APPLICATION OF CHLOROPHYLL FLUOROMETRY FOR MONITORING PHYTOREMEDIATION OF URANIUM MILL TAILINGS SITES

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Abstract

Plants are used increasingly as tools in controlling or remediating environmental contamination. There is growing evidence that the effectiveness of phytoremediation can be influenced by the health status of the plants. Plant stress is a measure of plant health status and can readily be monitored through chlorophyll fluorometry. This technology can be used to optimize the relationship between plant stress and environmental remediation. It was demonstrated in this context at the U.S. Department of Energy (DOE) Moab uranium mill tailings site. The site is a historic processing facility for uranium ore, which generated large quantities of material containing low levels of uranium and radon. The site has a number of environmental issues, including a high concentration ammonium plume in near-surface groundwater. Chlorophyll fluorescence measurements were made on plants across the ammonium plume. The results correlated well with ammonia detected in groundwater. Locations could be clearly identified where the ammonia-induced stress damaged the plants. It is suggested that control of ammonia distribution would increase the efficiency of Tamarisk hydrological control. This process can be monitored by chlorophyll fluorescence. Plants in the uranium-contaminated locations at Moab did show stress levels higher than those in the control areas but not at a serious level.

In this preliminary demonstration, chlorophyll fluorometry provided rapid, low-cost and valuable information concerning the status of plant communities that are important to the continued activities at these sites. This technology appears to be able to quantify gradients of stress associated with exposure to groundwater ammonium and/or uranium plumes.

Remedial activities and goal of monitoring

The Atlas Site, Moab, is located in southeastern Utah along the Colorado River and is an abandoned uranium milling site, which falls under the UMTRA Title I protocol. DOE – Grand

Junction oversees the remedial activities at this site under UMTRA. The Atlas Site consists of a large uranium tailings pile closed with an earthen cover layer.

Initial characterization of the site has identified a plume of ammonium migrating into the river. The final remedial action for the site has not been determined. Options for remediation of the ammonium plume include intercepting and removing it through vegetative uptake along the river bank or oxidizing the ammonia to nitrate.

Tamarisk (salt cedar, *Tamarix ramosissima*) is the dominant plant species growing along the Colorado River floodplain at the site and plays an important role in site hydrology and contaminant transport in the shallow aquifer (passive phytoremediation). Models show that these plants remove 25 % of plume water budget with a wide evapo-transpiration (ET) range of 0.7 m/year to 3.1 m/year. The working hypotheses for enhancing this ongoing process are the following:

- The existing Tamarisk population is significantly limiting the amount of ammonia reaching the Colorado River which could be enhanced by hydraulic gradient manipulation.

- Tamarisk can tolerate high concentration of ammonia but will also become stressed at levels as high as in the current plume. A moderate reduction in groundwater ammonia levels that moderates plant stress, or cultural methods to stimulate plant growth, could greatly increase productivity, transpiration rate and ammonia uptake by Tamarisk.

The goal of monitoring and characterization of the existing Tamarisk population by different plant diagnostic tools, including fluorometry, is to estimate current and potential ET and ammonia-uptake rates and give a basis for studying the response of Tamarisk to system manipulations (e.g. cutting, burning, irrigation or oxidation of ammonia).

Fluorometric measurements

The ability to monitor plant stress is a key factor for these activities and potential deployment of a subsequent phytoremediation strategy. Hence, we carried out *in situ* fluorometric samplings using the CFM-636973 field-portable chlorophyll fluorometer [1-4] to verify its potential applicability for monitoring and mapping plant stress distribution [5-7] over the Tamarisk population of the site.

The test area of Tamarisk can be seen in Fig. 1. The shaded (green) dots (10 altogether) of the marked locations were selected for collective samplings with other diagnostic groups. Of these, we selected 9 for fluorescence analysis (their locations are given in Tables 1 and 2 listing the measured characteristic fluorescence data).

In addition to the sampling points of Fig. 1, samples were taken in and around the uranium plume (Fig. 2 and Tables 1 and 2). Two zones were sampled: one with uranium concentration similar to the values around the ammonia plume, and the other with 3 times higher value in the uranium plume.

