Annals of West University of Timişoara, ser. Biology, 2025, vol. 28 (1), pp.39-46

COMPARATIVE EVALUATION OF STANDARDIZED METHODS FOR PHYTOTOXICITY TESTING

Bianca-Vanesa AGACHI[1,3], Constantina-Bianca VULPE[2,3]*, Cosmina-Alecsia COSMA[1], Diana ISTRATE[1], Andrijana Pujicic[1,3], Adina-Daniela IACHIMOV-DATCU[1,3]

[1]Department of Biology, Faculty of Chemistry, Biology, Geography, West University of Timisoara, Pestalozzi 16, 300115, Timișoara, Romania

[2]Department of Scientific Research in Biology, Advanced Environmental Research Institute, West University of Timisoara, Oituz, 4, 300086 Timisoara, Romania

[3]The Academy of Romanian Scientists, Ilfov 3, 050044, București, România

**Corresponding author's e-mail: constantina.vulpe@e-uvt.ro*

Received 7 July 2025; accepted 24 July 2025

ABSTRACT

The objective of this literature study is to review notions related to tests that are commonly used to assess the phytotoxicity of the various compounds. A comparative analysis of the tests was conducted, with emphasis placed on elements of originality. Considering the escalating prevalence of such analyses, necessitated by the proliferation of novel chemical compounds, the publication of such a paper which comprises the predominant tests that can be conducted using vegetal organisms is of paramount importance. Furthermore, a range of biochemical methods that can be utilized to complement the fundamental tests is emphasized to provide a comprehensive overview of the effects of the test compounds.

KEY WORDS*:* *OECD, Lemna, seedlings test, stress enzymes*

INTRODUCTION

Phytotoxicity describes the toxic effects that chemical substances exert on plant growth, physiology and development (Alghofaili et al. 2025; Dar et al. 2024; Huang et al. 2024; Klátyik et al. 2024). It is typically characterized by symptoms such as delayed germination, reduced root or shoot elongation, chlorosis, necrosis, and in severe cases, plant mortality (Hasanussaman et al. 2020, Lewis 1995). With the continual emergence of synthetic chemicals, pharmaceuticals, nanomaterials, and complex industrial byproducts, phytotoxicity testing has become a cornerstone of ecotoxicological assessments (Cestin et al. 2021; Tumurbaatar et al. 2024). Plants, as primary producers, serve not only as sensitive bioindicators of environmental stress but also as critical components of ecosystem function, necessitating robust methods to evaluate their responses to potential contaminants (Boxall et al. 2012, Rico et al. 2011, Wilkinson et al. 2022).

To ensure standardized and reproducible phytotoxicity evaluations, international regulatory bodies such as the Organisation for Economic Co-operation and Development (OECD) and the United States Environmental Protection Agency (EPA) have developed validated test guidelines targeting terrestrial and aquatic plant species. Among the most widely implemented protocols is OECD Test No. 208 (OECD 2006), the Seedling Emergence and Seedling Growth Test, which assesses the influence of chemicals on the early development of crop plants by monitoring parameters such as germination rate, biomass accumulation, and visible phytotoxic symptoms over a 14- to 21-day period (OECD 2006).

Complementary to this, OECD Test No. 227, the Vegetative Vigour Test, evaluates the impact of foliar application of test substances on mature, actively growing plants, focusing on growth metrics like shoot height and dry weight (OECD 2006). For aquatic environments, OECD Test No. 221 targets the growth inhibition of Lemna species (duckweed), which are highly responsive to a broad range of waterborne toxicants, providing valuable insights into the phytotoxicity of effluents and water-soluble compounds (OECD 2006).

