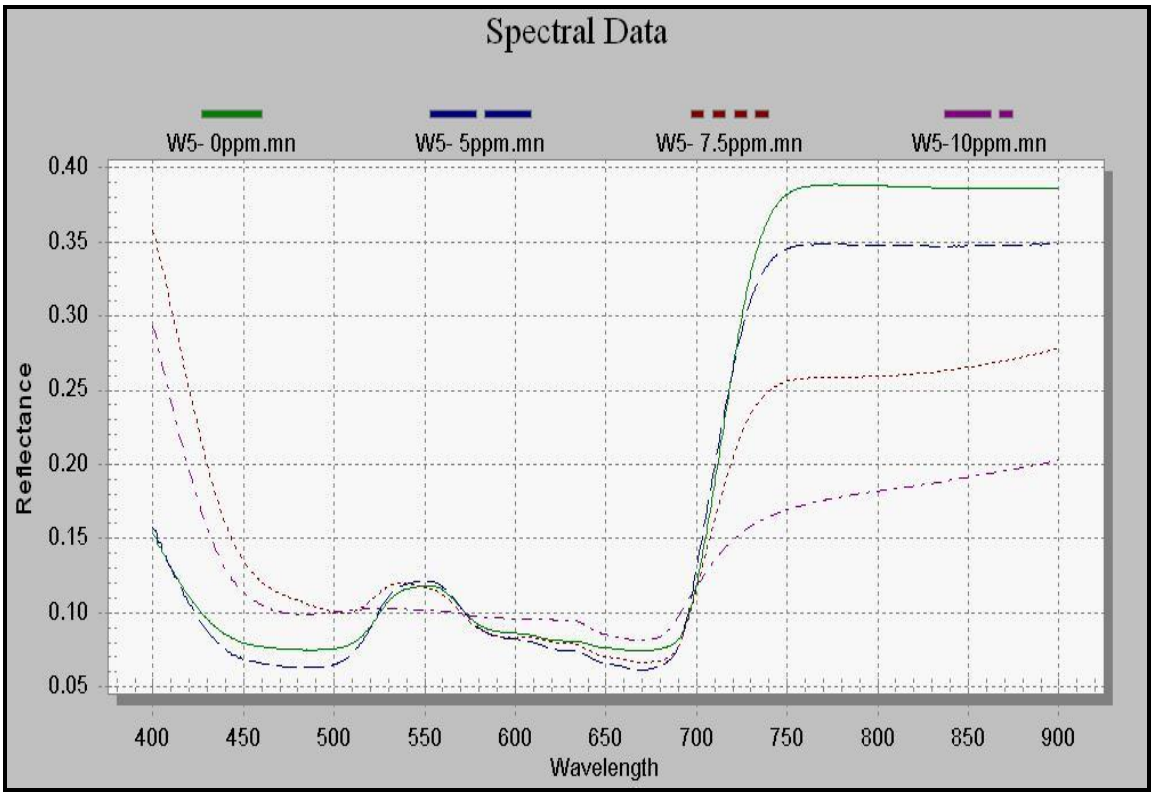

Figure 21. Effect of arsenic on the spectral reflectance curves of sunflower leaves at five weeks. The spectral curve represented in the comparison graph is mean curve for each treatment during experiment 2, week 5, 2010.

