Effect of Bioaccumulation of Cs and Sr Natural Isotopes on Foliar Structure and Plant Spectral Reflectance of Indian Mustard (*Brassica juncea*) – 8105

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ABSTRACT

The objectives of this study are: 1.) evaluate the capacity of Indian mustard (*Brassica juncea*) for uptake and accumulation of Cs and Sr natural isotopes; 2.) identify foliar structural and other physiological changes (biomass, relative water content, etc.) resulting from the accumulation of these two elements; and 3.) monitor Cs and Sr uptake and bioaccumulation process by spectral reflectance. Potted Indian mustard plants were exposed to different concentrations of Cs (50 and 600 ppm) and Sr (50 and 300 ppm) natural isotopes in solution form for 23 days. Bioaccumulation of Cs and Sr was found in the order of leaves > stems > roots for both Cs- and Sr-treated plants. The highest leaf Sr accumulation is observed to be 2708 mg kg^{-1}, and the highest leaf Cs accumulation is 12251 mg kg^{-1}. High translocation efficiency for both elements is documented by shoot/root concentration ratios greater than one. Relative water content (RWC) of the plants showed a significant ($p < 0.05$) decrease in Cs-treated plants. Cs accumulation also affected the pigment concentration and internal structure of the leaf and the spectral characteristics of plants. Within the applied concentration range, Sr accumulation resulted in no significant changes in RWC, structural and spectral characteristics of mustard plants. Cs shoot concentration showed significant negative correlation with relative water content RWC ($r = -0.88$) and Normalized Difference Vegetation Index (NDVI) ($r = -0.68$) of plant shoots. The canopy spectral reflectance and NDVI analysis clearly revealed ($p < 0.05$) the stress caused by Cs accumulation.

INTRODUCTION

Radionuclide contamination of the global environment has resulted from aboveground nuclear testing, weapons production, accidental spills and emissions from nuclear fuel cycle operations, mining and milling, etc. The presence of radionuclides in soil and water poses serious threats to populations inhabiting in or near the contaminated environments. Removal of the top soil layer (soil excavation) and transfer to designated repositories remains the most common method of treating contaminated soil. Because of the high cost, decontamination of large areas polluted with radionuclides, such as ^{137}Cs and ^{90}Sr, by conventional engineering methods remains an intractable problem. In addition, these methods often have an adverse effect on biological activity, soil structure and fertility, and disturb the ecosystem. Phytoremediation is an emerging technology that

uses various plants to degrade, extract, contain or immobilize contaminants from soil and water. Phytoextraction is the process of concentrating contaminants in the stems and leaves of metal accumulating-plants [1]. The development of phytoremediation is being driven primarily by the high cost of other soil remediation methods as well as the desire to use an environmentally benign process. Recently, Dushenkov published a review on trends in phytoremediation of radionuclides [2]. Several studies have demonstrated the phytoremediation potential of plants to accumulate ^{137}Cs and ^{90}Sr. ^{137}Cs and ^{90}Sr are regarded as important radionuclides in radioecology, because of their relatively high fission yields and influence on human health. Phytoremediation of ^{137}Cs was suggested to approach field deployment as a site-specific technology [3]. The phytoremediation potential of Alamo switchgrass and two other grass species for ^{137}Cs and ^{90}Sr was investigated by Entry *et al*. [4,5]. Lasat *et al*. reported their field study and green house study on phytoremediation of radiocesium-contaminated soil by three plant species: red root pigweed, Indian mustard and tepary bean [6,7]. Later Fuhrmann *et al*. published field study results of phytoextraction of ^{137}Cs and ^{90}Sr by the same three plant species [8]. Among trees, ^{90}Sr and ^{137}Cs uptake and distribution was studied in *Salix* species (osier willows), where ^{90}Sr accumulates mainly in leaves and ^{137}Cs mainly in roots [9].

The success of phytoextraction, as an environmental cleanup technology, depends on several factors, including the extent of soil contamination, bioavailability, and the plant ability to intercept, absorb, and accumulate contaminants in shoots [10]. The soil-to-plant transfer factor (TF) is regarded as one of the most important parameters in environmental impact assessment of radionuclides. Environmental processes affecting plant root uptake of radioactive trace elements has been reviewed by Ehlken and Kirchner [11].

Elucidation of plant-based remedial mechanisms may provide clues for optimizing the effectiveness of phytoremediation with appropriate agronomic practices, as pointed by Lasat [12]. It is extremely important to monitor and understand the processes of uptake, translocation, and accumulation of heavy metals and radionuclides by plants. Plants are known to slowly alter their response mechanisms to adapt to metal-contaminated environments. Most of these response mechanisms result in long-term metabolic and morphological changes. These include changes in pigment concentration, water content, dry weight and growth of plants.

Plants are not capable of distinguishing isotopes of the same elements. Radioactive isotopes are widely used as tracers in plant physiology and biochemistry. In some cases plants react analogously to ions with similar physicochemical properties. It is known that Sr is an analogue of Ca in living organisms [13] and the effect of K on ^{137}Cs accumulation in plants has also been reported [14]. It is important to understand the behavior of abundance natural isotopes in the environment (distribution, pathways, mobility, transfer, etc.) because the information can be used as an indicator of the long-term behavior and processes of radiological isotopes [15]. It is also of great significant to acquire important baseline data on the chemical and biological effects of Cs and Sr on plants.

