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Article

Automated data collection in acute and chronic phytotests

A.M. Sheromov , A.S. Olkova* , E.V. Tovstik 

Vyatka State University, Moskovskaya St. 36, Kirov, 610000 Russia

*morgan-abend@mail.ru

Abstract. The purpose of this work was to develop a phytobioassay algorithm including the automated measurement of plant morphometric parameters and primary data processing using the ImageJ software. The objects of the study were pictures of 7-day *Trifolium pratense* L. clover seedlings exposed to short-term (7 days) copper chloride solutions (5, 10 and 15 mg/L in terms of copper (II) ions) and pictures of the subflag leaf of *Hordeum vulgare* L. barley grown in soil contaminated with cadmium (19.2 ± 1.5 mg/kg) for 60 days. The experiments were performed in 3 replicates with $n = 20$. An algorithm for working with the pictures, including such steps as dividing a photo into color channels and image segmentation up to excluding 5% of extreme sample values, was proposed. In acute tests on *T. pratense*, the standard deviation (SD) made up 5–11% when measuring the length of roots and 3–7% for sprouts. In chronic experiments on *H. vulgare*, SD for linear parameters (leaf length and width) fluctuated from 11 to 21% and reached 33% for the leaf area. Conclusions were made about the suitability of the proposed algorithm for assessing acute phytotoxicity and the need to refine the methodological and statistical aspects of chronic phytotests. The main advantages of phytobioassay with data collection and processing in ImageJ are the possibility of delayed measurements of phytoobjects from a photo and assessment of the parameters of geometrically complex objects.

Keywords: bioassay, Image, ImageJ, heavy metals, toxicity, biological data processing

ORCID:

A.M. Sheromov, <https://orcid.org/0009-0005-8691-4042>

A.S. Olkova, <https://orcid.org/0000-0002-5798-8211>

E.V. Tovstik, <https://orcid.org/0000-0003-1861-6076>

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Научная статья**Автоматизированный сбор массива данных в острых и хронических фитотестах**А.М. Шеромов , А.С. Олькова* , Е.В. Товстик *Вятский государственный университет, 610000, Россия, г. Киров, ул. Московская, д. 36***morgan-abend@mail.ru*

Аннотация. Целью данной работы была разработка алгоритма фитотестирования, включающего автоматизированное измерение морфометрических параметров растений и первичную обработку данных средствами программы ImageJ. Материалами исследований служили фотоснимки 7-дневных проростков клевера (*Trifolium pratense* L.), подвергшихся краткосрочному воздействию (7 суток) хлорида меди в растворе (5, 10 и 15 мг/л в пересчете на ионы меди (II)), и фотоснимки подфлагового листа ячменя (*Hordeum vulgare* L.), выращенного в почве, загрязненной кадмием (19.2 ± 1.5 мг/кг) в течение 60 суток. Опыты выполнены в трехкратной повторности, $n = 20$. Предложен алгоритм работы с фотоснимками: от разделения снимка на цветовые каналы и сегментации изображения до исключения 5% экстремальных значений выборки. В острых испытаниях на *T. pratense* показано, что стандартное отклонение среднего составляет 5–11% при измерении длины корней, 3–7% – ростков. В хронических опытах на *H. vulgare* стандартные отклонения колебались от 11 до 21% для линейных показателей и достигали 33% для площади листа. Сделаны выводы о пригодности предложенного алгоритма для оценки острой фитотоксичности и необходимости доработки методических и статистических аспектов хронических фитотестов. Главными преимуществами фитотестирования со сбором и обработкой данных в ImageJ являются возможность отсроченных измерений фитообъектов по фото и оценка параметров геометрически сложных объектов.

Ключевые слова: биотестирование, Image, ImageJ, тяжелые металлы, токсичность, обработка биологических данных

ORCID:А.М. Шеромов, <https://orcid.org/0009-0005-8691-4042>А.С. Олькова, <https://orcid.org/0000-0002-5798-8211>Е.В. Товстик, <https://orcid.org/0000-0003-1861-6076>

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Introduction

Computer vision (CV) is currently replacing direct measurement methods of environmental objects with information extracted from photographs and videos (Olkova and Medvedeva, 2023). CV can increase the amount of acquired and processed data, as well as the number of measurable parameters. The method eliminates subjective assessments of biological objects, reduces research costs, and facilitates the development of new biomonitoring methods. CV enables the rapid, accurate, and reliable acquisition of environmental data (Lopez-Marcano et al., 2021).