Control samplings were carried out in a preserved area and in the nearby island in the Colorado River.

Results

Table 1 lists the reference names and the selected fluorescence markers calculated from the Kautsky-kinetics. Table 2 lists the GPS locations of all sampled zones. Fig. 1 and 2 map the locations and the respective fluorescence signatures of the sampled zones. Fig. 3 explains the samplings of upper and lower leaf levels of Tamarisk.

Figure 1. *Map of zone locations and the respective 690 nm fluorescence signatures for ammonia. Concentrations are given in mg/liter; fluorescence data are mean values for a given zone except for *Rfd which is median; H and L denote data read on upper and lower leaf levels.*

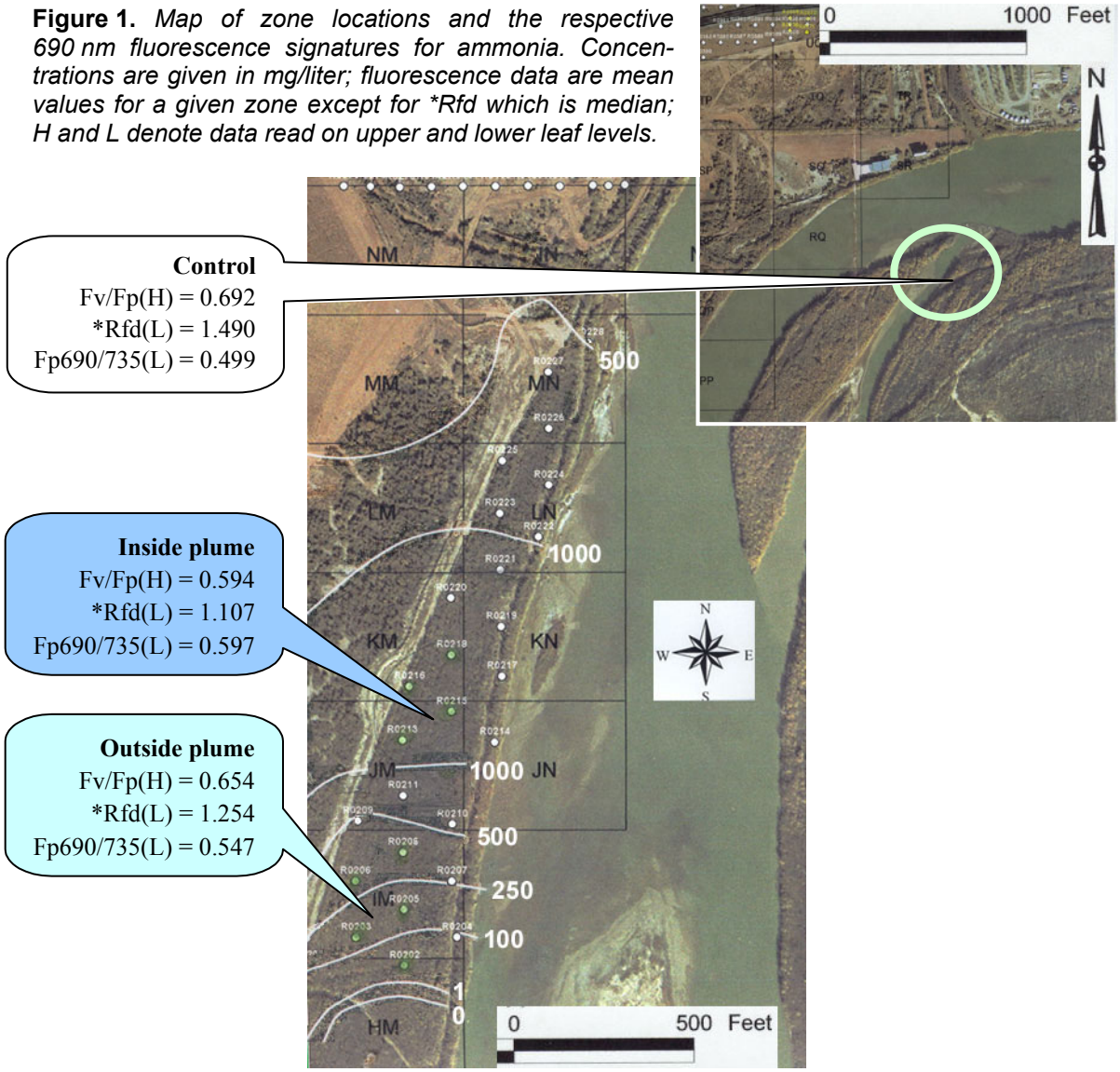

Table 1. *List of reference names, sampling time and selected fluorescence markers* Fv/Fp, Rfd *and* Fp690/Fp735 *(3, 4) for the tested zones at Moab/Atlas site. Naming convention: first 5 characters of the reference describes the zone (e.g.* `Ctr-I`, `R0202`*). The last letter defines the leaf level (*L *for lower and* H *for upper). Pale (orange and blue) shades mark low, saturated (dark) shades mark high contaminant concentration – controls are not shaded.*

#	Upper level (**H**) leaves Reference	Time	Fv/Fp 690nm	Fv/Fp 735nm	Rfd 690nm	Rfd 735nm	Fp690 Fp735	Lower level (**L**) leaves Reference	Time	Fv/Fp 690nm	Fv/Fp 735nm	Rfd 690nm	Rfd 735nm	Fp690 Fp735
1..3	`Ctr-I-H`	18:09						`Ctr-I-L`	18:15					
4..6	`CtrlA-H`	17:33	0,692	0,755	1,271	1,264	0,490	`CtrlA-L`	17:37	0,654	0,733	1,514	1,596	0,499
7..8	`CtrlB-H`	18:45						`CtrlB-L`	18:49					
