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To cite this article: V S Goryainov and A A Buznikov 2021 *J. Phys.: Conf. Ser.* **2103** 012155

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A study of the influence of copper sulfate on the spectral properties of common buckwheat (*Fagopyrum esculentum*)

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Abstract. The influence of pollutants on the spectral properties of common buckwheat (*Fagopyrum esculentum*) has been investigated insufficiently, compared to the cereals from the *Poaceae* family. A two-stage spectral survey has been carried out, growing common buckwheat in containers with set concentrations of copper(II) sulfate in soil both in laboratory conditions and in the open air. Spectral distributions of diffuse reflectance of the plants were registered in the range of 400 – 1100 nm, and spectral indices were calculated, using wavelengths corresponding to spectral features of plant pigments. Simultaneously, digital photos were taken to account for projective cover of the plants. Four spectral indices were found to depend quantitatively on pollutant concentration, three of them taking extreme values at the time of maximal projective cover. When growing buckwheat in the open air, lower temperatures and higher irradiation lead to closer values of spectral indices corresponding to different copper concentration, than in laboratory conditions. The results show the usability of common buckwheat as an indicator of soil pollution by copper.

1. Introduction

Heavy metals, including soluble compounds of mercury, lead and cadmium are among the common pollutants of soil, water and vegetation. The ability to form complexes and participate in biochemical redox reactions makes them dangerous to human health.

Numerous plant species have been described to show indicator properties, changing the distribution of their diffuse reflectance in the presence of certain pollutants in the soil. Many of these species belong to the *Poaceae* family, including wild common meadow-grass (*Poa pratensis*) [1] as well as agricultural cereals: wheat (*Triticum aestivum*) [2], maize (*Zea mays*) [3] and barley (*Hordeum vulgare*) [4].

Common buckwheat (*Fagopyrum esculentum*), however, is a pseudocereal from the *Polygonaceae* family, and its reflective spectra and indicator properties to date have been investigated to a much lesser extent [5, 6]. This may be due to the fact that cultivation of buckwheat is mainly concentrated in several countries of all the world: back in 2016, for example, more than half of the world buckwheat crop was harvested in Russia, with China providing 17% more.

Based on the above, buckwheat was chosen as an object of experiment to study the effect of soil contamination with copper on its spectra.



2. Methods and means of the experiment

Two stages of the experiment have been carried out using the same setup: the first one in laboratory conditions [7], the second one in the open air. In both stages, common buckwheat seeds were planted into 4 containers, in 3 of which soil had been previously treated with water solution of copper(II) sulfate to obtain copper concentration of 20, 40 and 60 mg/kg (per unit of soil mass). To the 1st container, no copper sulfate was added.

Buckwheat was grown under natural illumination in the 2nd stage of the experiment, which lasted from September to October at approximately 60 degrees northern latitude. In the 1st stage, both natural illumination and household LED lightning (at daytime) were used.

Reflection spectra of buckwheat plants were registered every 2 – 3 days using the "Raduga" spectrometer [1]. This device uses a Rowland circle optical setup with a concave reflection grating (120 lines/mm), providing a spectral resolution of 1 nm in the working range of 400 – 1100 nm. The angle of view is $12' \times 5^\circ$. A Toshiba TCD1304AP CCD array of 3648 elements works as a photodetector. The signal is then processed by a eZdsp F2802 (Texas Instruments) digital signal processor. To control the spectrometer and save the data obtained, the spectrometer is connected to the computer via a serial port.

During measurements, the spectrometer was mounted on a tripod, so that its field of view covered most of the soil's surface but remained within the container's dimensions. The largest of the two dimensions of the field of view was about 30 cm.

In the 1st stage of the experiment, 1 kW and 150 W incandescent halogen lamps were used alternately as a light source for measurements. In the first case, the CCD integration time was set to 50 ms; in the second case, to 80 ms. In the 2nd stage, measurements were taken under natural daylight, mostly with integration time of 20 ms.

In every measurement, the resulting spectrum was averaged over 16 consecutive repetitions.

Incandescent light used in the experiments differed from sunlight both in absolute radiant intensity and in spectral composition, with sunlight's properties depending on time of day and weather conditions. Therefore, the luminance spectra of the plants $L_O(\lambda)$ were compared to the reference luminance spectra $L_S(\lambda)$ of the light sources, registered before each series of measurements: for daylight, by using a white polytetrafluoroethylene (PTFE) reference, and for the lamps, by simply pointing the spectrometer at the filament.