Automation has recently become a key driver of methodological development in bioassay, bioindication, and environmental monitoring (Olkova and Tovstik, 2024). It is particularly important in phytotoxicity testing because the majority of existing methods (GOST R ISO 18763-2019¹, FR.1.31.2012.11560², MP 2.1.7.2297-07³) suggests using a ruler as the main measurement tool. However, manual measurement of plant growth parameters is not only labor-intensive but also prone to inaccuracies due to the complex objects geometry (Fiskesjö, 1985).

Global practice has accumulated experience in the automated assessment of plant morphological parameters for use in bioassay and bioindication (Li et al., 2020; Železnikar et al., 2025). However, specialized software products are often expensive and subject to copyright restrictions. ImageJ and its version, ImageJ Fiji, offer significant advantages in this regard: they are open source and do not infringe copyright.

ImageJ is JAVA image processing software that has become an essential tool for scientific research in natural sciences (Curtis et al., 2017). It is known for its extensibility via plugins. Users can create new plugins or record macros to customize the program's option for specific purposes (Kainz et al., 2015).

For decades, ImageJ has been a pioneer as an open-source scientific image analysis software (Schneider et al., 2012). A search for "ImageJ" in the ScienceDirect database with "natural sciences" filter yields around 14 thousand results between 2014 and 2024. Approximately 1000 of these publications belong to "phytotoxicity testing" specification. Furthermore, root length estimates obtained with ImageJ and commercial root measuring software WinRHIZO showed a high-accuracy correlation ($r = 0.986$) (Tajima and Kato, 2011).

However, further information analysis revealed the absence of specific methodology for phytotoxicity testing using ImageJ tools. Leaf length and its fractal dimension turned to be a good indicator of plant responses to environmental toxicants (Bialowiec et al., 2010). In a study of C. Eichberg et al. (2024), the phytotoxicity of antibiotics was assessed based on the responses of *Carum carvi* L., *Centaurea jacea* L., *Dactylis glomerata* L., and *Silene latifolia* (Poir.). Germination was determined visually, roots length of 7-day-old seedlings were measured using ImageJ tools by 5 (average 3.0 ± 1.7) out of 50 randomly selected specimens. The 3D root model built with VG StudioMAX 3.2 and ImageJ (Skeletonise 3D plugin) was used to determine the root length, root tortuosity and root plate tilt in field tests of biochar on maize crops (Ruan et al., 2024).

The aim of this work was to develop a phytotoxicity testing algorithm consisting of automated plant measurement and primary data processing using ImageJ.

Materials and methods

To obtain photographic data and test the ImageJ software, two independent heavy metal toxic effect experiments were performed.

Acute toxicity experiments were conducted on 7-days red clover (*Trifolium pratense* L.) seeds by short-term exposure to various concentrations of copper chloride solution (5, 10, 15 mg/L or 5, 10, 15 × MAC of Cu^{2+})⁴. Drinking water served as a control and was used to prepare plant test solutions. Seeds (20 seeds) were germinated in Petri dishes under laboratory conditions: a temperature of 20 ± 2 °C and a light intensity of 250 lux. The analysis was made in triplicate.

¹ GOST R ISO 18763-2019. Soil quality. Determination of the toxic effects of pollutants on germination and early growth of higher plants.

² FR.1.31.2012.11560. Methodology for measuring the biological activity of humic substances using phytotesting ("Phytoscan").

³ MR 2.1.7.2297-07. Methodological recommendations. Justification of the hazard class of industrial and consumer waste based on phytotoxicity ("Phytotest").

⁴ SanPiN 1.2.3685-21. Hygienic standards and requirements for ensuring the safety and (or) harmlessness of environmental factors for humans.

Chronic toxicity data was obtained in barley (*Hordeum vulgare* L.) experiments with a cadmium sulfate hydrate ($3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$) as a reference toxicant. The toxicant solution was added to containers with sandy loam texture Albeluvisol. Pollution levels were referenced to the total metal forms APC (1 mg/kg)⁵ in the absence of other environmental standards for cadmium in Russia. The resulting content was $19.2 \pm 1.5 \text{ mg/kg}$ ($19.2 \times \text{APC}$). The control consisted of soil with no cadmium added. Plants grew for 60 days from May to July under a temperate continental climate (Kirov region, Russia). The soil was maintained near field capacity with a tap water. At the tillering stage, the subflag leaf was excised from 20 plants in both the experimental and control groups. This leaf was selected due to the correlation of its area (Bosieva et al., 2021) and pigment content (Noskova et al., 2019) with grain yield, which is a key indicator of plant development.