9..11	`Lo-U--H`	15:40	0,582	0,635	1,707	1,676	0,534	`Lo-U--L`	15:45	0,456	0,548	1,098	1,109	0,535
12..14	`Hi-U--H`	16:07	0,447	0,533	1,735	1,893	0,472	`Hi-U--L`	16:25	0,447	0,540	1,610	1,812	0,493
15..17	`R0202-H`	09:23	0,699	0,755	1,216	1,211	0,569	`R0202-L`	09:19	0,705	0,764	1,119	1,049	0,557
18..20	`R0203-H`	09:59	0,649	0,726	1,398	1,451	0,535	`R0203-L`	09:54	0,682	0,753	1,135	1,165	0,554
21..23	`R0205-H`	10:34	0,675	0,747	1,322	1,367	0,563	`R0205-L`	10:30	0,615	0,697	1,231	1,266	0,554
24..26	`R0206-H`	11:14	0,652	0,736	1,480	1,611	0,526	`R0206-L`	11:10	0,631	0,720	1,333	1,408	0,524
27..29	`R0208-H`	11:50	0,597	0,700	1,369	1,633	0,543	`R0208-L`	11:46	0,621	0,702	1,398	1,425	0,546
30..32	`R0212-H`	12:44	0,554	0,657	1,145	1,368	0,604	`R0212-L`	12:40	0,498	0,615	1,086	1,365	0,621
33..35	`R0213-H`	13:25	0,559	0,644	1,825	1,933	0,582	`R0213-L`	13:29	0,495	0,602	1,680	1,898	0,636
36..38	`R0215-H`	14:00	0,586	0,694	1,027	1,190	0,581	`R0215-L`	14:04	0,590	0,694	1,104	1,293	0,580
39..41	`R0216-H`	14:35	0,678	0,752	1,033	1,106	0,512	`R0216-L`	14:39	0,650	0,726	0,950	1,043	0,550

Table 2. *List of sample references (zones) and their GPS locations*

Reference	Elevation, m	N, deg	W, deg
Ctr-I	no data		
CtrlA	1214	38, 60154	109, 58410
CtrlB	no data		
Lo-U	1215	38, 60380	109, 58779
Hi-U	1208	38, 60242	109, 58844
R0202	1207	38, 59399	109, 59235
R0203	1208	38, 59419	109, 59267
R0205	1208	38, 59418	109, 59223
R0206	1198	38, 59449	109, 59295
R0208	1202	38, 59489	109, 59247
R0212	1203	38, 59549	109, 59182
R0213	1202	38, 59572	109, 59231
R0215	1209	38, 59596	109, 59184
R0216	1199	38, 59618	109, 59223