To compare and analyze the spectral properties of buckwheat plants, spectral distributions of the diffuse reflectance (or the spectral luminance factor) were calculated using the following formula:

$$R(\lambda) = \frac{L_O(\lambda) - L_D(\lambda)}{L_S(\lambda) - L_D(\lambda)}, \quad (1)$$

where $L_O(\lambda)$ is the monochromatic luminance of the object at wavelength λ , $L_S(\lambda)$ is the monochromatic luminance of the light source at the same wavelength, as stated above, and $L_D(\lambda)$ is the supposed monochromatic luminance corresponding to the noise level in the CCD channels, which detect the radiation with the wavelength λ , with the integration time used.

To assess the condition of the plants, spectral indices $R(\lambda_1)/R(\lambda_2)$ were used, i.e. ratios of diffuse reflectances at two wavelengths λ_1, λ_2 , selected to correspond to the positions of minima and maxima of absorption by plant pigments and water.

Along with the spectral survey, photos of the plants were taken regularly from approximately the same angle, using a downward-looking digital camera, to assess the influence of the projective cover on the distribution of spectral reflectance. The pixels in the photos were then split into two clusters (leaves and background), based on the brightness of the green component, using the k-means method [8]. Both photo analysis and calculation of spectral reflectance distributions were carried out using the R language for statistical computing [9].

3. Results

Figure 1 shows the time dependence of projective cover for buckwheat grown in laboratory conditions (a) and in the open air (b), that is, the fraction of pixels from the container's digital photo, which have been placed into the "leaves" cluster by the k-means algorithm. The markers in the lines denote the copper concentration in soil for the corresponding container.

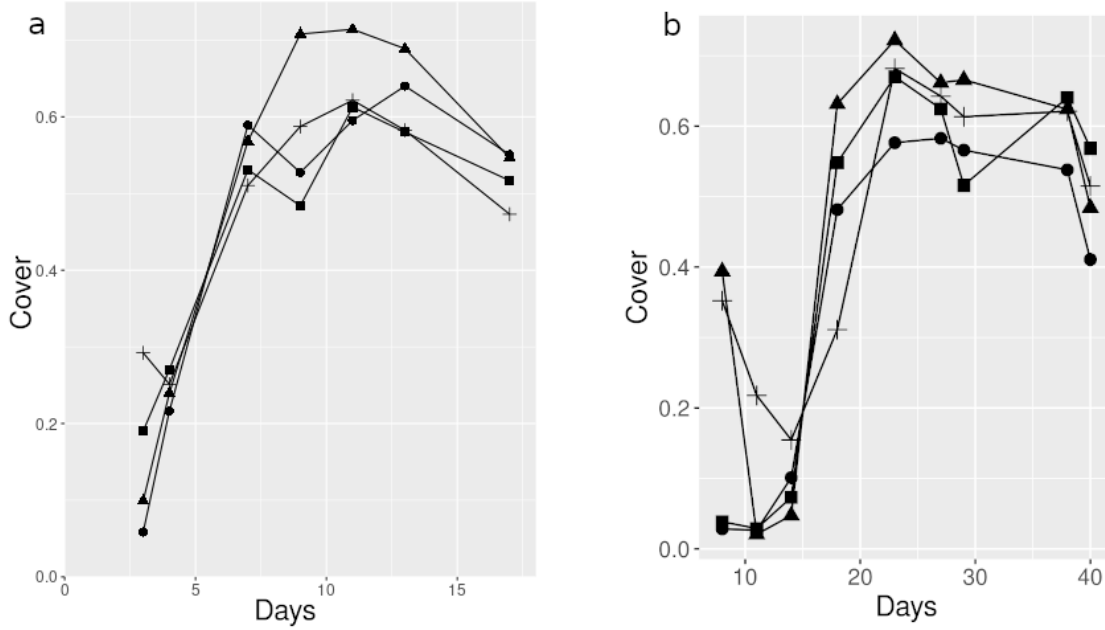


Figure 1. Time dependence of projective cover for buckwheat grown in laboratory conditions (a) and in the open air (b). Line markers' shape shows the copper concentration in soil, mg/kg: ● – 0; ▲ – 20; ■ – 40; + – 60.

For comparison, four examples of spectral distributions of luminance ("raw" spectra, $L_O(\lambda) - L_D(\lambda)$) are given in figure 2, registered in the laboratory stage of the experiment on the 5th and 9th day after planting the seeds, for copper concentration of 0 and 60 mg/kg.