Clover seedlings and barley leaves were photographed using a 20.1 MP smartphone camera. Image processing was performed using the ImageJ software⁶. The program was used to measure the root and shoot lengths of a clover seedling and the length and width of barley leaves. The area information was obtained through image segmentation. Results were presented as mean \pm standard deviation. Data series were compared using one-way ANOVA analysis with STATGRAPHICS Centurion XV.I software (StatPoint Inc., USA).

Results and discussion

Primary data collection

The general algorithm for processing photographs and primary data includes the following steps:

1. ImageJ launch and image upload (File > Open).
2. Split of the input image into Red channel, Green channel and Blue channel (Image > Color > Split Channels) and selection of the channel with the highest contrast (minimize/close the windows with the less contrast).
3. Conversion of the image into a pure black and white bitmap (Process > Binary > Make Binary).
4. Setting the scale (Analyze > Set Scale) to convert pixels into needed measurement unit. Selection of an object of known size with the Straight Line tool, setting the scale & units of measurement in the pop-up window. Typically, a ruler is used as a scaling reference.
5. Use of the “Segmented Line” and “Magic Wand” tools to measure the length (Fig. 1A) and area (Fig. 1B) of the object. The user manually places marker points for length measurement by left-clicking. The number of marker points depends on the curvature of the object. Points must be placed on the curves. The object outline for area measurements is selected automatically. If necessary, the “Magnifying Glass” tool can be used to zoom in and out of the image.
6. Multiple images from a single experimental series are processed sequentially, and the unit scale is calibrated for each image. The data are automatically merged into the output table, which can be viewed by clicking the “Results” button (Window > Results).
7. Use of the “Analyze > Measure” command or the “Ctrl + M” combination to output the individual measurement results to the data table (Fig. 2).
8. Data export to Excel for further analysis.

The ImageJ output table shows the initial mathematical processing of the measurements, indicating the minimum and maximum values (Fig. 2A).

When processing the data in Excel, the extreme 5% of the values were excluded to obtain a 10% trimmed mean, which is more accurately represents the “average” of the dataset (Fig. 2B). A similar approach using trimmed means has been used in the analysis of morphological traits in some medical research (Gattavov et al., 2007).

⁵ SanPIN 1.2.3685-21. Hygienic standards and requirements for ensuring the safety and (or) harmlessness of environmental factors for humans.

⁶ Image J. Web page. URL: <https://imagej.net/ij/> (accessed: 11.09.2025).