The EPA also offers parallel guidelines for phytotoxicity assessment under its Office of Chemical Safety and Pollution Prevention. Notably, EPA 850.4100 outlines the Seed Germination and Root Elongation Test, suitable for evaluating toxicity in soils and leachates using endpoints such as germination percentage and root length. EPA 850.4150, the Vegetative Vigor Test, measures sublethal effects on plant biomass and growth rates, while EPA 850.4230, akin to OECD 208, focuses on Seedling Emergence under exposure to soil-applied chemicals. These assays are designed for use with both crop species and native plants, ensuring ecological relevance and regulatory compliance. (EPA 2012)

In recent years, bibliometric tools such as VOSviewer have gained traction in environmental research, offering visual and quantitative analysis of scientific literature. By mapping co-occurrence networks of keywords, authorship clusters, and citation linkages, VOSviewer enables researchers to identify prevailing methodologies, influential publications, and research gaps within a given field. While bibliometric studies using VOSviewer are well-established in domains such as neuropsychiatry and public health, their application in phytotoxicology remains relatively underexplored. A bibliometric assessment of phytotoxicity testing can offer valuable insights into methodological trends and innovation potential within this dynamic area of environmental science (Tang et al. 2023).

This study aims to present a comparative analysis of phytotoxicity test methods with a focus on standardized protocols such as OECD and EPA guidelines, supplemented by biochemical approaches that enhance mechanistic interpretation. Through a synthesis of methodological strengths and limitations, as well as insights drawn from bibliometric mapping, this review seeks to provide a comprehensive perspective on current and emerging strategies for assessing phytotoxicity using plant-based systems.

MATERIALS AND METHODS

The main scope of this paper was to comparatively analyze different methods that were successfully proven to be appropriate for phytotoxicity assays. A specific literature investigation was accomplished during 2025 using Google Academic (https://scholar.google.com/) and Web of Science (https://www.webofscience.com/wos/woscc/basic-search), and articles in other languages, not English, were excluded. Data processing was accomplished using Microsoft Office Excel.

Moreover, a logical graphical representation was created using VOSviewer website (https://www.vosviewer.com/), in order to integrate the obtained material, resulting in a network map that presents the links between numerous notions related to phytotoxicity, based on co-citation or co-occurrence of phrases in the examined data corpus.

RESULTS AND DISCUSSIONS

Starting with 2000 and continuing until 2024, there are over 68400 research articles related to phytotoxicity available on Google Scholar (GS), with a clear growing interest in the past years (Figure 1).

The VOSviewer co-occurrence map shows that phytotoxicity is the central theme, with a related focus on plant growth, soil, and oxidative stress. Frequent terms like tolerance, stress, and responses highlight interest in how plants cope with toxic conditions. Keywords such as photosynthesis, germination, and phytoremediation point to effects on key plant functions and potential for environmental remediation (Figure 2).

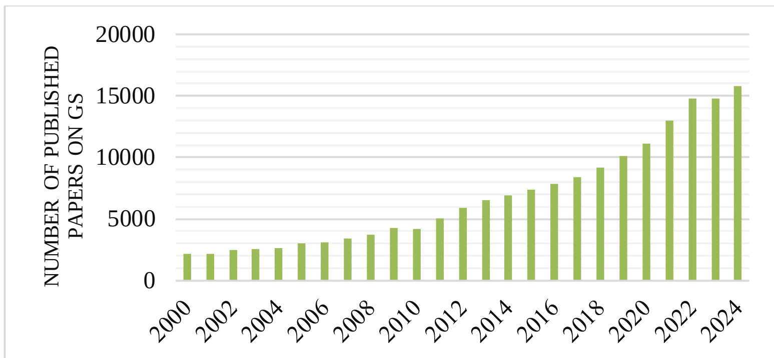

FIG. 1. The amount of phytotoxicity-related papers published between 2000 and 2024