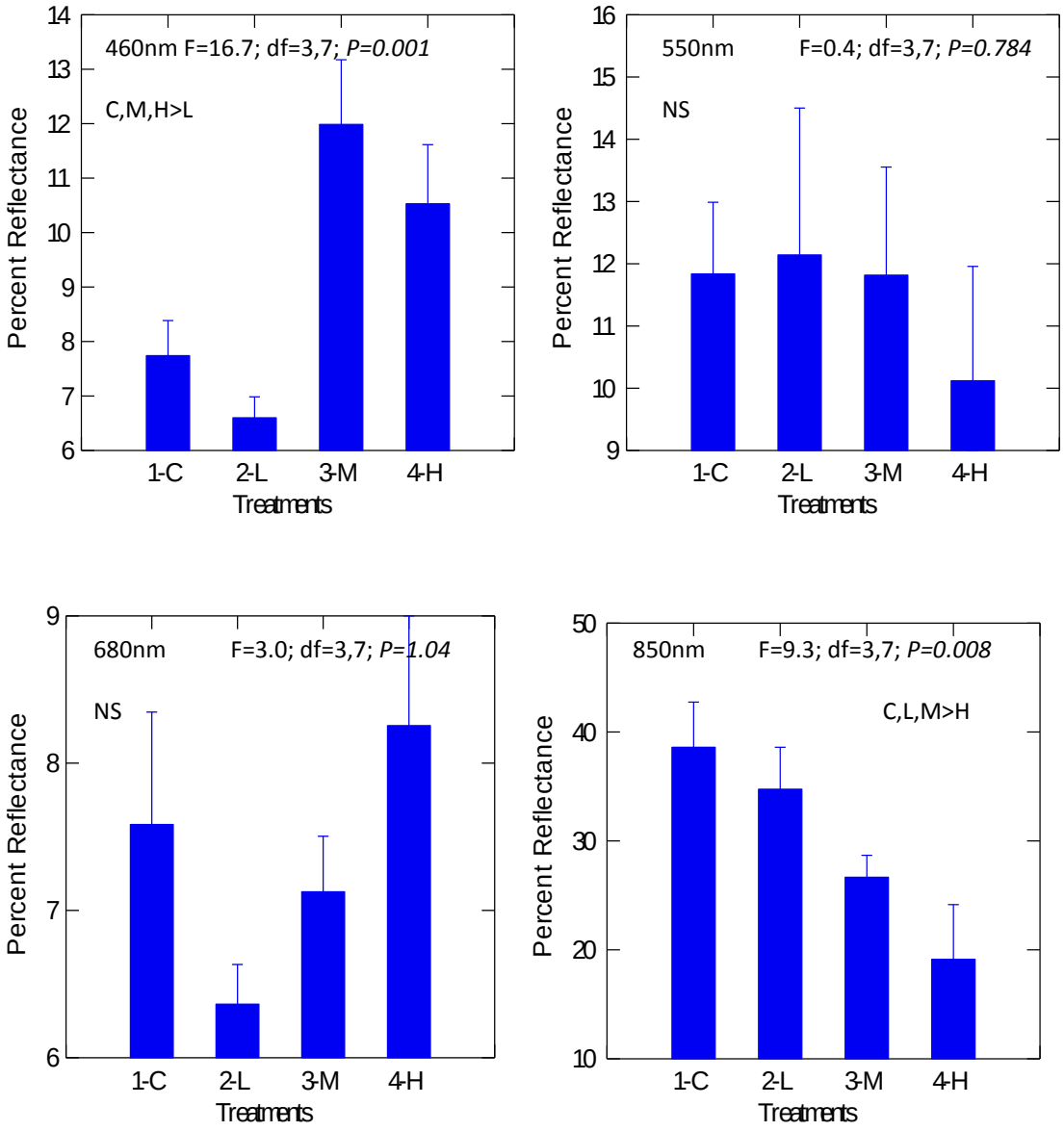

Figure 22. Spectral reflectance measurements for sunflower foliage in the blue (460nm), green (550nm), red (680nm) and near-infrared (850nm) regions of the spectrum; Experiment 1, week 5 post treatment, 2010. Arsenic treatment groups: control group (1-C) 0ppm, low (2-L) 5ppm, medium (3-M) 7.5ppm, and high (4-H) 10ppm.