Plant reflectance is governed by leaf surface properties and internal structure as well as by the concentration and distribution of foliar pigments and biochemical components [16-18]. Hence changes in plant structure result in changes in plant reflectance. Thus analysis of remotely sensed reflected light can be used to assess both the biomass and the physiological status of plants [18, 19]. Several studies have demonstrated that by analyzing the spectral reflectance of plants, the changes caused by metal stress can be identified [20-22].

Indian mustard (*Brassica juncea*), a high biomass producing plant, has been used as a model system to investigate the physiology and biochemistry of metal and radionuclide accumulation in plants [3,7,8,23-26]. In our group, phytoremediation potential of Indian mustard plants for both Cr^{6+} and Cr^{3+} forms of Cr contaminants [27] and for Zn, Cd and As contaminants has been studied [28].

In the study reported here, we evaluated the uptake and accumulation capacity of Indian mustard plants for natural abundance Cs and Sr isotopes. To by-pass soil-sorption and other bioavailability determining factors, we used potting mix and both Cs and Sr were provided as nitrate solutions. The focus of the current study was to investigate the impact of plant-accumulated Cs and Sr on foliar internal structure and spectral reflectance characters of plant canopy. Our objectives are: 1.) evaluate the capacity of Indian mustard for uptake and accumulation of natural abundance Cs and Sr isotopes; 2.) identify foliar structure and other physiological changes (biomass, relative water content, etc.) resulting from the accumulation of these two elements; and 3.) monitor Cs and Sr uptake and accumulation processes by spectral reflectance. The resulting information will contribute to determine whether the spectral reflectance of plants can be used as an inexpensive, non-destructive monitoring method for assessing the physiological status of plants in Cs- and Sr-contaminated environments; and hence whether remote-sensing technology can be used for long term monitoring of the bioavailability and mobility of these radionuclide contaminants.

MATERIALS AND METHODS

Plant Culture and Phytoextraction Experimental Design

The mustard seeds were a commercial variety purchased from Siegers Seed Co. (Holland, MI) and the soil used for the pot study was Miracle-Gro Potting Mix (Marysville, OH). The seeds were sown in plastic pots, each containing approximately 2.0 kg of potting mix. The plants were kept outdoors in an enclosed area except during extreme weather conditions. The seedlings were thinned to two plants per pot at the 2-3-leaf stage. Modified Hoaglands Nutrient solution [29] was supplied to the plants daily after the plants attained 3-4 leaves. The composition of the nutrient solution was 0.5 mM $Ca(NO_3)_2$, 3.1 mM NH_4NO_3, 0.01 mM KH_2PO_4, 50.0 μM KCl, 0.2 μM $CuSO_4$, 12.0 μM H_3BO_4, 0.1 μM $NiSO_4 \cdot 6H_2O$, 2.0 μM $MnSO_4 \cdot H_2O$, 0.5 μM $ZnSO_4 \cdot 7H_2O$, and 0.2 mM $MgSO_4$. Metal treatments with five replicates in each group were supplied with 50 ppm (Cs50), 600 ppm (Cs600) of $CsNO_3$ solution and 50 ppm (Sr50) and 300 ppm (Sr300) of

$Sr(NO_3)_2$ solution. The Cs and Sr solutions were prepared from standard solutions supplied by Spex Certi Prep (Metuchen, NJ).

All the treatment groups along with the control (T0) group were arranged in a completely randomized design. The metal treatment at the rate of 100 ml pot^{-1} day^{-1} was started at five weeks after plant emergence. The metal treatments were supplemented with nutrient solution to avoid any water or nutrient deficiencies. Twenty-three days after the start of metal treatment, all the plant groups (n = 25) were harvested for metal accumulation analysis and microscopic studies.

Experimental Set-Up for Spectral Reflectance

A Fieldspec Pro spectroradiometer (350–2500 nm) from ASD Inc. (Boulder, CO) was used to collect reflectance spectra with quartz-tungsten-halogen (QTH) lamps in laboratory. Diffused light from two 100W Lowell Pro-Lights was used to illuminate the plant canopy at 45° angles in the laboratory. The foreoptics were aligned vertically and the height of the foreoptics from the plant canopy level was adjusted so that the whole canopy filled the field-of-view (FOV) of the instrument. The experimental setup was put on top of a four-wheel wagon and a black cloth was used as a non-reflective background for spectral collection. Calibration spectra using a white Lab-Sphere Spectralon panel were acquired before canopy spectra were recorded. Diffuse reflectance spectra (350 nm to 2500 nm) of all the plant canopies (n = 25) were collected regularly with laboratory illumination and with solar radiation during cloud free days. Spectra collection was started prior to the beginning of the metal treatment procedure. Both outdoor and laboratory spectra were recorded throughout the metal treatment process for all the mustard plants (n =25), while only laboratory spectra were collected on cloudy days.

