

RESEARCH ON THE EFFECT OF HUMIC SUBSTANCES-BASED PREPARATIONS
IN PROMOTING SOIL BIODEGRADATION PROCESSESOleh Kibarov[✉], Ganna Trokhymenko^{*}, Vladyslav Nedoroda^{*}*Admiral Makarov National University of Shipbuilding,
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Abstract. The paper presents an analysis of the market of fertilizers and organic additives that promote the biodegradation of herbicide residues in the soil and ensure stable growth and development of agricultural crops. The work also presents studies of the action of biostimulants based on humic substances in promoting the activation of plant defense mechanisms when combating stress in conditions unfavorable for growth. To study the effect of such biofertilizers on the growth and development of cereal crops under stressful conditions of exposure to glyphosate, the phyto-indicator *Sorghum bicolor subsp. Drummondii* was used, as well as all known types of fertilizers based on humic substances. These include liquid organic experimental fertilizers based on humic acids with an increased composition of fulvic acids, as well as the more popular potassium humate and inoculants based on them, which include strains of bacteria of the genus *Bacillus* and ascomycete fungi *Trichoderma*. The main research methods are experiment, comparison, and analysis.

Keywords: bioremediation, growth stimulants, herbicides, fulvic acids, humic acids.

1. Introduction

The increased usage of herbicides for agricultural weed control has led to an annual global herbicide consumption of about one million tons. The extensive

usage of herbicides has sparked worries about how their residues may affect soil ecology and human health. Herbicides harm the soil microbiome and associated ecosystem functioning in addition to inflaming the human small and large intestines. The future of sustainable agriculture and the welfare of society depend on thorough research into how pesticide residues alter soil microorganisms and functions. Soil ecosystems are multifaceted and multifunctional by nature. Climate regulation, primary productivity, carbon sequestration, nutrient supply and cycling, soil biodiversity maintenance, water purification and regulation, and more are all functions of soil. Long-term environmental and human issues including pesticide use, pollution buildup, climate change, and intensified agricultural land use are all exacerbated by these functions (Alister et al., 2020).

Humic compounds, protein hydrolysates, seaweed extracts, and microbes are examples of biostimulants that have demonstrated the ability to enhance plant growth, boost crop output and quality, and bioremediate soils. However, trying to understand the underlying mechanisms of commercially available biostimulants is difficult due to their heterogeneous composition and multimolecular structure. Recent molecular research has started to identify the pathways that particular products stimulate at the cellular and gene levels, however the majority of studies have concentrated on the broad impacts of biostimulants on crops. Improved crop protection and soil bioremediation methods could result from a better understanding of molecular impacts.

The sorption and desorption of pesticides are the primary variables influencing their fate in the environment. Improper sorption-desorption mechanisms can lead to decreased microbial activity and increased pesticide volatilization or leaching. To combat these issues, fulvic acid—an organic molecular chain with carboxyl and phenolic functional groups—is employed as a sorption-desorption agent. Numerous polar or ionic pesticides react favorably with fulvic acid. For instance, the nitrogen of the imidacloprid molecule can establish robust hydrogen bonds with the phenolic groups of fulvic acid. Fulvic acid can also create a potent sorption mechanism with carbamates (carbaryl and carbofuran), phenoxyacetic acids, and imidacloprid (Zhang et al., 2012). These strong interactions allow fulvic acid to protect and buffer pesticide molecules, increase their solubility, and reduce the required dosage of pesticide by 20–30 % (Ćwieląg-Piasecka et al., 2018).

Plant nutrition management can be enhanced using fulvic acid as a biostimulant to improve nutrient availability and uptake.

While chemical fertilizers increase productivity, they also contribute to environmental pollution and climate change. Organic biostimulants, such as fulvic acid, serve as non-toxic chelating and water-binding agents that improve nutrient uptake and plant productivity. Fulvic acids easily chelate essential nutrients (Zn, Fe, Mg, Ca) and transfer them to plants. These acids naturally occur in lignite, soil, and peat, and form a complex mixture with phenolate and carboxylic groups through organic matter decomposition. Humic acids, which have lower molecular weights and higher oxygen content than fulvic acids, are abundant in these mixtures (Canellas et al., 2015).