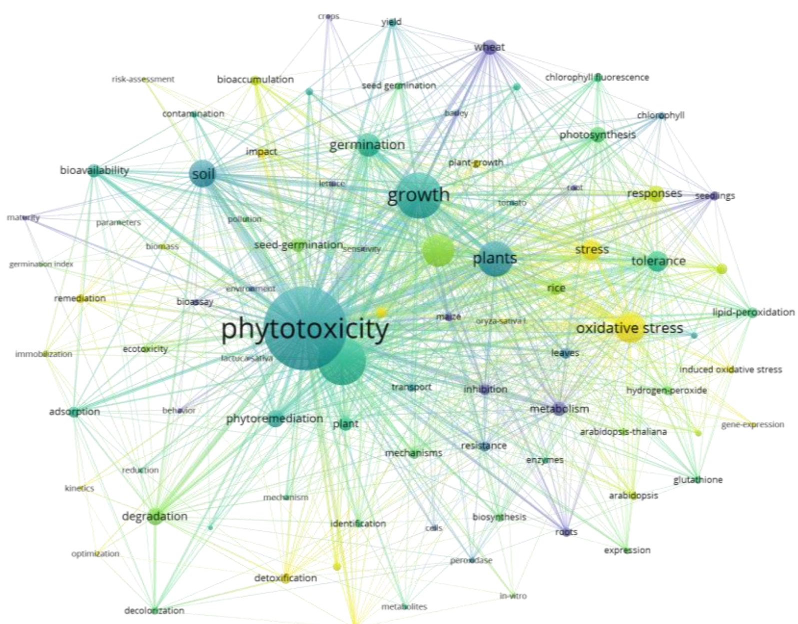

FIG. 2. Map of the co-occurrence network of keywords in Web of Science articles related to phytotoxicity

In order to accomplish quantitative analysis, a series of analytical techniques can be utilized (Table 1). The data highlights that plant toxicity tests vary by growth stage, environment, and plant type—ranging from simple germination tests to mature plant assessments in soil, soilless, or aquatic systems. While some methods overlap, each offers specific insights based on species and exposure conditions.

TABLE. 1: Comparative table of standardized tests used to assess phytotoxicity

Test	Short Description	Similarities and differences	References
OECD 208 Seedling Emergence and Seedling Growth Test	Evaluates the effects of substances on the germination and growth of seedlings in soil.	Similar to EPA 850.4230; both test for emergence in soil. OECD 208 allows for a wider choice of species and can use natural or artificial soil.	OECD (2006), *Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264070066-en.
OECD 221 *Lemna sp.* Growth Inhibition Test	Evaluates the toxicity of various substances to genus Lemna, which includes small aquatic plant species.	Developed on aquatic species. Similarities with OECD 201, developed on *Freshwater Alga and Cyanobacteria*	OECD (2006), *Test No. 221: Lemna sp. Growth Inhibition Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264016194-en.
OECD 227 Vegetative Vigour Test	Test on already developed plants, in the vegetative phase.	Similar to EPA 850.4150; both evaluate mature plants. OECD 227 is applied in controlled, soilless environments, usually by foliar application.	OECD (2006), *Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264067295-en.
EPA 850.4100 Seed Germination / Root Elongation Test	Laboratory test for effects on germination and root elongation.	Similar to OECD 208, but simpler and shorter, performed in inert media (e.g. Petri dish). Used as a screening test.	U.S. Environmental Protection Agency. Seedling Emergence and Seedling Growth; OCSPP 850.4100; EPA: Washington, DC, USA, 2012. Available online: www.epa.gov
EPA 850.4150 Vegetative Vigor	Evaluates the effects of substances on the growth of developed plants.	Similar to OECD 227; uses standardized species, usually with foliar application.	U.S. Environmental Protection Agency. Vegetative Vigor; OCSPP 850.4150; EPA: Washington, DC, USA, 2012. Available online: www.epa.gov
EPA 850.4230 Seedling Emergence	Tests seedling emergence and growth in soil	Almost equivalent to OECD 208; species specific to EPA requirements are used	U.S. Environmental Protection Agency. Early Seedling Growth Toxicity Test; OCSPP 850.4230; EPA: Washington, DC, USA, 2012. Available online: www.epa.gov

Phytotoxicity tests are often complemented by a range of additional analyses, including microscopic, morphometric, gravimetric, and biochemical methods.