Microscopy

Leaf and stem samples from 15 mustard plants treated with T0, Cs600 and Sr300-treatments were collected on the last day of metal treatment (23^{rd} day). The leaf samples were 5 mm in length and excised from the middle portion of the lamina in between the second and third leaf vein counting from the leaf midrib. The samples (n =15) were collected from the third leaf counting from the stem-root intersection. Stem samples (n =15) were excised from 2 cm above the stem–root intersection. All the plant samples were immediately fixed in formaldehyde-acetic acid (FAA) and prepared for light microscopy (LM). The plant samples were alcohol dehydrated, paraffin embedded, and ultramicrotomed and subjected to safranin (0.1%) – fastgreen (0.2%) or toluidine blue (1%) staining to observe the structural changes [30].

Chemical Analysis

All the potted (n =25) mustard plants were cut about 2 cm above soil at the end of the pot study. The leaves, stems and roots were harvested and dried at 80° C in an oven for 48 hours, plant roots were washed with deionized water before being put into oven. Dry leaves, stems and roots were then ground and weighed. Plant samples (approximately 0.5

g) were digested with concentrated HNO_3 and H_2O_2 [31]. The digested solution was filtered and then analyzed for Sr concentration using inductively coupled plasma-atomic emission spectrometry (ICP-AES). Cs concentrations were determined by atomic absorption spectrometry (AA).

Spectral and Statistical Analysis

The reflectance spectra for canopies of each treatment groups were averaged to overcome individual variations. Normalized difference vegetative index (NDVI) was calculated with the averaged spectra (n =5) for each treatment. NDVI [32], a widely used vegetative index to monitor plant stress, is given by NDVI = $(R_{810} - R_{680})/ (R_{810} + R_{680})$. All the spectral analysis was performed using Microsoft Excel spreadsheets. Changes in spectral reflectance along with metal accumulation in shoots and roots and plant physiological characteristics (dry weight and relative water content) were used to characterize the phytoextraction process. Relative water content (RWC) is defined as (fresh weight – dry weight)/ fresh weight. Pearson's correlation coefficients were calculated and Duncan's multiple range tests were performed using SAS statistical analysis software (SAS Institute Inc., NC).

RESULTS

Cs and Sr Accumulation and Plant Characteristics

Mustard plants in all Cs- and Sr-treated groups grew steadily and chlorosis was not visually observed during the treatment process. Cs and Sr accumulation in leaves, stems and roots, shoot/root ratios, fresh weight, dry weight, RWC, and NDVI values of the plants are summarized in Table 1 for Cs- and Sr-treated groups. The metal accumulation in plant leaves, stems and roots increased significantly ($p<0.05$) with an increase in applied metal solution concentration in all the Cs- and Sr- treated groups. Accumulation of Cs and Sr remained high in leaves followed by stems and roots for Cs- and Sr-treated plants.

The ratios of shoot to root metal concentrations were calculated to indicate the translocation efficiency of Cs and Sr from root to shoot. As Cs and Sr (Table 1) concentrations were higher in stems and leaves over roots, the shoot/root ratios remained greater than one for both Cs- and Sr-treated plants. This indicates that mustard has high Cs and Sr uptake efficiency when the contaminants are bioavailable. The fresh weight and dry weight of plants showed a significant ($p<0.05$) decrease in both Cs600- and Sr300-treated groups when compared to the control group (T0). RWC of plants showed significant ($p<0.05$) decrease in only Cs600-treated plants (Table 1).

Structural Changes

The Cs600-treated (Fig. 1B) plants show significant foliar structural changes compared to the control-T0 (Fig. 1A) plants. No structural changes in leaves were observed in Sr- (Fig. 1C) treated plants compared to control (Fig. 1A) plants. The light micrographs

Table I. Cs and Sr accumulation in leaves, stems and roots (in mg kg^{-1} dry weight), shoot/rooot ratio, fresh weight, dry weight, relative water content (RWC) and NDVI of plants treated with Cs (n=5) and Sr (n=5) at the end of the experiment. The given values are means ± standard error of five replicates

Treatment	Leaf conc. (mg kg^{-1})	Stem conc. (mg kg^{-1})	Root conc. (mg kg^{-1})	Shoot /Root Ratio	Fresh wt. (g)	Dry wt. (g)	RWC (%)	NDVI
Cesium								
T0	nd	nd	nd	nd	173 a (± 12)	9.4 a (± 1)	95 a (± 0.002)	0.818 a (± 0.01)
Cs50	1929 b (± 295)	1145 b (± 192)	535 b (± 127)	2.7	145 a (± 25)	8.3 a (± 2)	94 a (± 0.003)	0.805 a (± 0.01)
Cs600	12251 a (± 932)	8163 a (± 590)	6794 a (± 621)	1.4	95 b (± 12)	7.1 b (± 0.8)	93 b (± 0.002)	0.736 b (± 0.02)
Strontium								
T0	68 c (± 3)	71 c (± 3)	44 c (± 2)	1.5	173 a (± 12)	9.4 a (± 1)	95 a (± 0.002)	0.818 a (± 0.01)
Sr50	457 b (± 22)	517 b (± 33)	318 b (± 29)	1.5	175 a (± 15)	9.2 a (± 0.8)	95 a (± 0.001)	0.794 a (± 0.01)
Sr300	2708 a (± 190)	2660 a (± 254)	1194 a (± 98)	2.1	126 b (± 16)	6.9 b (± 1)	95 a (± 0.001)	0.798 a (± 0.02)

† Means followed by a different letter are significantly different at the 0.05 probability level, grouped into classes a and b; nd, not detected.

obtained from the leaf samples of Cs600-treated plants show changes in the distribution of chloroplasts in palisade and spongy parenchyma cells (Fig. 1B) compared to the control (Fig. 1A) leaves. The microscopic observations were based on all the replicates (n=5) within each treatment group. Light micrographs of Cs600-treated plant stems (Fig 2B) showed thickly stained areas along the walls of xylem and phloem vessels compared to control (Fig 2A) stems.