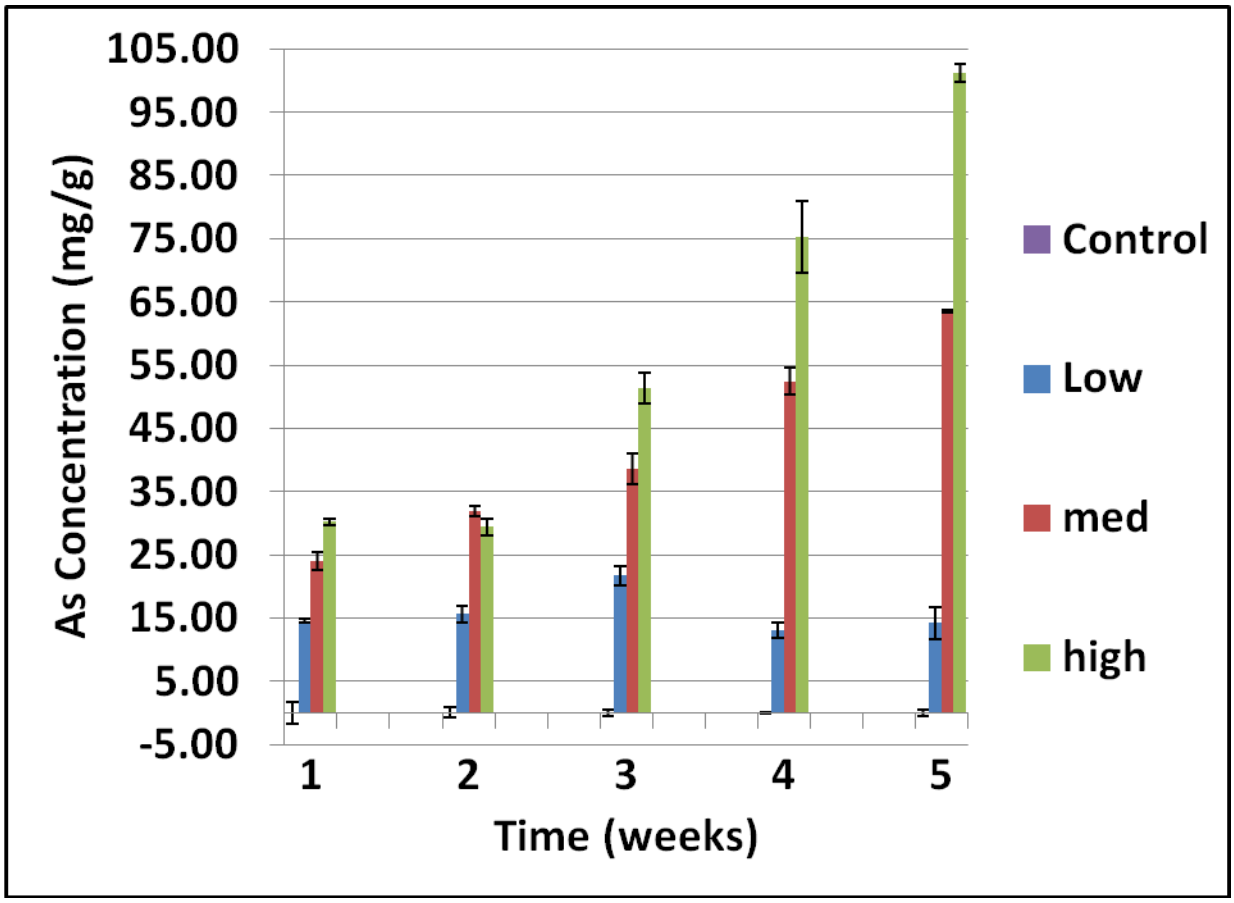

Figure 23. Arsenic concentration in the leaves of wild sunflower leaves. Bars represent mean of three replicates. Experiment 2, 2010.