Fulvic and humic substances are promising in enhancing plant resistance to abiotic stress. Studies show that applying seaweed extract and humic acid to pre-treat certain grasses improved leaf hydration under drought, increased root and shoot growth, and boosted antioxidant activity. Treating bell pepper (*Capsicum annuum*) with humic acid under salinity reduced Na uptake and increased N, S, Cu, Fe, Mg, Ca, Mn, and K in roots and shoots, indicating protection under moderate salinity stress (Çimrin & Türkmen, 2010). Similarly, applying humic acids to beans (*Phaseolus vulgaris*) under high salinity increased proline accumulation and reduced membrane leakage, indicating improved stress adaptation (Aydin et al., 2012).

Fulvic acid also acts as a pollutant remover when mixed with pesticides. When droplet pesticides land on soil, fulvic acid can emulsify and disperse them, altering water surface tension and enabling ion exchange reactions. As a colloid with large surface area, it binds pesticides strongly, reducing their harmful effects on soil microbes and crops. Under certain conditions, fulvic acid can even degrade pesticide residues, further protecting the ecosystem.

The term “humates” refers to sodium or potassium salts of humic acids, forming the chemical basis of humus. Humus maintains soil biochemical equilibrium and fertility. Fertilizers made from soft brown coal or peat are rich in humic substances and fulvic acids, vital for soil health. For example, preparation of potassium humates involves grinding coal, mixing with KOH, and separating the solid phase. This concentrated humic fertilizer is rich in humic and fulvic acids and trace elements. Using potassium humate on soil improves water retention, boosts beneficial microbes, and enhances nutrient availability. It chelates essential minerals, improving plant growth and soil structure. Studies show that potassium humate significantly enhances soil composition by binding particles, improving drainage, and reducing compaction. Its organic matter content supports diverse soil microorganisms, accelerating nutrient cycling and improving fertility (El-Beltagi et al., 2023). Humate application also lowers soil Salinity and retains moisture, reducing the need for irrigation.

Best practices for potassium humate involve using it as a soil enhancer during planting (mixing with topsoil) and as a foliar spray to improve nutrient uptake. It should be sprayed in the early morning or late evening with water to prevent evaporation.

To further enhance humic biostimulants, they are often combined with soil microbes. Common beneficial strains include *Bacillus* and *Trichoderma*. Although the exact plant-microbe interactions under stress are not fully understood, many microbes can act as biostimulants in challenging environments. Genera such as *Pseudomonas*, *Rhizobium*, *Bacillus*, *Azotobacter*, *Azospirillum*, and *Bradyrhizobium* contain strains adapted to saline, alkaline, acidic, or arid soils. These microbes modify their cell walls and accumulate solutes (e.g., exopolysaccharides, lipopolysaccharides) to survive stress, forming protective biofilms on roots and retaining water. Inoculating soil with plant growth-promoting rhizobacteria (PGPR) can enhance plant stress tolerance by improving hydration and nutrient uptake (Selvakumar & Joshi, 2009).

For example, *Rhizobium* strains can produce exopolysaccharides that help maintain root-zone hydration under drought or salinity (Abd El-Ghany et al., 2020).