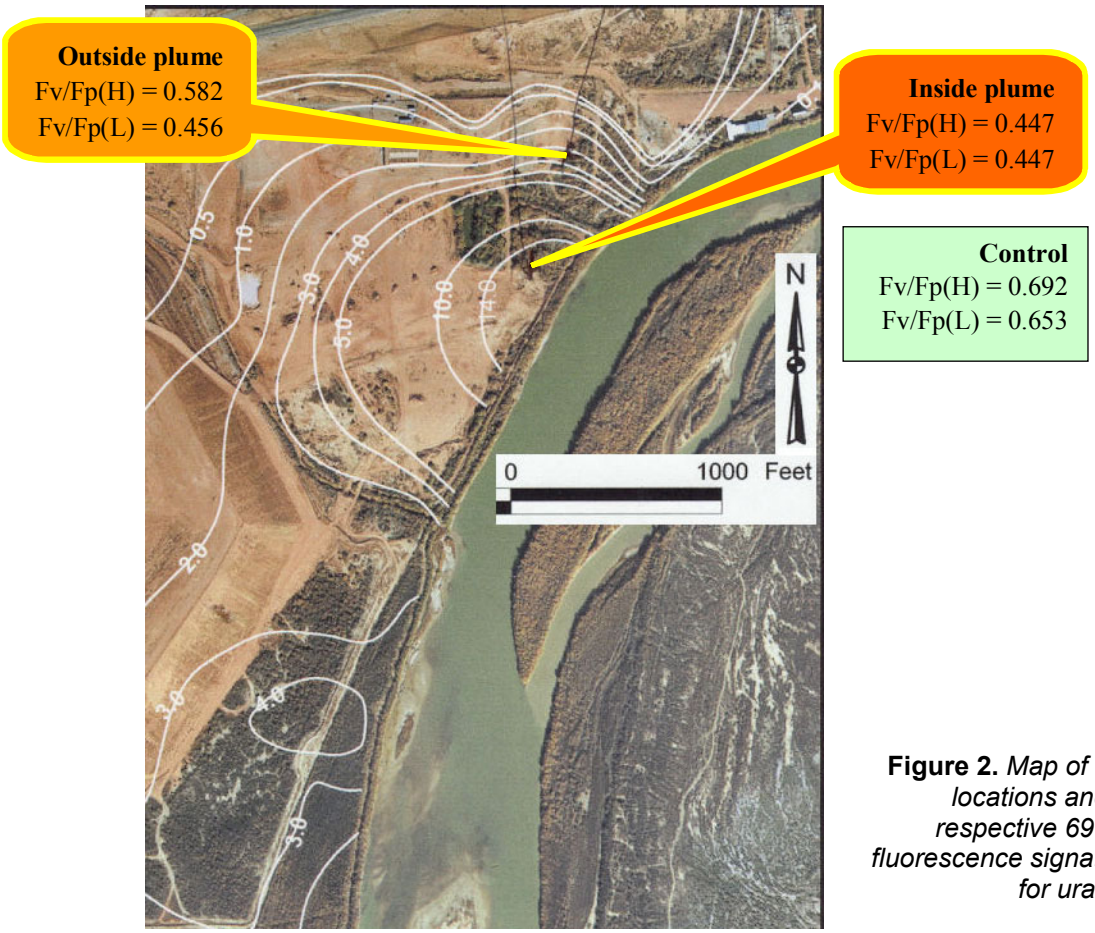

Figure 2. *Map of zone locations and the respective 690 nm fluorescence signatures for uranium*

Comparison of the *control*, *in-plume* and *off-plume* test groups was carried out for finding statistically significant differences to identify signatures of contamination effect in fluorescence data. Table 3 gives a detailed report for ammonia affected Tamarisk population by running *SigmaStat*™ (SPSS Inc.), an advisory statistical evaluation software. Based on a set of criteria, *One Way Analysis of Variance* (ANOVA) tests were used in most cases.