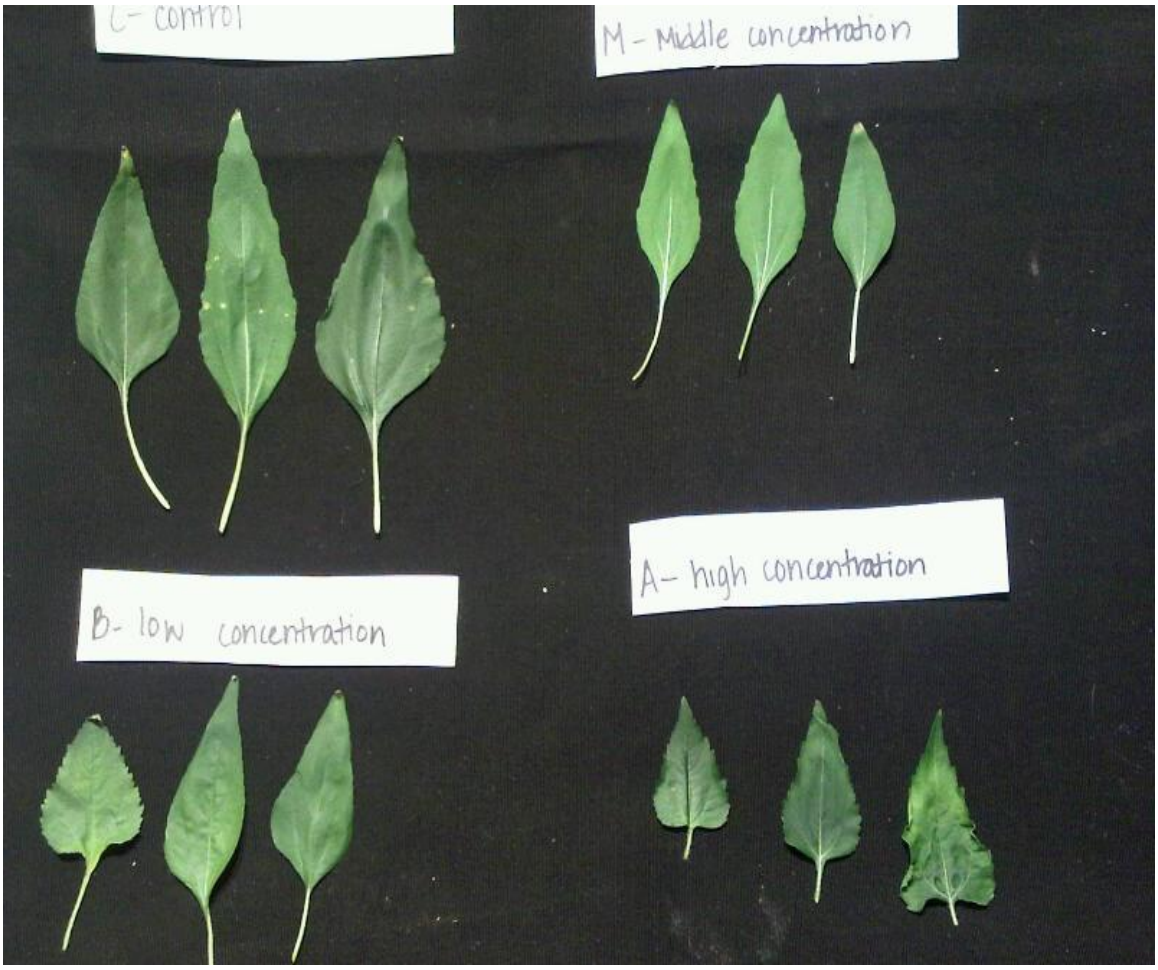

Figure 24. Effect of arsenic treatments on wild sunflower *(Helianthus annuus)* leaves. The image shows the effect arsenic treatments on untreated control, 5 ppm (low concentration), 7.5 ppm (medium concentration), and 10 ppm (high concentration), on the size and appearance of leaves at five weeks of application.

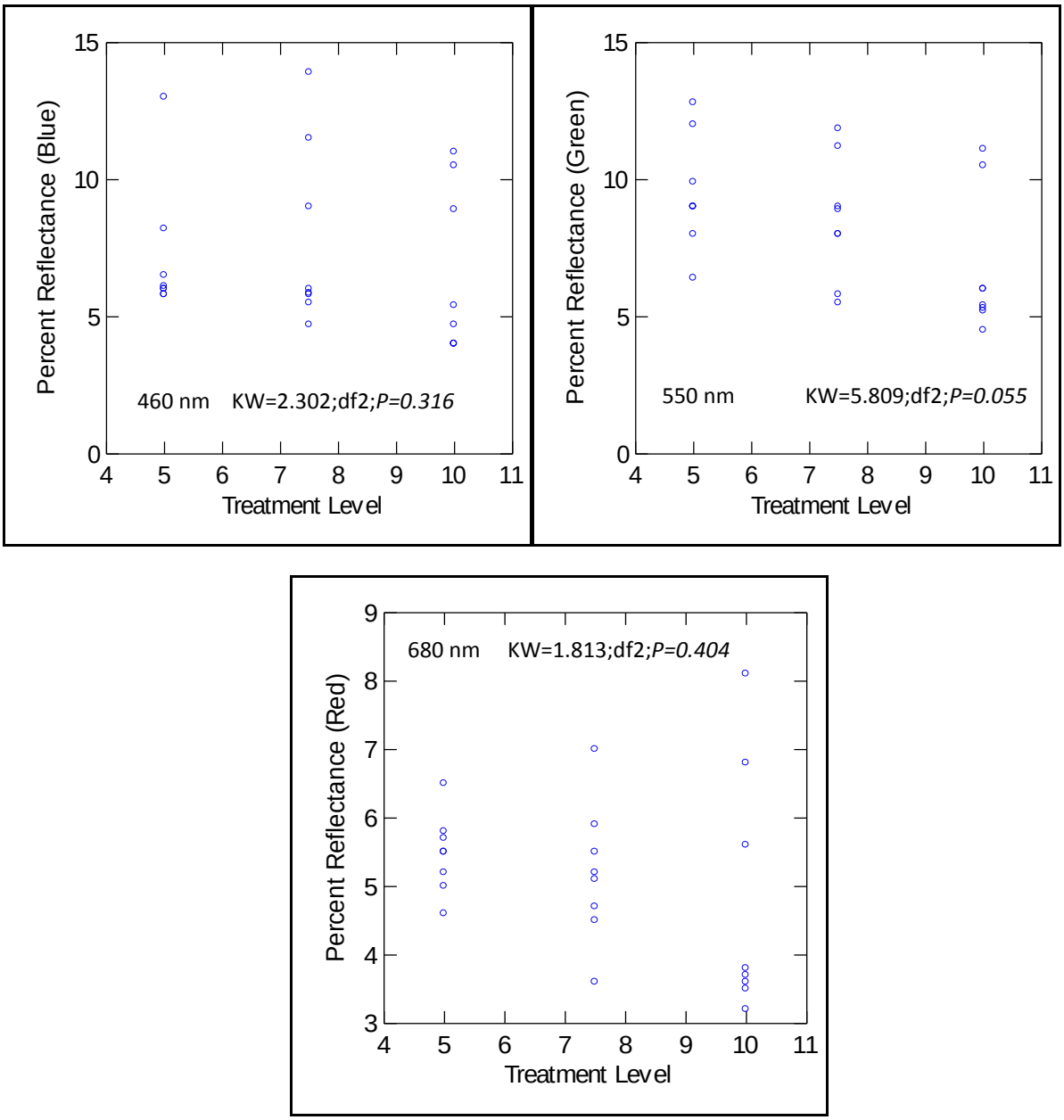

Figure 25. Non-parametic analysis of Experiment 1 and Experiment 2 combined data of spectral reflectance by sunflower foliage at three regions of the visible spectra (blue, green, and red) vs arsenic treatment levels (5ppm, 7.5ppm and 10ppm).