Field studies show that inoculating crops with nitrogen-fixing or salt-tolerant bacteria improves stress resistance. Inoculation of maize with *Azotobacter* strains under salt stress increased availability of P and N and helped the plant exclude Na. For wheat under salt stress, saline-tolerant *Azotobacter* improved grain yield and nitrogen content (Kaushal, 2015). When two strains of *Rhizobium leguminosarum* (one salt-tolerant, one sensitive) were inoculated into pea and faba bean, plants with the tolerant strain fared better under salt stress (Ihsan and Hussein, 2005). Inoculating chickpeas and faba beans with *Azospirillum brasilense* enhanced root colonization, nodulation, and salt tolerance. Another bacterium, *Azotobacter chroococcum*, showed salt tolerance; inoculating crops with it on saline soils increased yields of peas, potatoes, rice, wheat, and cotton, as well as root and shoot growth (Hamaoui et al., 2001). These findings highlight that selecting the right biostimulant—based on composition, soil type, and stress factors—is crucial for enhancing plant stress resilience.

2. Materials and Methods

The experiment analyzed the effect of humic substance-based biostimulants on the growth of *Sorghum bicolor subsp. Drummondii* (Sudan grass) and their interaction with glyphosate herbicides. The treatments included the following fertilizers and inoculants:

- BioFulvo – a liquid organic fertilizer containing 150–200 g/L fulvic acids, 10 g/L humic acids, and *Bacillus amyloliquefaciens* ssp. *plantarum*. It is produced from processed organic waste (bran, grain waste, straw, etc.) and is rich in low-molecular fulvic acids. It also contains *Bacillus* strain 531 (a heavy-metal-resistant biofertilizer).
- Stubble Destroyer – a biological inoculant with live cells and spores of *Bacillus subtilis* and *Bacillus licheniformis* ($\geq 1.0 \times 10^9$ CFU/cm³), spores of *Trichoderma viride* and *T. lignorum*, and their metabolites. Contains ≥ 100 g/L humic substances.
- Potassium Humate – a concentrated organo-mineral humic fertilizer with 70 g/L humic acids, 34 g/L fulvic acids (total 104 g/L humic substances), and macro- and micronutrients (N, K, B, Co, Cu, P, Zn,

Fe, Mn, Mo). It is used for seed treatment, foliar feeding, and root feeding to stimulate rapid growth.

The herbicides used were:

1. “Urahan Forte” – 500 g/L glyphosate.
2. “Federal” – 480 g/L glyphosate (isopropylamine salt) + 60 g/L dicamba.

At the first stage, all soil samples were treated with the fertilizers at the following dilutions:

- “BioFulvo” at 1:500, 1:100, 1:10
- Stubble Destroyer at 1:500, 1:100, 1:10
- Potassium Humate at 1:500 and 1:300

Several control soil samples received no fertilizer. After two weeks, all soil samples were sown with *S. bicolor subsp. drummondii* seeds and watered every three days. This established the baseline growth under the different biostimulant treatments.

One week after sowing, above-ground parts of the plants were treated with the herbicides. A week later (day 14 after sowing), plants were again treated with the fertilizers. Thus, the experimental scheme involved alternating fertilization and herbicide stress to assess how pre-treatment with humic biostimulants affected plant recovery and growth.

Throughout the experiment, plant growth was measured by sprout height at specified intervals (days 8, 14, 18 after sowing). Mortality rates were also recorded under combined herbicide and biostimulant treatments. Control (water-only) samples were used for comparison.

3. Results and Discussion

Potassium humate treatments (especially 1/500 dilution) increased early growth by about 20 % compared to control, indicating a significant stimulation of sprouting. In contrast, the microbial inoculant (Stubble Destroyer) at high concentrations (e.g. 1/10) appeared to inhibit growth by 25–50 %. This adverse effect might be due to the instability or over-concentration of microbial spores causing stress to seedlings.

The BioFulvo treatment (rich in fulvic acids) increased average height by about 5 % over control, reflecting its biostimulant role.

On the 8th day after sowing, plant growth measurements yielded the results summarized in Table 1.