As shown in Table 3, the test first calculates the basic descriptive statistical data (mean, median, standard deviation, standard error of mean, etc.) and checks whether the distribution of data can be considered to be normal. If so, mean values are used for comparison otherwise the medians are used (the latter give a weaker condition for significant difference).

Figure 3. *Explanation of taking leaf samples from lower (L – left) and upper (H – right) leaf levels*

Three characteristic fluorescence markers have been analyzed, *Fv/Fp*, *Rfd* and the fluorescence ratio *Fp690/Fp735* calculated at the peak fluorescence. According to the literature and our preliminary studies [2, 4], these ones are accepted and sensitive markers of stress physiology ranked on physiological bases. If *Fv/Fp* is the only to change, this refers to a photoinhibition of photosystem II (PSII) caused by strong illumination or other influencing (moderate stress) factors. The entire PSI and PSII membrane system remains intact. If *Rfd* changes also, this suggests that the control system is not functioning (blocking or damage of the PSI+II system). If all three parameters, including *Fp690/Fp735*, changes, that is there is a decrease of the photosynthetic pigment contents, this shows the serious damage of the PSI+II system.

We have run all three tests for both the upper and lower leaves at 690 nm. Investigation of fluorescence signatures at 735 nm can be also carried out, however it is known from the literature that the 690 nm fluorescence is tightly related to the stress effects in plants.

Table 4 lists the statistical report for Tamarisk samples taken at the uranium plume. As there is no significant difference for *Rfd* and *Fp690/Fp735* among the zones, only the *Fv/Fp* table is presented.

Conclusions

According to the first evaluation of the fluorescence data at the Moab/Atlas site we can conclude the following:

- The effect of elevated ammonia level on Tamarisk can be clearly identified on the plants' fluorescence signatures. All 3 tested markers (*Fv/Fp*, *Rfd* and *Fp690/Fp735*) significantly distinguishes non-stressed control plants from the population existing in the ammonia plume.

- The *Fp690/Fp735* ratio, related to the chlorophyll content of the leaves, significantly differs in each group showing that some loss of pigments is present outside the ammonia plume. An increased loss in photosynthetic pigments is present in the zone being in the center of the plume, which can, at places, be visually observed as yellowing or paling.

- Deterioration of Tamarisk population in the center of the ammonia plume is well observed in the *Rfd* and *Fv/Fp* fluorescence markers with a confidence level of <0.001 (that is the probability of falsely distinguishing the stressed group is less than 0.1 %).

This sharp difference was visually observed on site as shorter shoot length and reduced leaf density.

- Fluorescence signatures of the off-plume zone (R0202-08) show that this population slightly differs from the control one and strongly differs from the in-plume population. This suggests that one may find an optimum ammonia gradient and a maximum ammonia level which does not affect fatally the existing population. If so, this population can effectively add to the enhanced phytoremediation and control of ammonia discharge.

Table 3. *Statistical analyses of* Fv/Fp*,* Fp690/Fp735*, and* Rfd *at 690 nm to find signatures of ammonia effect on Tamarisk*

Fv/Fp (690 nm) **One Way Analysis of Variance (ANOVA)** Wednesday, June 19, 2002, 12:02:27

Normality Test: Passed (P=0.073) **Equal Variance Test:** Passed (P=0.704)

Group Name	N	Missing	Mean	Std Dev	SEM
Ctrl-H	8	0	0.692	0.0424	0.0150
Outside-H	15	0	0.654	0.0478	0.0124
Inside-H	12	0	0.594	0.0608	0.0176

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0.0499	0.0249	9.346	<0.001
Residual	32	0.0854	0.00267		
Total	34	0.135			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P=<0.001). Power of performed test with alpha = 0.050:0.957

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparison	Diff of Means	p	q	P	P<0.050
Ctrl-H vs. Inside-H	0.0979	3	5.873	<0.001	Yes
Ctrl-H vs. Outside-H	0.0375	3	2.347	0.236	No
Outside-H vs. Inside-H	0.0604	3	4.268	0.013	Yes

Fp690/Fp735 **One Way Analysis of Variance (ANOVA)**

Normality Test: Passed (P=0.016) **Equal Variance Test:** Passed (P=0.613)