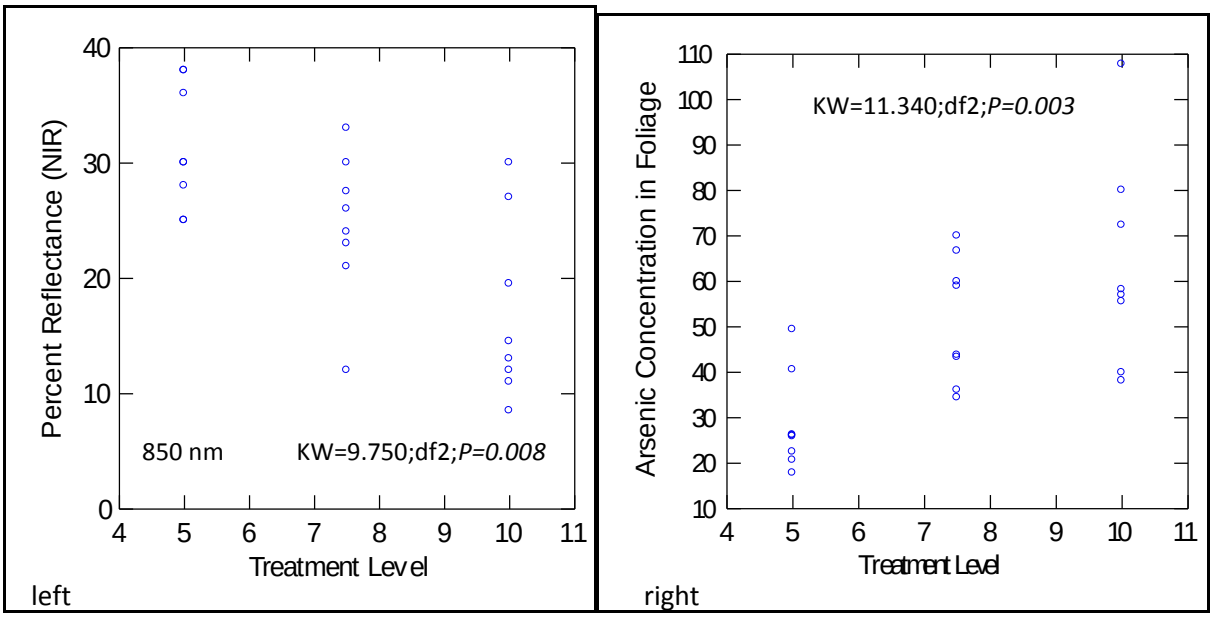

Figure 26. Non-parametric analysis of Experiment 1 and Experiment 2 using combined data of NIR spectral reflectance by sunflower foliage (left) and arsenic concentration (right) vs arsenic treatment levels (5ppm, 7.5ppm and 10ppm).

CHAPTER IV

SUMMARY AND CONCLUSIONS

Because of its toxicity, carcinogenicity, and widespread occurrence in the environment, the development of technologies to detect sites contaminated by significant levels of arsenic is likely to remain a priority in the foreseeable future. Although conventional chemical analyses will remain fundamentally important in the identification of arsenic-contaminated soil *per se*, several types of field analyses involving one or more plant species grown on contaminated soil may provide an effective surveillance method to detect As-contamination provided that the plant(s) in question accumulate arsenic in measureable quantities in foliage and other tissues, and that accumulated arsenic causes one or more physiological changes that can be detected and quantified.

Results of this study demonstrated the feasibility of using spectral reflectance by foliage of common sunflower as an "indicator" of arsenic contamination. In both experiments, arsenic concentrations in leaf tissues were directly proportional to arsenic concentrations in hydroponic solutions in which such plants were grown. Although the effect(s) of arsenic accumulation had minimal impact on reflectance of visible (blue, green and red) wavelengths, the effects on NIR reflectance were substantial and resulted in a progressive decrease in reflectance as arsenic

concentrations in foliage increased. While the underlying physiological mechanisms were not identified in this study, there clearly appears to be a reciprocal relationship between arsenic concentrations in sunflower foliage (which tends to increase with increases in exposure levels) and reflectance of NIR wavelengths (which tend to decrease with increases in exposure levels and hence, with foliar concentrations of arsenic).