Table 1

Phytoindicator growth on the 8th day after sowing (in cm)

Drugs	Control/ Water	«BioFulvo» Drug	Stubble destroyer	Potassium humate
Concentrations		1/500	1/500	1/500
	3	5	3	7.7
	5	12.5	6	9.5
	5.1	9	5	9.1
	3	1.5	5.5	3.8
	4.5	4.5	6.5	4
	6	5	10	4.9
	6.2	5.3	1.5	7
	9.5	6.3	2.5	4.2
	10.5	9.5	4.1	10
Concentrations		1/100	1/100	1/300
		3	7	4.5
		3	5.5	5
		4.5	7	6
		7.5	3.3	8
		8.5	4.5	6.5
		10	4	8.5
		3	6.8	6
		1.5	3.9	5.5
		2	10.4	10
Concentrations		1/10	1/10	1/300
		8	2	3.5
		6.7	2	4.2
		9	6.3	4
		6.5	1.5	1
		6.5	4.2	5.1
		4	7.1	5.3
		7.9	-	6.3
		7.5	3	10
		8.3	3.4	12.2

The better performance of potassium humate may be attributed to its additional nutrients (K and trace elements) that strengthen the root system and

stress resistance. The general results of the progression of sprout growth on the 8th day is shown in Fig.1.

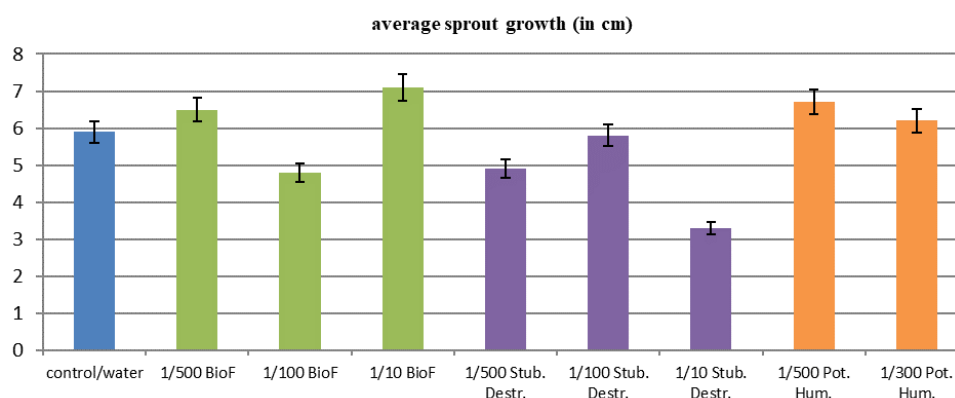


Fig. 1. Phytoindicator growth on the 8th day after sowing (cm)

A week after sowing, the above samples were treated with herbicides. The results of the growth of the phyto-indicator a week after herbicide treatment shown in Table 2.

Table 2

Phytoindicator growth on the 14th day after sowing (cm)

Drugs	Control/ Water	«BioFulvo» Drug	Stubble destroyer	Potassium humate
Concentrations		1/500	1/500	1/500
	6.8	9.5	8	9.2
	9	13.5	12	11.3
	9.3	15	9.5	13
With “Urahan F.”	4.5	3.2	7	4.5
	6	3	8.2	4.5
	7	7.8	11.2	8.9
With “Federal”	9	–	–	7
	11.3	–	–	5.5
	12	–	–	13.5
Concentrations		1/100	1/100	1/300
		5.3	12	10
		9.2	13	12
		12.5	12.5	12
With “Urahan F.”		9.2	3	6.5
		9.5	4.8	6
		12	4	9.5
With “Federal”		–	6.8	–
		–	–	–
		–	11	6
Concentrations		1/10	1/10	1/300
		14.5	5.1	13.1
		8.7	6	11.5
		14	8.7	13.7
With “Urahan F.”		7	3.5	6.5
		6.8	5	5
		7	7.1	5
With “Federal”		8	–	7.5
		9	–	11.5
		11	–	13

After one week of herbicide stress, growth was generally suppressed. In the most extreme case (herbicide + inoculant 1/10), plant growth was nearly halted. The highest growth at this stage was in control (no herbicide) and in treatments with BioFulvo (1/10) and potassium humate, which suggests some protective effect. Fig. 2 illustrates the relative heights under herbicide treatment.