Microscopic evaluations typically involve the analysis of features such as stomatal density and diameter, as well as the number and turgidity of trichomes (Mushtaq et al. 2020; Yadav et al. 2021). Morphometric methods include measurements of root and stem length (Begum et al. 2012), but also determination of root growth index (de Souza et al. 2025), while gravimetric analyses commonly involve the determination of fresh and dry weights but also sample humidity (Amin et al. 2013). Moreover, there are numerous studies in which germination tests are done (Bone et al. 2023; Garrido et al. 2025), due to the fact that these offer responses to different chemicals quite quick. Frequently assessed biochemical parameters include photosynthetic pigments content (Li et al. 2022), completed by other indices as photosynthetic rate (Chohra et al. 2025), concentrations of reducing sugars, starch, and proteins (Chatteriee et al. 2003; Hasan et al. 2021), as well as the enzymatic activity of key enzymes involved in oxidative stress (such as catalase, guaiacol peroxidase, glutathione peroxidase, and superoxide dismutase) (Li et al. 2022).

CONCLUSIONS

The growing number of publications on phytotoxicity between 2000 and 2024 reflects an increasing global concern regarding the impact of environmental pollutants, natural compounds, and synthetic substances on plant health. Bibliometric analysis confirms that phytotoxicity remains a central theme in plant stress research, particularly in relation to plant growth, soil interactions, and oxidative responses.

Phytotoxicity testing has evolved to incorporate a wide array of techniques tailored to different plant species, developmental stages, and environmental contexts. The integration of classical tests—such as seed germination and root elongation—with complementary microscopic, morphometric, gravimetric, and biochemical analyses offers a comprehensive understanding of toxicological effects. These multidimensional approaches enable the evaluation of physiological, structural, and metabolic responses of plants to various stressors.

Notably, key biochemical markers such as photosynthetic pigments, sugars, and antioxidant enzyme activities serve as reliable indicators of phytotoxic stress. The use of advanced visualization tools like VOSviewer further enhances our ability to detect emerging research trends and connections among key terms and concepts in the field.

Altogether, this work underscores the importance of integrated phytotoxicity assessment frameworks that not only inform ecological risk evaluation but also guide the development of sustainable strategies for environmental protection and phytoremediation.

ACKNOWLEDGMENT

This research was funded by a grant entitled "Integrating EXperimental and INformatics methodologies in TOXICological impact assessment: Investigating the effects of triazole fungicides on the environment and human health, acronym EX-IN-TOX, 04.2025-12.2026, funded by the Academy of Romanian Scientists through the Academy of Romanian Scientists Research Projects Competition for Young Researchers "AOȘR-Teams-IV" Edition 2025-2026 " Digital Transformation in Sciences".