Another potentially important effect of arsenic accumulation by common sunflower involves what appears to be a noticeable difference in size of leaves among plants exposed to various levels of arsenic (Fig. 24). This was particularly evident in the last two samples (weeks 4 and 5) of Experiment 2 and involved a progressive reduction in average leaf size among plants exposed to increasingly high levels of arsenic. Again, although the underlying physiological mechanisms were not identified, these results have significant implications in the use of remote sensing technology to detect "indicator" plants. NIR reflectance tends to increase with increases in biomass and conversely, tends to decrease as biomass decreases (Campbell 2007). Thus, fewer or smaller leaves on individual plants would be expected to result in a reduction of NIR reflectance greater than that resulting from relatively high foliar concentrations in foliage alone. If exposure to relatively high levels of arsenic is indeed associated with a reduction in average leaf size, then the combined effects of reduced biomass and reduction of NIR reflectance from individual leaves may greatly facilitate the detection of plants growing on As-contaminated soil using multispectral and/or hyperspectral imagery. This in turn, would greatly facilitate the identification of probable sites of contamination over large geographic areas that would be difficult, if not impossible, to survey using conventional methodology.

Data collected during the study demonstrated the feasibility of using spectral measurements of an "indicator" plant (common sunflower) as an adjunct to conventional

chemical analysis in the detection of arsenic contamination. If our conclusions are borne out in future research, the combined use of conventional chemical analyses and various types of remote sensing technology will facilitate surveys of large geographic areas that would be difficult to accomplish by other means. Future research that should be prioritized include promising areas: speciation of arsenic accumulated in the foliage, changes in spectra in comparison to other stress factors, the possibility to detect specific areas using hyperspectral technology, the effect of other organic and inorganic arsenic species on the leaf spectra of plants growing on soil and/or hydroponic systems, etc.

REFERENCES

Abbas-Mohamed, H. H. and Meharg, A. A. (2008). Arsenate, arsenite and dimethyl arsinic acid (DMA) uptake and tolerance in maize (Zea mays L). *Plant and Soil,* 304.1/2: 277-289.

Abedin, M. J., Cottep-Howells, J., and Meharg, A.A. (2002). Arsenic uptake and accumulation in rice (*Oryza sativa L*.) irrigated with contaminated water. *Plant and Soil*, 240, 311-319.

Abedin, M.J. (2002). Uptake kinetics of arsenic species in rice plants. *Plant Physiology*, 128(3): 1120-1128

Agency for Toxic Substances and Disease Registry (ATSDR).(2007). Toxicological Profile for Arsenic (Update). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Baskin, J. M.and Baskin, C.C. (1985). Annual Dormancy Cycles in Buried Weed Seeds: A Continuum. *Bioscience,* 35, no. 8: 492-498.

Bleeker, P. M., Schat, H., Vooijs, R., Verkleij, J., Ernst W. (2003). Mechanisms of arsenate tolerance in *Cytisusstriatus*.*New Phytologist*, 157, 33-38

Carbonell-Barrachina, A.A., Burlo-Carbonell, F., and Mataix-Beneyto, J. (1995). Arsenic uptake, distribution and accumulation in tomato plants: effect of arsenic on plant growth and yield. *J. Plant Nutrition*, 18, 1237–1250.

Calabrese, E. J., Stanek, R. C., and Roberts, J. (1997). Soil ingestion: a concern for acute toxicity in children. *Environ Health Perspectives,* 105(12): 1354–1358.

Campbell, J. B. (2007). Introduction to Remote Sensing. The Guilford Press, New York.

Chandler, J.M. and Jan, C.C. (1985). Comparison of Germination Techniques for Wild Helianthus Seeds. *Crop Science,* 25:356-358

Cullen, W.R., and Reimer, K.J. (1989). Arsenic Speciation in the Environment. *Chem. Rev*, 89: 713-784.

Davey, J.C., Bodwell, J, E., Gosse, J.A. and. Hamilton, J.W. (2007) Arsenic as an Endocrine Disruptor: Effects of Arsenic on Estrogen Receptor–Mediated Gene Expression In Vivo and in Cell Culture *Toxicological Sciences*, 98(1):75-86;

De La Rosa, Guadalupe et al. (2006). Spectroscopic Study of the Impact of Arsenic Speciation on Arsenic/ Phosphorus Uptake and Plant Growth in Tumbleweed (Salsola kali)."*Environmental Science & Technology* 40.6: 1991-1996

Duester, L., Van der Geest, H.G., Mowleken, S., Hirner, A.V., and Kueppers, K. (2010). Comparative phytotoxicity of methylated and inorganic arsenic-and antimony species to *Lemna minor*, *Wolffiaarrhiza and Selenastrum capricornutum.Microchemical Journal*, ***In Press, Corrected Proof***,

Everitt, J. H., Summy, K. R., Escobar, D. E., and Davis, M. R. (2003) An Overview of Aircraft Remote Sensing in Integrated Pest Management. *Subtropical Plant science* 55:59-67.

Everitt, J.H., Drawe, D.L., andLonard, R.I. (1999)Field guide to the broad leaved herbaceous plants of South Texas used by livestock and wildlife. TexasTechUniversity Press, Lubbock.