Spectral Analysis

Spectral measurements of canopies of each treatment group were obtained regularly during the metal treatment process and were averaged (n =5) to overcome individual variations. Also the NDVI of each treatment group were calculated throughout the metal treatment process. The NDVI values calculated from the averaged canopy level spectra of the last day of metal treatment show significant (p<0.05) decrease for plants treated with

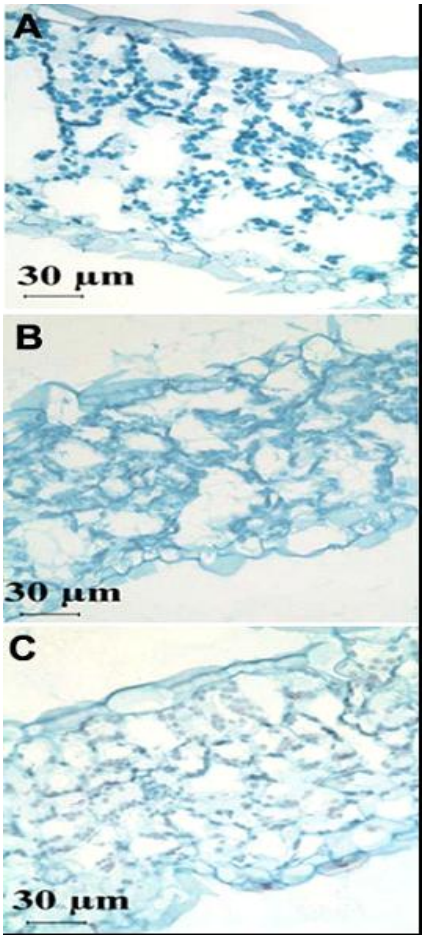

Figure 1. Light micrographs showing leaf transverse sections of control (A), Cs600- (B) and Sr300- (C) treated plants stained with safranin-fastgreen. The leaves of Cs600-treated plants (B) show large intercellular spaces decrease in number of chloroplasts compared to control plants (A). The leaves of Sr300- (C) treated plants show no such structural changes compared to their control plants (A). The samples were collected from the last day (23rd day) of metal treatment.

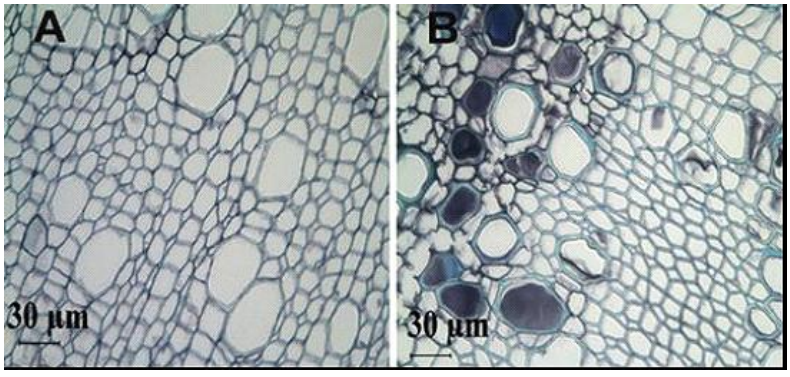

Figure 2. Light micrographs showing the transverse section of stem of control (A) and Cs600-treated plants (B) stained with toluidine blue. The Cs600- treated stems showed thickly stained areas surrounding the vascular bundles of stems (B) compared to control stems (A).

Cs600 (Table 1). No significant changes were observed in RWC and NDVI values of Sr-treated plants.

Pearson's correlation coefficients were calculated in order to identify the significant correlations between spectral indices, metal accumulation, and physiological characters of the plants. NDVI showed significant negative correlation with Cs leaf (r = -0.68*; $p<0.05$) concentrations. No such correlations were seen for NDVI with metal accumulation in leaves of Sr-(r = -0.03) treated plants. RWC of Cs-treated plants showed significant ($p<0.01$) negative correlation with Cs concentrations in stems (r = -0.88**) and roots (r = -0.88**) of Cs-treated plants. No such correlations were found for RWC with metal accumulation in Sr-treated plants.

The spectral measurements of canopies of Cs-treated plants show a consistent and systematic difference from untreated plants starting from the 18^{th} day of the metal treatment. No significant decrease in spectral reflectance was observed in Sr-treated plants. Fig. 3 shows the canopy level spectral reflectance of Cs- and Sr-treated plants obtained on the last day of metal treatment. During the metal treatment process, the Cs-treated plants started to show a decrease in reflectance in 800-1300 nm, 1470-1850 nm and 2000-2400 nm regions from 18^{th} day onwards. The spectral changes in Sr- (Fig. 3B) treated plants were not significantly different from control (T0) plants. The canopy-level spectra were also confirmed and found to be in consistent with the leaf-level spectra (data not shown) obtained from single leaf layer lying on a dark background from the last day of treatment for all Cs- and Sr-treated groups. Since Indian mustard is a leafy plant the contribution of soil reflectance to the plant canopy level reflectance is minimum. Only laboratory spectral results are shown in this paper; the outdoor results are consistent with the laboratory data except that there is more noise (due to atmospheric interference).