REFERENCES

- Alghofaili F., Tombuloglu H., Almessiere M.A., Tombuloglu G., Akhtar S., Turumtay E.A., Baykal A., Turumtay H. 2025. Phytotoxicity and growth enhancement properties of magnesium and zinc co-doped aluminum oxide nanoparticles on barley (*Hordeum vulgare* L.). *Environ Sci Pollut Res* **32**: 9593–9613.
- Amin H., Arain B.A., Amin F., Surhio M.A. 2013. *Phytotoxicity of Chromium on Germination, Growth and Biochemical Attributes of Hibiscus esculentus L. American Journal of Plant Sciences*, **4**: 2431–2439. https://doi.org/10.4236/ajps.2013.412302
- Begum P., Ikhtiari R., Fugetsu B., Matsuoka M., Akasaka T., Watari F. 2012. *Phytotoxicity of multi-walled carbon nanotubes assessed by selected plant species in the seedling stage. Applied Surface Science,* **262**: 120–124. https://doi.org/10.1016/j.apsusc.2012.03.028.
- Bona D., Lucian M., Feretti D., Silvestri S., Zerbini I., Merzari F., Messineo A., Volpe M. 2023. Phytotoxicity and genotoxicity of agro-industrial digested sludge hydrochar: The role of heavy metals, *Science of The Total Environment* **871**: 162138, https://doi.org/10.1016/j.scitotenv.2023.162138.
- Boxall A.B.A., Rudd M.A., Brooks B.W., Caldwell D.J., Choi K., Hickmann S., Innes E., Ostapyk K., Staveley J.P., Verslycke T., Ankley G.T., Beazley K.F., Belanger S.E., Berninger J.P., Carriquiriborde P., Coors A., DeLeo P.C., Dyer S.D., Ericson J.F., … Van Der Kraak G. 2012. *Pharmaceuticals and personal care products in the environment: What are the big questions? Environmental Health Perspectives*, **120**: 1221–1229. https://doi.org/10.1289/ehp.1104477
- Ceschin S., Bellini A., Scalici M. 2021. Aquatic plants and ecotoxicological assessment in freshwater ecosystems: a review. *Environmental Science and Pollution* Research **28**: 4975–4988. https://doi.org/10.1007/s11356-020-11496-3
- Chohra H., Lee, K.-A., Choe H., Cho J.Y., Kantharaj V., Cheong M.S., Kim Y.-N., Lee Y.B. 2025. Dose-Dependent Physiological Response to Transient Bioaccumulation of Tetracycline in Kimchi Cabbage (*Brassica campestris* L.). *Antibiotics* **14**: 501. https://doi.org/10.3390/antibiotics14050501
- Chatterjee J., Chatterjee C. 2003. Management of phytotoxicity of cobalt in tomato by chemical measures. *Plant Science*, **164**: 793–801. https://doi.org/10.1016/s0168-9452(03)00066-9
- Dar F.A., Tahir I., Rehman R.U., Alharby H.F., Alzahrani Y., Alsamadany H., Hakeem K.R. 2024. Exogenous silicon alleviates aluminum phytotoxicity in Fagopyrum esculentum Moench by modulating physiological and antioxidant responses. *South African Journal of Botany*, **167**: 367-384, https://doi.org/10.1016/j.sajb.2024.01.073.
- de Souza, L.G.X.; Teran, F.J.C.; Cuba, R.M.F.; Chaves, A.R.; da Silva, K.C. 2025. Interaction of Microplastics with Emerging Organic Pollutants: A Study on Atrazine Adsorption and Phytotoxicity. *Toxics* **13**: 257. https://doi.org/10.3390/toxics13040257
- Garrido R.M., Dayan F.E., Ozanique P.R., Regasini L.O., Kolb R.M. 2025. Hydroxychalcones as Herbicides. *Agronomy* **15**: 572. https://doi.org/10.3390/agronomy15030572
- Hasan M., Mehmood K., Mustafa G., Zafar A., Tariq T., Hassan S.G., Loomba S., Zia M., Mazher A., Mahmood N., et al. 2021. Phytotoxic Evaluation of Phytosynthesized Silver Nanoparticles on Lettuce. *Coatings* **11**: 225. https://doi.org/ 10.3390/coatings11020225
- Hasanuzzaman M., Mohsin S.M., Bhuyan M.H.M.B., Bhuiyan T.F., Anee T.I., Masud A.A.C., Nahar K. 2020. *Phytotoxicity, environmental and health hazards of herbicides: challenges and ways forward*. In:

Agrochemicals Detection, Treatment and Remediation: Pesticides and Chemical Fertilizers, 55–99. https://doi.org/10.1016/B978-0-08-103017-2.00003-9