Feldmann, J. 2001. An appetite for arsenic.*Chemistry in Britain*, 37, 31-32

Foley, S., Rivard, B., Sanchez-Azofeifa, G.A. and Calvo, J. (2006) Foliar spectral properties following leaf clipping and implications for handling techniques. *Remote Sensing of Environment*, Volume 103, Issue 3, Spectral Network, 265-275,

Gausman, H.W., Allen W.A., and Cardenas, R. (1969).Reflectance of cotton leaves and their structure.*Remote Sensing of Environment*, 1:19–22.

Gates, H.J., Keegan, J.C. Schleter, and Weidner, V.R. (1965) Spectral properties of plants, Applied *Optics* 4, 19:11–20.

Hernandez, L. F. and Orioli, G,A., (1985)Imbibition and germination rates of sunflower (*Helianthus annuus* L.) seeds according to fruit size. *Field Crop Research*, 10: 355-360.

Hoagland, D. R. and Arnon, D. I. (1950) The water culture method for growing plants without soil. Calif. Agric. Exp. Sta. Circ. 347.

Horler, D., Barber, N., and Barringer, H. (1980)Effects of Heavy Metals on the Absorbance and Reflectance Spectra of Plants. *International Journal of Remote Sensing* 1, 121-136.

ImamulHuq, S.M., Rahman, A., Sultana, S., Naidu, R. (2003) Extent and severity of arsenic contamination in soils of Bangladesh. In: F. Ahmed, Ali, Z.A., (Eds.), Fate of arsenic in the environment. BUET_UNU Int. Symp. Dhaka, Bangladesh 69-84.

Jensen, J. R. (2007). Remote Sensing of the Environment: An Earth Resource Perspective, 2nd Ed. Prentice-Hall, Upper Saddle River, NJ.

Kristen, K. Arsenic in Old Herbicides Comes Back to Haunt Denver. (2000). *Environmental Science and Technology*, 34:376A

Lillesand, T. M., Kiefer, R. W., and Chipman, J. W. (2004).Remote Sensing and Image Interpretation, 5th Ed. John Wiley & Sons, New York.

Lee, L., and Herbert, B. (2003). Geogenic and Anthropogenic Sources of Arsenic and Associate Trace Metals in Texas Groundwater. Statistical Analysis for Toxicology, 10 Nov. 2003. *The Society of Environmental Toxicology and Chemistry*. College Station, Texas: TexasA&MUniversity.

Ma, L.Q, Komar, K.M., Tu, C., Zhang, W.H, Cai, Y, Kennelley, E.D. (2001). A fern that hyperaccumulates arsenic - a hardy, versatile, fast-growing plant helps to remove arsenicfrom contaminated soils. *Nature* 409, 579-579

Maiti, R. K., et al. (2006). Development and Standarization of a Simple Technique for Breaking Seed Dormancy in Sunflower (*Helianthus annuus L.*).*Helia* 29.45 117-125.

Marin, A.R., Masscheleyn, P.H., Patrick Jr., W.H. (1992). The influence of chemical form and concentration of arsenic on rice growth and tissue arsenic concentration.*Plant and Soil* 139, 175–183.

Marin, A.R., Pezeshki, S.R., Masscheleyn, P.H. Choi, H.S. (1993). Effect of dimethylarsinic acid (DMAA) on growth, tissue arsenic andphotosynthesis of rice plants.*Journal of Plant Nutrition*, 16:865–880.

Maruthi, S., Han, F.X., Diehl, S.V., Monts, D.l. and Su, Y. (2007).Monitoring the Effectss of Arsenic and Chromium Accumulation in Chinese Brake Fern (*PterisVittata*).*International Journal of Remote Sensing*, 28: 1055-1067.

Meharg, A. A. and Hartley-Whitaker, J.(2002). Arsenic uptake and metalbolism in arsenic resistant and non resistant plant species.*New Phytologist*, 154: 29-43.

Meharg, A.A., Macnair, M.R. (1992). Suppression of the high-affinity phosphate-uptake system – a mechanism of arsenate tolerance in (*Holcuslanatus L).Journal of Experimental Botany*, 43: 519–524.

Meharg, A.A, Macnair M.R. (1994). Relationship between plant phosphorus status and the kinetics of arsenate influx in clones of Deschampsiacespitosa *(*L.)Beauv that differ in their tolerance to arsenate. *Plant and Soil* 162: 99-106.

Melamend, D. (2004). Monitoring Arsenic in the Environment: a Review of Science and Technologies for Field Measurements and Sensors.US Environmental Protection Agency. Washington, D.C.

Misra, A., and Sharma, S. (2006) Critical Fe concentration and productivity of Java citronella.*Rev. Bras. Pl. Med., Botucatu,* 8:54-58.

Monni, S, Bucking. H., Kottke, I.(2002). Ultra structural element localization by EDXS in *Emperum nigrum*.*Micron*, 33:339-351.