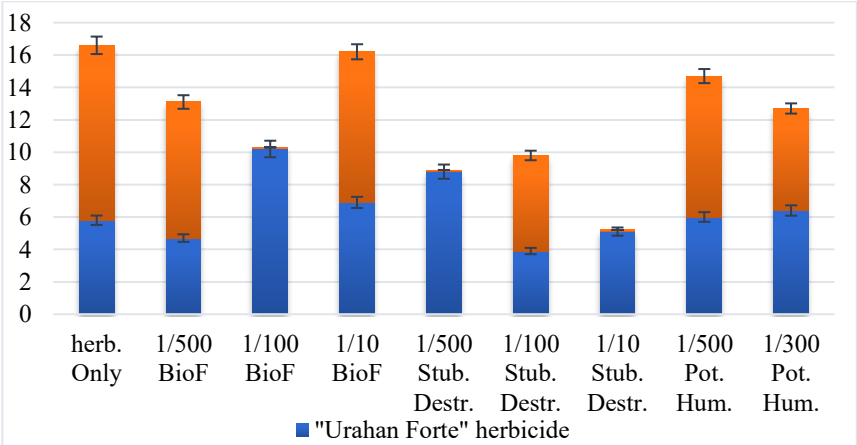


Fig. 2. Growth ratio of herbicide-treated plants (cm)

One week later (day 18), plants received a second round of fertilizers. By day 18, potassium humate treatments showed the greatest recovery, with the highest average growth among all samples

(Table 3). BioFulvo also improved growth but to a lesser extent. The combined use of potassium humate likely helped plants adapt to the adverse conditions.

Table 3

Phytoindicator growth on the 18th day after sowing (cm)

Drugs	Control/ Water	“BioFulvo” Drug	Stubble destroyer	Potassium humate
Concentrations		1/500	1/500	1/500
	9.2	13.1	10.5	14
	9.5	16.5	12	15.2
	12.2	18	10.5	15.3
With “Urahan F.”	–	–	–	–
	–	–	–	–
	–	–	–	8.9
With “Federal”	–	–	–	–
	–	–	–	–
	–	–	–	–
Concentrations		1/100	1/100	1/300
		10	15.2	12.2
		10.2	14.5	12.9
		12.5	16	16.1
With “Urahan F.”		–	–	7.2
		–	4.8	–
		–	–	–
With “Federal”		–	–	–
		–	–	–
		–	–	–
Concentrations		1/10	1/10	1/300
		14.5	6.1	13.5
		14	6	12.5
		14	8.7	18
With “Urahan F.”		–	3.5	7.2
		–	5	6.9
		7	–	5
With “Federal”		–	–	–
		–	–	–
		–	–	–

By day 18, the highest single-sprout heights in controls were observed with: (1) Potassium humate 1/500–22.5 cm; (2) BioFulvo 1/500–19.5 cm; (3) BioFulvo 1/100–19.0 cm. Mortality rates under herbicide + biostimulant treatments were: Federal herbicide – 100 % mortality; Urahan Forte – 73 % mortality; with potassium humate 1/300 – 33 % mortality, 1/500 – 66 %; with BioFulvo 1/100 – 66 %, 1/10 – 66 %; with Stubble destroyer 1/100 – 66 %, 1/10 – 33 %; all others – 100 %.

These results show that humic-based biostimulants promoted activation of plant defense mechanisms under stress. The potassium humate treatments, rich in K and trace elements, provided the strongest protection, likely by strengthening root systems and

triggering stress-resistance pathways. The fulvic-rich BioFulvo also benefited early vegetative growth via its high biological activity and ability to increase membrane permeability for nutrients and metabolites. The mixed bacterial/fungal inoculant (Stubble Destroyer) did not consistently improve growth; indeed, some *Trichoderma* strains appeared to reduce growth when overdosed, indicating that such biostimulants require careful dosage and context-specific use.