- Huang J., Huang M., Guan Z., Chen L., Chen J., Lv L., Liu M. 2024. Phytotoxicity of HNTs to rice (*Oryza sativa* L.): Effects on rice growth and development, Chemosphere, **368**: 143735, https://doi.org/10.1016/j.chemosphere.2024.143735.
- https://scholar.google.com/
- Klátyik S., Takács E., Barócsi A., Lenk S., Kocsányi L., Darvas B., Székács A. 2024. Hormesis, the Individual and Combined Phytotoxicity of the Components of Glyphosate-Based Formulations on Algal Growth and Photosynthetic Activity. *Toxics* **12**: 257. https:// doi.org/10.3390/toxics12040257
- Lewis M.A. 1995. *Use of freshwater plants for phytotoxicity testing: A review. Environmental Pollution*, 87: 319–336. https://doi.org/10.1016/0269-7491(94)P4164-J
- Li X., Riaz M., Song B., Liu H. 2022. *Phytotoxicity response of sugar beet (Beta vulgaris L.) seedlings to herbicide fomesafen in soil. Ecotoxicology and Environmental Safety*, **239**: 113628. https://doi.org/10.1016/j.ecoenv.2022.113628
- Mushtaq W., Siddiqui M.B., Alharby H.F., Hakeem K.R. 2020. Assessment of the Contribution of Foliar Trichomes towards Allelopathy. *Phyton-International Journal of Experimental Botany* **89**(2): 292-301. https://dx.doi/10.32604/phyton.2020.08740
- OECD (2006), Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264070066-en.
- OECD (2006), Test No. 221: *Lemna sp.* Growth Inhibition Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264016194-en.
- OECD (2006), Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264067295-en.
- OECD (2011), *Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264069923-en.
- Rico C.M., Majumdar S., Duarte-Gardea M., Peralta-Videa J.R., Gardea-Torresdey J.L. 2011. Interaction of nanoparticles with edible plants and their possible implications in the food chain. *Journal of Agricultural and Food Chemistry*, 59: 3485–3498. https://doi.org/10.1021/jf104517j
- Tang Y., Lu X., Hu M. 2023. A bibliometric analysis of publications on obsessive–compulsive disorder using VOSviewer. *Frontiers in Psychiatry*, **14**: 1136931 https://doi.org/10.3389/fpsyt.2023.1158478
- Tumurbaatar S, Kováts N, Hubai K. 2024. Phytotoxicity Testing of Atmospheric Polycyclic Aromatic Hydrocarbons. *Atmosphere* **15**(9): 1143. https://doi.org/10.3390/atmos15091143
- U.S. Environmental Protection Agency. Early Seedling Growth Toxicity Test; OCSPP 850.4230; EPA: Washington, DC, USA, 2012. Available online: www.epa.gov
- U.S. Environmental Protection Agency. Seedling Emergence and Seedling Growth; OCSPP 850.4100; EPA: Washington, DC, USA, 2012. Available online: www.epa.gov
- U.S. Environmental Protection Agency. Vegetative Vigor; OCSPP 850.4150; EPA: Washington, DC, USA, 2012. Available online: www.epa.gov
- Wilkinson J.L., Boxall A.B.A., Kolpin D.W., Leung K.M.Y., Lai R.W.S., Galban-Malag C., Adell A.D., Mondon J., Metian M., Marchant R.A., Bouzas-Monroy A., Cuni-Sanchez A., Coors A., Carriquiriborde P., Rojo M., Gordon C., Cara M., Moermond M., Luarte T., … Teta C. 2022. *Pharmaceutical pollution of the world's rivers. Proceedings of the National Academy of Sciences of the United States of America*, **119**: e2113947119. https://doi.org/10.1073/pnas.2113947119
- Yadav V., Arif N., Kováč J., Singh V.P., Tripathi D.K., Chauhan D.K., Vaculík M. 2021. *Structural modifications of plant organs and tissues by metals and metalloids in the environment: A review. Plant Physiology and Biochemistry*, **159**: 100–112. https://doi.org/10.1016/j.plaphy.2020.11.047