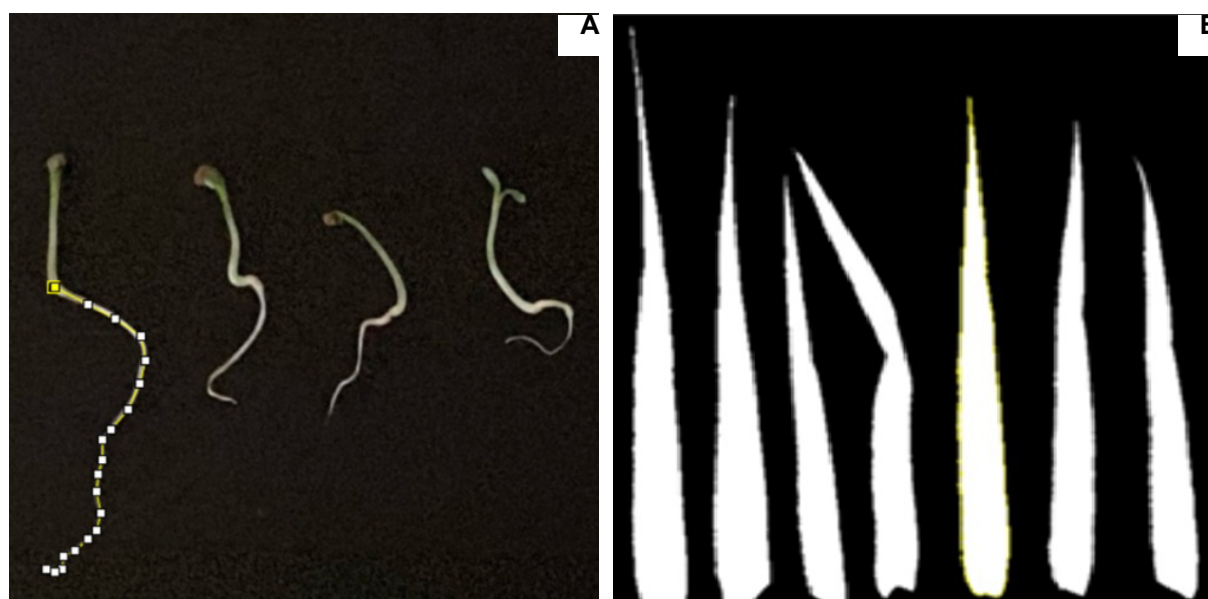


Fig. 1. Placement of marker points on a clover seedling root for length measurement (A) and the resulting binary image for barley leaf area measurement (B) in ImageJ software.

Evaluation of acute and chronic plant test results

The algorithm was approbated in two standard phytotoxicity experiments (Fig. 3, Table 1). It is known that small seeds are more susceptible to toxicity than larger ones because of the limited nutrient reserves (Terekhova et al., 2014). However, despite high sensitivity of small seeds, measuring their linear dimensions is difficult due to their small size and curved roots.

The standard deviation of automated root length measurements (excluding extreme values) ranged from 5% to 11% of the mean; for stem measurements, the standard deviation was 3% to 7%. This represents a fairly good result for morphological parameters of plants. Low concentrations of copper (II) ions (5 and 10 × MAC) did not affect root length ($p > 0.05$), while high concentrations (15 × MAC) stimulated root growth. The observed copper effect (hormetic effect) is consistent with biological action data (Zhivukhina and Ertikeeva, 2019) and indicates the reliability of the results obtained using the software.

In chronic effects experiment with Cd, we measured both leaf area and linear parameters (leaf length and width at the widest part) (Table 1).

Chronic toxicity data showed high variability compared to acute one: standard deviations for linear parameters ranged from 11% to 21%, while for the area they reached 33%. This is an acceptable result for 20 samples (Lyubimov and Balina, 2005). All evaluated parameters demonstrated a trend toward increased responses to cadmium exposure, but no significant differences were found between the experimental variables. It is likely that higher concentrations would have revealed more consistent trends, but this was not part of the study's objectives.

Nevertheless, it can be said that the results obtained using the proposed algorithm are consistent with literature data. It is known that the phytotoxicity threshold for cadmium in soil can be quite high – 25 mg/kg (Krylenkin et al., 2023). Even when the cadmium content in soil increases to 45 mg/kg, the toxic effects may occur only in roots, where cadmium accumulates at concentrations 10 times higher than in aboveground organs (Sterckeman and Thomine, 2020; Vassilev et al., 1998).

The main achievement of this experiment is the automated plant morphological data collection. Automated measurement of each plant part is the most promising approach because the manual process is labor-intensive. Measurement accuracy can be improved through increasing the number of measured objects.