The NDVI results obtained during the metal treatment period are given in Fig. 4 for Cs- and Sr-treated plants. The Cs600-treated (Fig. 4A) plants show significant ($p < 0.05$) decrease in NDVI compared to control (T0) plants from the 18^{th} day onwards. The Sr-treated plants (Fig. 4B) show no significant difference in NDVI from untreated plants during the metal treatment process. The stress caused by Cs accumulation increased along with the progress of metal treatment. We believe that the stress indicated by NDVI (Fig. 4A) in Cs-treated plants is due to changes in distribution of chloroplasts as observed in LM micrographs (Fig 1B) and other physiological and morphological changes (Table 1) in mustard plants.

DISCUSSION AND CONCLUSIONS

Our results provide important baseline data on the chemical and biological effects of Cs and Sr on plants. Cs accumulation resulted in significant changes in the structural (Fig. 1B) and growth characteristics (Table 1) of mustard plants. The spectral (Fig. 3) and NDVI (Fig. 4) results clearly indicate that the response of mustard plants to Cs was different from Sr-treated plants (Table 1). The distribution of Cs- and Sr- concentration in mustard plants followed the order leaves > stems > roots, which agrees well with other studies of ^{135}Cs and ^{90}Sr accumulation by plants [3,7,8]. Fricks *et al*. [9] reported

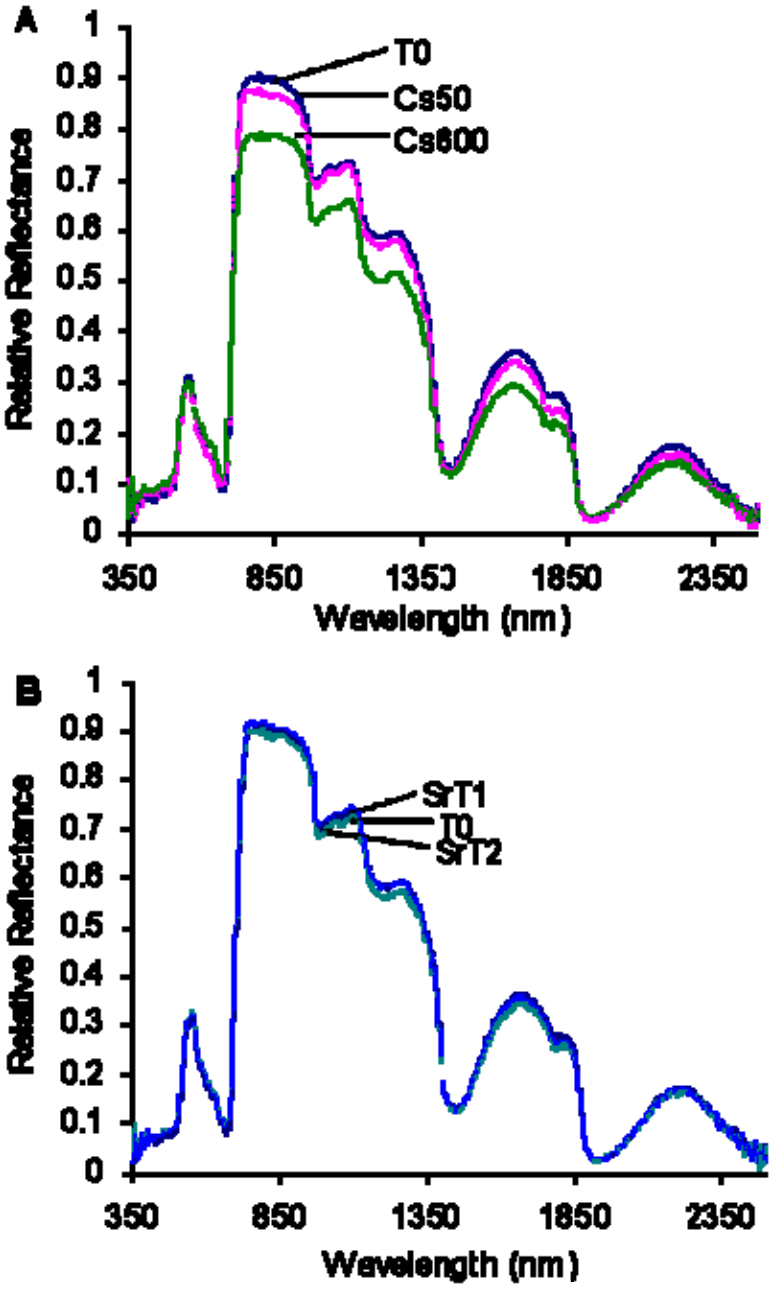

Figure 3. Averaged canopy level spectral reflectance of Cs- (A) and Sr- (B) treated plants on last day of the metal treatment process. Bars are ± standard error of the mean (n = 5).