As mentioned earlier, only the averaged luminance spectra were written to files, without saving the data for individual repetitions. This prevents the random error of the measurements from being estimated. However, to assess the method's accuracy, the spectrometer's level of dark noise can be used as a measure of its instrumental error. For this purpose, the following expression in partial derivatives was used:

$$\begin{aligned} \theta_{R(\lambda)} &= \left| \frac{\partial R(\lambda)}{\partial L_O(\lambda)} \right| \theta_{L_O(\lambda)} + \left| \frac{\partial R(\lambda)}{\partial L_S(\lambda)} \right| \theta_{L_S(\lambda)} + \left| \frac{\partial R(\lambda)}{\partial L_D(\lambda)} \right| \theta_{L_D(\lambda)} = \\ &= (|a_{L_O(\lambda)}| + |a_{L_S(\lambda)}| + |a_{L_D(\lambda)}|) L_D(\lambda), \end{aligned} \quad (2)$$

where $\theta_{L_O(\lambda)}$, $\theta_{L_S(\lambda)}$ and $\theta_{L_D(\lambda)}$ are instrumental error values characteristic for corresponding luminance spectra. The second equality holds, since the threshold sensitivity of the spectrometer is considered to be limited only by the dark noise, which is regarded as constant for all measurements with the same integration time. Following this approach, the relative error averaged over the working spectral range $\overline{\theta_{R(\lambda)}/R(\lambda)}$ was calculated to be about 3% at 20 ms integration time, 15% at 50 ms, and 6% at 80 ms.

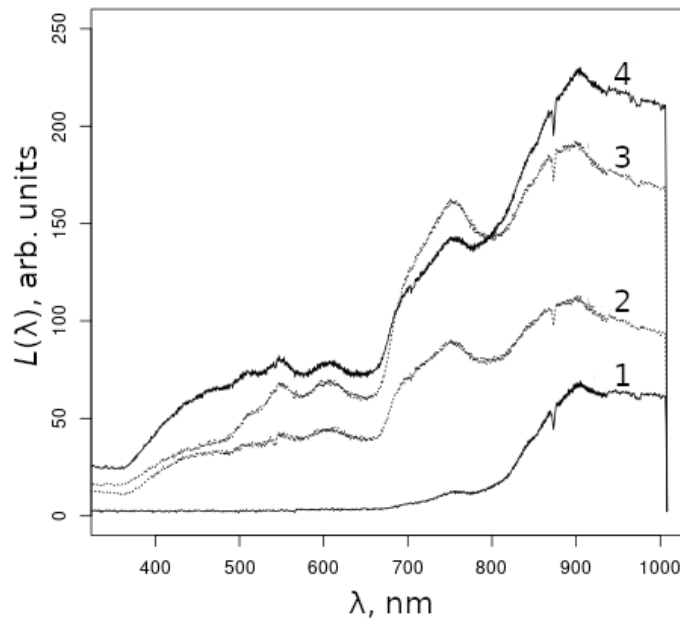


Figure 2. Spectral distributions of luminance $L(\lambda)$: 1 – 0 mg/kg, day 5; 2 – 60 mg/kg, day 5; 3 – 60 mg/kg, day 9; 4 – 0 mg/kg, day 9.

Table 1. Spectral features forming the informative indices of common buckwheat [10].

λ , nm	Feature description
435	Chlorophyll <i>a</i> absorption maximum
485	β carotene absorption minimum, and an intersection of its spectrum with the absorption spectrum of chlorophyll <i>b</i>
500	An intersection between absorption minima of chlorophyll <i>a</i> and <i>b</i> , and an absorption maximum of β carotene
550	An intersection between absorption minima of chlorophyll <i>a</i> and <i>b</i>
620	An intersection between absorption spectra of chlorophyll <i>a</i> and <i>b</i>
670	Chlorophyll <i>a</i> absorption maximum
735	Chlorophyll <i>a</i> and <i>b</i> absorption minima

Analyzing the data obtained, 20 spectral indices were considered, with wavelengths corresponding to characteristic points in the spectra of plant pigments [10]. Some of these indices were found to remain nearly constant during the whole vegetation process and to be independent from the soil pollution level. The other indices showed an upward or downward trend, with a possible switch to the opposite one with the plants starting to wither, and also differed sharply for different containers. The second type of indices was considered as potentially informative regarding soil pollution. As a result, 4 of such indices were selected: $R(435)/R(620)$, $R(670)/R(500)$, $R(550)/R(485)$ and $R(900)/R(735)$. Table 1 describes the spectral features affecting these indices.

As a characteristic example, figure 3 shows the time dependence of one of these indices, $R(550)/R(485)$, for different pollutant concentrations, obtained in the laboratory stage of the experiment.