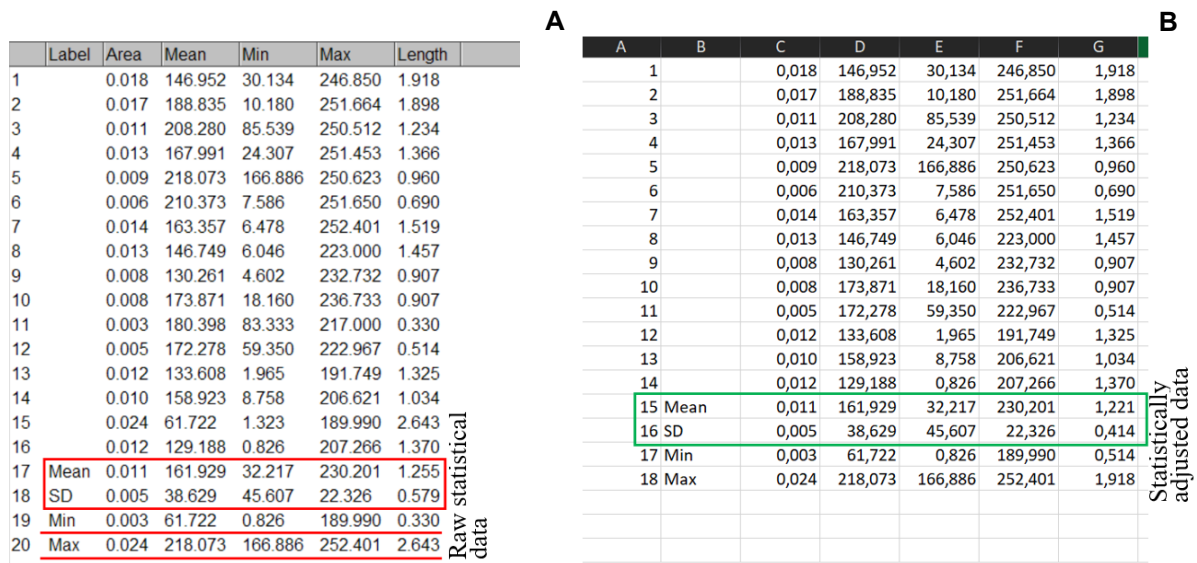


Fig. 2. ImageJ data table (A), export to Excel (B) with clarification of the final result as M ± SD.

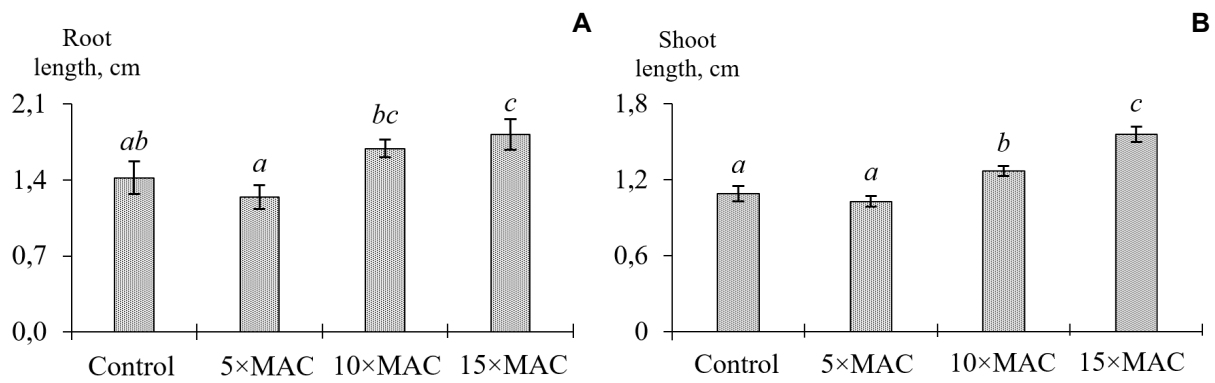


Fig. 3. Effect of copper (II) ions on the root length (A) and shoot length (B) of a clover seedling.

Table 1. Size of *H. vulgare* subflag leaves under chronic cadmium exposure.

Dose level	Length, cm	Width, cm	Area, cm ²
Control	10.9 ± 1.2	0.77 ± 0.09	4.8 ± 0.8
Cadmium	12.4 ± 2.5	0.81 ± 0.12	5.9 ± 1.9

Conclusion

Using photographs to obtain plant information is not only convenient but also effective. Experimental data can be readily verified or reused for comparison with other subjects. We have demonstrated that the ImageJ-based processing of photographs of plant test objects can replace manual measurement with acceptable accuracy. This software can also assess certain plant features that are particularly difficult to measure manually. For example, image magnification and binarization allow us to measure the length of curved clover roots and determine the area of secondary flag leaves of barley.

Acute exposure of germinated clover seeds to copper (II) ions showed that 20 seeds per experiment in 3 replicates were sufficient to obtain reliable differences between different samples. At the same time, the chronic exposure accounted for the adaptation process and variability of individual characteristics of each seedling. That is why 20 specimens per experimental variant proved to be insufficient.

The novelty of this work lies in the detailed algorithmic approach for phytotoxicity experiments. The proposed algorithm is applicable to both acute and chronic experiments. The algorithm's broad applicability is ensured by its reliance on fundamental plant test parameters: seedling root and shoot length, and leaf area. These are the most commonly used evaluation metrics in plant testing worldwide.

The proposed algorithm for processing plant images and preprocessing in ImageJ has been shown to be effective in practice. It can be used to determine acute phytotoxicity of mediums and objects. Assessing chronic phytotoxicity is a more complex process and requires further methodological and statistical development.

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