accumulation of ^{90}Sr mainly in leaves and ^{137}Cs in roots of *Salix* species. However in our study both Cs- and Sr-accumulation were found to be at higher concentrations in leaves over other plant parts for Indian mustard. It has been widely reported that the response to certain metals by different plants varies. Accumulation of Cs in the leaves (1929 mg kg^{-1}) and stems (1145 mg kg^{-1}) of mustard plants was higher than Sr accumulation in leaves (457 mg kg^{-1}) and stems (517 mg kg^{-1}) when applied at equal concentrations of 50 ppm

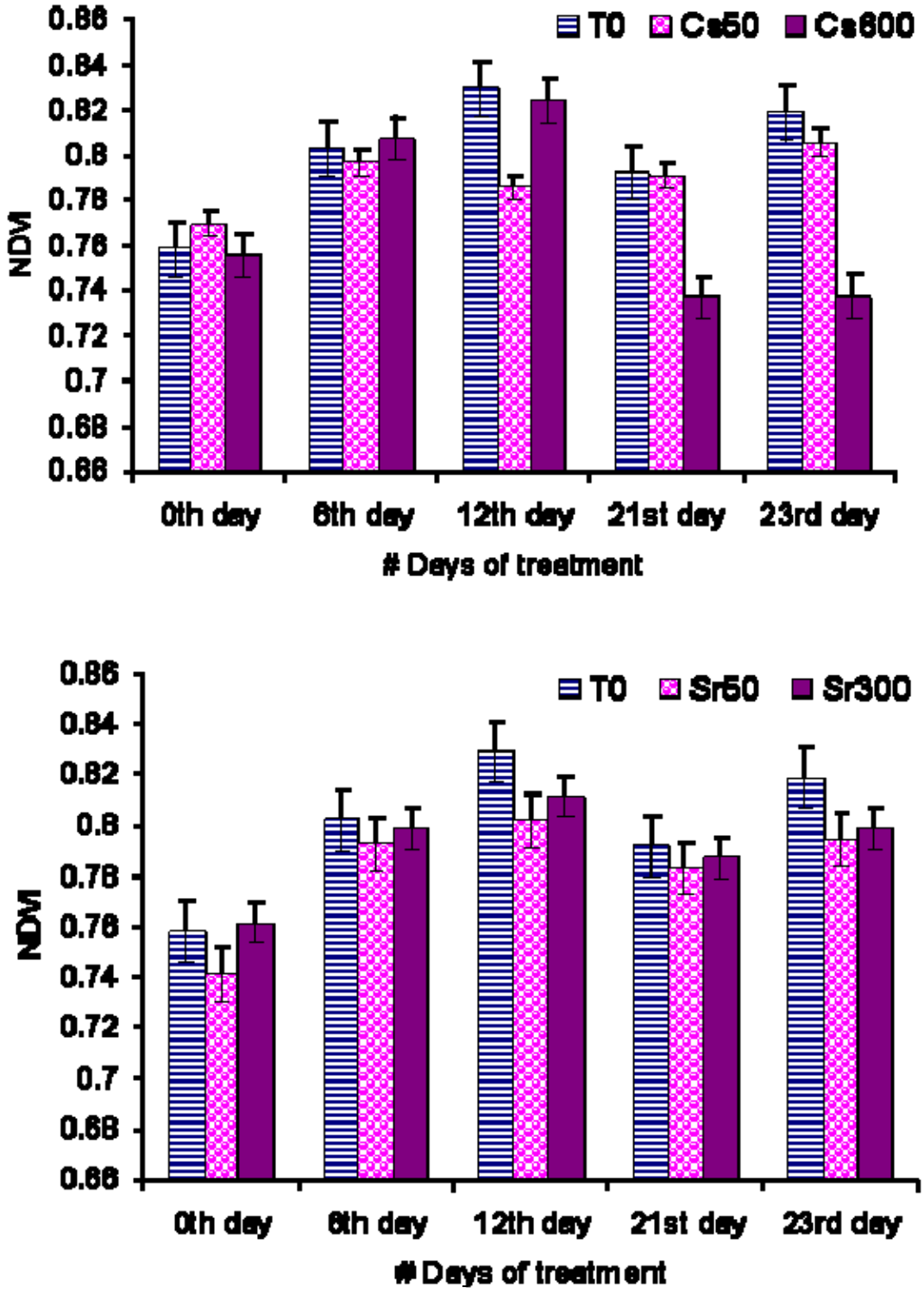

Figure 4. Changes in NDVI of plants during the Cs- (A) and Sr- (B) treatment process. Bars are ± standard error of the mean (n = 5).

each in Sr50- and Cs50-treated plants. However higher accumulation of Sr over Cs in *Salix* plants [9] and Alamo switch grass [4] were reported. Accumulation of Cs resulted in a significant decrease in spectral reflectance of plants at both canopy and leaf level. No significant decrease in spectral reflectance was observed in Sr-treated plants. This agrees with the significant changes in structural and physiological characters of Cs-treated plants.