Group Name	N	Missing	Mean	Std Dev	SEM
Ctrl-L	8	0	0.499	0.0505	0.0179
Outside-L	15	0	0.547	0.0314	0.00810
Inside-L	12	0	0.597	0.0471	0.0136

Statistically significant difference (P=<0.001). Power of performed test with alpha = 0.050:0.996. Tukey Test:

Comparison	Diff of Means	p	q	P	P<0.050
Inside-L vs. Ctrl-L	0.0983	3	7.280	<0.001	Yes
Inside-L vs. Outside-L	0.0496	3	4.334	0.012	Yes
Outside-L vs. Ctrl-L	0.0486	3	3.756	0.032	Yes

Rfd (690 nm) One Way Analysis of Variance (ANOVA)

Normality Test: Failed (P=0.008), ANOVA on Ranks begun:

Rfd (690 nm) **Kruskal-Wallis One Way Analysis of Variance on Ranks**

Group	N	Missing	Median	25%	75%
Ctrl-L	8	0	1.490	1.353	1.630
Outside-L	15	0	1.254	1.155	1.338
Inside-L	12	0	1.107	0.967	1.222

H=7.980 with 2 degrees of freedom (P=0.019). The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P=0.019).

All Pairwise Multiple Comparison Procedures (Dunn's Method):

Comparison	Diff of Ranks	Q	P<0.05
Ctrl-L vs Inside-L	13.167	2.815	Yes
Ctrl-L vs Outside-L	8.717	1.943	No
Outside-L vs Inside-L	4.450	1.121	No

- We found changes of all three fluorescence signatures (*Fv/Fp*, *Rfd* and *Fp690/Fp735*). According to the ranking of these markers, this shows a serious damage of the plants' PSI+II system in most locations of the in-plume population.

Table 4. *Statistical analysis of* Fv/Fp (690 nm) *parameter to find signatures of uranium effect on Tamarisk*

Fv/Fp (690 nm) **One Way Analysis of Variance (ANOVA)** Thursday, June 20, 2002, 16:15:17

Normality Test: Passed (P>0.200) **Equal Variance Test:** Passed (P=0.106)

Group Name	N	Missing	Mean	Std Dev	SEM
Ctrl-H	8	0	0.692	0.0424	0.0150
Ctrl-L	8	0	0.653	0.0556	0.0197
Lo-U--H	3	0	0.582	0.0597	0.0345
Hi-U--H	3	0	0.447	0.0525	0.0303
Lo-U--L	3	0	0.456	0.169	0.0976
Hi-U--L	3	0	0.447	0.170	0.0980

Source of Variation	DF	SS	MS	F	P
Between Groups	5	0.292	0.0583	7.937	<0.001
Residual	22	0.162	0.00735		
Total	27	0.453			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P=<0.001). Power of performed test with alpha = 0.050:0.993

All Pairwise Multiple Comparison Procedures (Bonferroni t-test):

Comparison	Diff of Means	t	P	P<0.050
Ctrl-H vs. Ctrl-L	0.0385	0.898	1.000	No
Lo-U--H vs. Lo-U--L	0.126	1.805	1.000	No
Hi-U--H vs. Hi-U--L	0.000	0.000	1.000	No
Ctrl-H vs. Lo-U--H	0.110	1.890	1.000	No
Ctrl-H vs. Hi-U--H	***0.245***	***4.216***	***0.005***	***Yes***
Lo-U--H vs. Hi-U--H	0.135	1.929	1.000	No
Ctrl-L vs. Lo-U--L	0.197	3.403	0.038	Yes
Ctrl-L vs. Hi-U--L	***0.206***	***3.553***	***0.027***	***Yes***
Lo-U--L vs. Hi-U--L	0.00867	0.124	1.000	No

- The high concentration area of the uranium-plume zone shows up clearly in the *Fv/Fp* signature compared to the control area. This area showed the lowest *Fv/Fp* values recorded while measuring Tamarisk. As uranium is the only influencing factor in the uranium-plume, this suggests that it initiates a strong photoinhibition in plants without actually damaging them. These differences were observed as plant stress without visual cues (the plants in the uranium plume did not appear to be stressed). Further tests should be carried out to verify this phenomenon.

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