Ng, J.C, Wang, J.P, Shraim, A. (2003) A global health problem caused by arsenic from naturalsources.*Chemosphere* 52:1353-1359.

Nahar, N., Faisal Hossain, and M. DelawerHossain. (2007): Health and Socio-Economic Effects of Groundwater Arsenic Contamination in Rural Bangladesh: New Evidence from Field Surveys. *Journal of Environmental Health,* 12:19.

Nriagu, J. (1994) Arsenic in the Environment. John Wiley, N. Y.

Onken, B. M., Hossner, L.R. (1995) Plant uptake and determination of arsenic species in soil solution under flooded conditions. *Journal Environmental Quality*, 24:373-381.

Peñuelas, J and Filella, I. (1998) Visible and near-infrared reflectance techniques for diagnosing plant physiological status, *Trends in Plant Science*, 3:151–156.

Pickering, I. J., Prince, R.C., George, M. J., Smith, R.D. (2000). Reduction and coordination of arsenic in Indian mustard. *Plant physiology*, 122:1171-1177.

Raab, Andrea, et al. (2005). Uptake, translocation and transformation of arsenate and arsenite in sunflower (Helianthus annuus): formation of arsenic–phytochelatin complexes during exposure to high arsenic concentrations. *New Phytologist*, 168.3 551-558.

Raab, Andrea, et al. (2007) Can arsenic–phytochelatin complex formation be used as an indicator for toxicity in Helianthus annuus L. *Journal of Experimental Botany* 58.6: 1333-1338.

Rahman, M.A., Rahman, M.M., Miah, M.A.M., Khaled, H.M. (2004). Influence of soil arsenic concentrations in rice (OryzasativaL.). *J. Subtropical Agriculture Res. Dev*. 2, 24–31.

Rahman, M.A. Hasegawa, H., Mahfuzur, M., Rahman, M. Nazrul Islam, M.A. MajidMiah, A. Tasmen, (2007). Effect of arsenic on photosynthesis, growth and yield of five widely cultivated rice (Oryza sativa L.) varieties in Bangladesh, *Chemosphere*, 67(6): 1072-1079.

Sinclair, T.R., Hoffer, R.M., and Schreiber, M.M. (1971). Reflectance and internal structure of leaves from several crops during a growing season, *Agronomy Journal*,63: 864–868

Sokal, Robert R and Rohlf, F. James. (2000) Biometry. The Principles and Practice of Statistics in Biology Research. Third Edition. W.H. Freeman and Company, NY.

Stilwell, David E. (2002) Excerpts on Uptake of Arsenic by Plants Grown Near CCA Preserved Wood. Bull. Environmental Contamination Toxicology.

Summy, K. R., C. R. Little, R. A. Mazariegos, J. V. French, P. Haslem, J. H. Everitt, and W. Scott, Jr.(2005). Detection of stressors in glasshouse crops using color infrared imagery: II Damage caused by insect and mite pests. Proc. 20th Biennial Workshop on Aerial Photography, Videography, and High-Resolution Digital Imagery for Resource Management. American Society for Photogrammetry and Remote Sensing, Weslaco,TX,

Thornton, I. (1994). Sources and Pathways of arsenic in south-west England: Health Impication in Arsenic exposure and health, chapter 6, pp 61-70 W. Chappell (Ed), Science and Technology Letters, Northwood, England.

Tu, C., Ma, L.Q., Bondada, B. (2002) Arsenic accumulation in the hyperaccumulator Chinese Brake and its utilization potential for phytoremediation. *Journal of Environmental Quality,* 31, 1671-1675

Tu, C, Ma, L.Q., Zhang, W., Cai, Y., Harris, W.G. (2003) Arsenic species and leachability in the fronds of the hyperaccumulator Chinese brake (*Pterisvittata L.*). *Environmental Pollution,* 124, 223-230

Turpeinen, R, Pantsar-Kallio M, Haggblom M, Kairesalo T. (1999).Influence of microbes on the mobilization, toxicity and biomethylation of arsenic in soil. *Science of the Total Environment* 236: 173–180.

Twarakavi, N.K.C. and Kaluarachchi, J.J. (2006). Arsenic in ground waters of conterminous United States: Assessment, health risk, and cost.

USEPA (2002) Arsenic treatment technologies for soil, waste, and water. http://www.clu-in.org/arsenic

Wang, J., Zhao, F.J., Meharg, A.A, Raab, A., Feldman, J., McGrath, S.P. (2002) Mechanisms of arsenic hyperaccumulation in *Pterisvittata*. Uptake kinetics, interactions with phosphate,and arsenic speciation. *Plant Physiology,* 130:1552-1561.

World Health Organization (WHO).Arsenic and arsenic compounds. Geneva: World Health Organization, 2001. 521 p. (Environmental health criteria no. 224).

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