Our results show NDVI (Fig. 4A) could be used to monitor plant stress caused by metal accumulation. NDVI results clearly reveal the stress caused by deformation of chloroplasts and decrease in biomass of plants (Table 1) with an increase in Cs concentration in Cs600-treated plants. NDVI is sensitive to chlorophyll concentration, internal structure of leaf [33] and biomass of plants [18]. The decrease in chloroplast distribution, RWC and changes in internal structure of leaf due to excess Cs accumulation resulted in decrease in spectral reflectance and in NDVI value of the Cs600-treated plants when compared with the control group (T0). These results are also confirmed by significant negative correlation of NDVI ($r = -0.68^*$) with Cs leaf concentration in Cs-treated plants. In the case of Sr-treated plants, no significant changes in chloroplast distribution, internal structure, RWC and other growth characters of plants were observed at the concentration levels tested. Thus accumulation of Sr caused no significant decrease in spectral reflectance and NDVI of Sr-treated plants. Consequently no significant correlations of NDVI with Sr- ($r = -0.03$) leaf concentration were observed in Sr- treated plants.

Our results indicate that accumulation of Sr and Cs is higher in leaves and stems when these contaminants were continuously bioavailable. In our experiment, Indian mustard plants accumulated high concentration of natural abundance Sr isotope in shoots (leaves and stems) without detectable stress. The highest leaf Sr accumulation is observed as 2708 mg kg^{-1}. High translocation efficiency is documented by shoot/root Sr concentration ratio greater than one (Table 1). These results indicate that Indian mustard plants maybe a good candidate for Sr phytoremediation. Uptake and accumulation of high concentration of Cs resulted in foliar internal structural change in Cs-treated plants. NDVI analysis revealed stress caused by Cs accumulation. With highest leaf Cs accumulation being 12251 mg kg^{-1}, and shoot/root Cs concentration ratio greater than one, plus the fact that Indian mustard is a high biomass producing plant, the potential of Indian mustard plant as a candidate for Cs phytoremediation still warrants consideration. However, Cs and Sr were made continuously available to the plants as nitrate solution (a bioavailable form) in our experiment; further studies under field condition are needed before any conclusion about field applicability can be made.

Spectral and growth characteristics indicate that Cs has a profound impact on the physiology and internal structure of mustard plants. The spectral results revealed that Cs accumulation at higher concentration affects the internal structure and number of chloroplasts in leaves. Our results suggest that it is feasible to use plant spectral reflectance for long-term monitoring of physiological stress caused by natural abundance Cs contaminants during the process of phytoextraction. However, further lab scale and field scale research is necessary to extend the application of this research to radioactive

contaminated sites. Currently, research in our laboratory is focused on monitoring the impact of different forms of heavy metals and radionuclides on physiological status of plants.

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REFERENCES

1. I. RASKIN and B.D. ENSLEY, *Phytoremediation of Toxic Metals: Using Plants to Clean the Environment*, John Wiley & Sons, New York (2000).
2. S. DUSHENKOV, "Trends in Phytoremediation of Radionuclides," *Plant and Soil* **249**, 167-175 (2003).
3. S. DUSHENKOV, A. MIKHEEV, A. PROKHNEVSKY, M. RUCHKO, and B. SOROCHINSKY, "Phytoremediation of Radiocesium-Contaminated Soil in the Vicinity of Chenobyl, Ukraine," *Environmental Science & Technology* **33**, 469-475 (1999).
4. J.A. ENTRY and L.S. WATRUD, "Potential Remediation of ^{137}Cs and ^{90}Sr Contaminated Soil by Accumulation in Alamo Switch Grass," *Water, Air, and Soil Pollution* **104**, 339-352 (1998).
5. J.A. ENTRY, L.S. WATRUD, and M. REEVES, "Influence of Organic Amendments on the Accumulation of ^{137}Cs and ^{90}Sr from Contaminated Soil by Three Grass Species," *Water, Air, and Soil Pollution* **126**, 385-398 (2001).
6. M.M. LASAT, W.A. NORVELL, and L.V. KOCHIAN, "Potential for Phytoextraction of ^{137}Cs from a Contaminated Soil," *Plant and Soil*. **195**, 99-106 (1997).
7. M.M. LASAT, M. FUHRMANN, S.D. EBBS, J.E. CORNISH, and L.V. KOCHIAN, "Phytoremediation of a Radiocesium-Contaminated Soil: Evaluation of Cesium-137 Bioaccumulation in the Shoots of Three Plant Species," *Journal of Environmental Quality* **27**, 165-169 (1998).
8. M. FUHRMANN, M.M. LASAT, S.D. EBBS, L.V. KOCHIAN, and J. CORNISH, "Uptake of Cesium-137 and Strontium-90 from Contaminated Soil by Three Plant Species; Applications to Phytoremediation," *Journal of Environmental Quality* **31**, 904-909 (2002).
9. Y.V. FRICKS, K. ROSEN, and L. SENNERBY-FORSSE, "Uptake and Distribution of Cs and Sr in *Salix viminalis* Plants," *Journal of Environmental Radioactivity* **63**, 1-14 (2002).
10. W.H.O. ERNST, "Bioavailability of Heavy Metals and Decontamination of Soil by Plants," *Applied Geochemistry* **11**, 163-167 (1996).