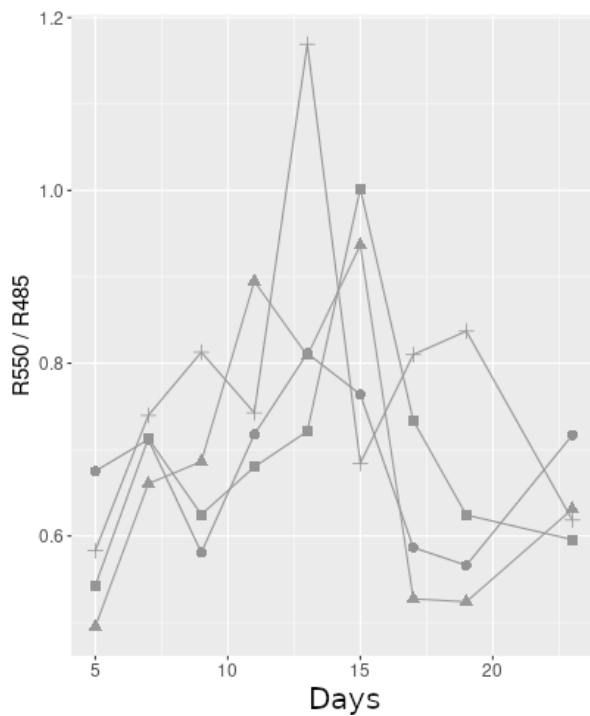


Figure 3. Time dependence of the $R(550)/R(485)$ spectral index. Line markers' shape shows the copper concentration in soil, mg/kg: ● – 0; ▲ – 20; ■ – 40; + – 60.

4. Discussion

In both stages of the experiment, plant growth and withering were observed: in laboratory conditions, plants withered due to lack of photosynthetically active radiation, and in the open air, due to night freezes. Both graphs in figure 1 show gradual increase and decrease of projective cover. At room temperature, buckwheat plants develop faster, as similar values of projective cover are observed 8 days after the planting in figure 1, *a*, and 14 days after the planting in figure 1, *b*.

High cover percentage in two of the containers before day 14 in figure 1, *b* can be attributed to the imperfection of the k-means algorithm, as the central coordinates of the "leaves" and "background" clusters turn out to be nearly the same at such points.

Figure 2 shows that the projective cover has a significant impact on reflection spectra of common buckwheat, especially during the first days of its growth. Accounting for the projective cover is necessary when performing spectral measurements both in field and laboratory conditions.

All the spectral indices, except $R(900)/R(735)$, showed the presence of extrema corresponding to ceasing of plants' development and start of withering. For various pollutant concentrations, such an extremum can be shifted in time both forwards and backwards about the projective cover maximum, as figure 3 shows.

The $R(900)/R(735)$ index showed high values at the beginning of plant growth, especially in clean soil. During the laboratory phase, at day 5, this index was equal to 5.27 for the "0" container, and 2.8 for the "20" container, while at higher pollutant concentrations its value was nearly the same (1.03 and 1.05 for 40 and 60 mg/kg). Later on, the index for the clean soil container decreased rapidly, still exceeding its values for other containers. Taking into account the influence of projective cover, the effect can be possibly explained by copper acting as a stimulant for germination and seedling growth. This allows proposing to use the $R(900)/R(735)$ index as an indicator of soil pollution by copper at early stages of buckwheat growth.

The extreme values of the first 3 spectral indices showed to depend qualitatively on copper concentration. Thus, in laboratory stage the minimal values of the $R(435)/R(620)$ index were

1.70, 1.32, 1.28 and 1.26 in ascending order of pollutant concentration. In the same conditions, the maximal values of the $R(670)/R(500)$ index were 0.78, 0.88, 0.95 and 1.01, and the maximal values of the $R(550)/R(485)$ were 0.81, 0.94, 1.00 and 1.17.

During the second stage of the experiment, the minimal value of the $R(435)/R(620)$ index was 1.36 for clean soil, and for copper concentration of 60 mg/kg it was 0.86. Nevertheless, the $R(670)/R(500)$ index was equal to 0.71 and 0.73 for clean soil and maximum pollution respectively, while the $R(550)/R(485)$ index was equal to 0.88 and 1.02. On the whole, the variability of spectral indexes under the influence of soil pollution turned out to be less than in the first, laboratory stage of the experiment. This can be attributed to lower temperatures and higher irradiation affecting the plants' biochemistry.

The found dependencies between the values of spectral indexes and the level of soil pollution have a qualitative character. To clarify the quantitative nature of these dependencies, chemical analysis of soil and plant tissues of buckwheat is required along with spectral surveys, to assess the copper content more precisely.

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