11. S. EHLKEN, and G. KIRCHNER, "Environmental Processes Affecting Plant Root Uptake of Radioactive Trace Elements and Variability of Transfer Factor Data: a Review," *Journal of Environmental Radioactivity* **58,** 97-112 (2002).
12. M.M. LASAT, "Phytoextraction of Toxic Metals: a Review of Biological Mechanisms," *Journal of Environmental Quality* **31**, 109-120 (2002).
13. KABATA-PENDIAS and H. PENDIAS, *Trace Elements in Soils and Plants*, CRC Press, Boca Raton, FL (1989).
14. J.F. SEEL, F.W. WHICKER, and D.C. ADRIANO, "Uptake of ^{137}Cs in Vegetable Crops Grown on a Contaminated Lakebed," *Health Physics* **68**,793-799 (1995).
15. F. VERA TOME, M.P. BLANCO RODRIGUEZ, and J.C. LOZANO, "Soil-to-Plant Transfer Factors for Natural Radionuclides and Stable Elements in a Mediterranean Area," *Journal of Environmental Radioactivity* **65**, 161-175 (2003).
16. B.R. VAZQUEZ DE ALDANA, B. GARCIA CRISDO, A. GARCIA CIUDAD, and M.E. PEREZ CORONA, "Estimation of Mineral Content in Natural Grasslands by Near Infrared Reflectance Spectroscopy," *Communications in Soil Science and Plant Analysis* **26,** 1383-1396 (1995).
17. J. PENUELAS and I. FILELLA, "Visible and Near Infrared Reflectance Techniques for Diagnosing Plant Physiological Status." *Trends in Plant Science* **3,** 151-156 (1998).
18. W.R. RAUN, G.V. JOHNSON, H, SEMBIRING, E.V. LUKINA, J.M. LARUFFA, W.E. THOMASON, S.B. PHILLIPS, J.B. SOLIE, M.L. STONE, and R.W. WHITNEY, "Indirect Measures of Plant Nutrients," *Communications in Soil Science and Plant Analysis* **29**, 1571-1581 (1998).
19. A.F.H. GOETZ, 'Progress in Hyperspectral Imaging of Vegetation', in *Optics in Agriculture: 1990-2000* (J.A. Deshazer and G.E. Meyer, eds.) SPIE Optical Engineering Press, pp. 20-36 (2001).
20. D.N.H. HORLER and J. BARBER, "Effects of Heavy Metals on the Absorbance and Reflectance Spectra of Plants," *International Journal of Remote Sensing* **1**, 121-136 (1980).
21. M.L. LABOVITZ, E.J. MASUOKA, R. BELL, A.W. SIEGRIST, and R.F. NELSON, "The Application of Remote Sensing to Geobotanical Exploration for Metal Sulfides- Results from the 1980 Field Season at Mineral, Virginia," *Economic Geology* **78**, 750-760 (1983).
22. N.M. MILTON, C.M. AGER, B.A. EISWERTH, and M.S. POWER, "Arsenic and Selenium Induced Changes in Spectral Reflectance and Morphology of Soybean Plants," *Remote Sensing of Environment* **30**, 263-269 (1989).
23. P.B.A.N. KUMAR, V. DUSHENKOV, H. MOTTO, and I. RASKIN, "Phytoextraction: the Use of Plants to Remove Heavy Metals from Soils." *Environmental Science & Technology* **29,** 1232-1238 (1995).
24. D.E. SALT, R.C. PRINCE, I.J. PICKERING, and I. RASKIN, "Mechanisms of Cadmium Mobility and Accumulation in Indian Mustard." *Plant Physiology* **109,** 1427-1433 (1995).
25. U. KRAMER, J.D. COTTER-HOWELLS, J.M. CHARNOCK, A.J.M. BAKER, and A.C. SMITH, "Free Histidine as a Metal Chelator in Plants that Accumulate Nickel," *Nature* **379**, 635-638 (1996).

26. J.W. HUANG, J. CHEN, W.R. BERI, and S.D. CUNNINGHAM, "Phytoremediation of Lead-Contaminated Soil: Role of Synthetic Chelates in Lead Phytoextraction," *Environmental Science & Technology* **31**, 800-805 (1997).
27. F.X. HAN, B.B. MARUTHI SRIDHAR, D.L. MONTS, and Y. SU, "Phytoavailability and Toxicity of Trivalent and Hexavalent Chromium to *Brassica juncea* L. Czern," *New Phytologist* **162**, 489-499 (2004).
28. B.B. MARUTHI SRIDHAR, *Monitoring Plant Spectral Reflectance and Internal Structure Changes during Phytoremediation Processes for Selected Heavy Metals*, PhD dissertation, Mississippi State University (2004).
29. D.R. HOAGLANG and D.I. ARNON, *The Water–Culture Method for Growing Plants without Soil*, California Agricultural Experiment Station Circular **347** (1950).
30. J.E. SASS, *Botanical Microtechniques*, Iowa State University Press, Ames, IA (1958).
31. M.L. JACKSON, *Soil Chemical Analysis*, Prentice Hall, Englewood Cliffs, NJ. (1958).
32. J.W. ROUSE, R.H. HAAS, J.A. SCHELLE, D.W. DEERING, and J.C. HARLAN, *Monitoring the Vernal Advancement and Retrogradation (Green Wave Effect) of Natural Vegetation*, Type III Final Report, NASA Goddard Space Flight Center, Greenbelt, Maryland, MD (1974).
33. J.L. ADAMS, W.D. PHILPOT, and NORVELL, "Yellowness Index: An Application of Spectral Second Derivatives to Estimate Chlorosis of Leaves in Stressed Vegetation," *International Journal of Remote Sensing* **20**, 3663-3675 (1999).