4. Conclusions

Applying biostimulants based on humic substances to crops may improve their ability to withstand environmental stressors. The present study identified

specific treatment combinations that significantly enhanced Sorghum growth under glyphosate stress. A deeper understanding of the mechanisms by which these biologic stimulants act—alone or in combination with microbes—will be needed to optimize their use. Our findings suggest that selecting the right type and concentration of humic-based fertilizer is crucial. In this experiment, a fulvic/humic fertilizer (BioFulvo) enhanced early growth, and potassium humate provided better protection against herbicide stress due to its nutrient content. The bacterial/fungal inoculant showed mixed results, highlighting that such treatments must be tailored to the crop, soil, and purpose.

In summary, humic substance-based preparations significantly influenced plant growth and stress adaptation. Fulvic acid-rich fertilizers improved cell permeability and growth in early stages, while potassium humate (with K and minerals) better stimulated protective functions and stress resistance. The presence of microbial inoculants (e.g. *Trichoderma*, *Bacillus*) can enhance effects but requires careful selection and dosage. These results underscore the promise of humic biostimulants in bioremediation and sustainable agriculture, provided their use is optimized based on environmental conditions and crop needs.

References

- Abd El-Ghany, M. F., & Attia, M. (2020). Effect of exopolysaccharide-producing bacteria and melatonin on faba bean production in saline and non-saline soil. *Agronomy*, 10(3), 316. doi: <https://doi.org/10.3390/agronomy10030316>
- Ali, S., Akhtar, M. S., Siraj, M., & Zaman, W. (2024). Molecular communication of microbial plant biostimulants in the rhizosphere under abiotic stress conditions. *International Journal of Molecular Sciences*, 25(22), 12424. doi: <https://doi.org/10.3390/ijms252212424>
- Alister, C., Araya, M., Cordova, A., Saavedra, T., & Kogan, M. (2020). Humic substances and their relation to pesticide sorption in eight volcanic soils. *Planta Daninha*, 38, e020171636. doi: <https://doi.org/10.1590/s0100-83582020380100021>
- Aydin, A., Kant, C., & Turan, M. (2012). Humic acid application alleviates salinity stress of bean (*Phaseolus vulgaris* L.) plants by decreasing membrane leakage. *African Journal of Agricultural Research*, 7(7), 1073–1086. doi: <https://doi.org/10.5897/AJAR10.274>
- Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, 57, 233–266. doi: <https://doi.org/10.1146/annurev.arplant.57.032905.105159>
- Boretska, I., Dzhura, N., & Podan, I. (2022). Impact of oil contamination and humates on the growth of Poaceae. *Environmental Problems*, 7(2), 62–70. doi: <https://doi.org/10.23939/ep2022.02.062>
- Bravin, M. N., Michaud, A. M., Larabi, B., & Hinsinger, P. (2010). RHIZOtest: A plant-based biotest to account for rhizosphere processes when assessing copper bioavailability. *Environmental Pollution*, 158(10), 3330–3337. doi: <https://doi.org/10.1016/j.envpol.2010.07.029>
- Canellas, L. P., Olivares, F. L., Okorokova-Façanha, A. L., Façanha, A. R., & Piccolo, A. (2015). Humic and fulvic acids as biostimulants in horticulture. *Scientia Horticulturae*, 196, 15–27. doi: <https://doi.org/10.1016/j.scienta.2015.09.013>
- Çimrin, K. M., Türkmen, Ö., Turan, M., & Tuncer, B. (2010). Phosphorus and humic acid application alleviate salinity stress of pepper seedlings. *African Journal of Biotechnology*, 9(36), 5845–5851.
- Ćwieląg-Piasecka, I., Medyńska-Juraszek, A., Jerzykiewicz, M., Dębicka, M., Bekier, J., Jamroz, E., & Kawalko, D. (2018). Humic acid and biochar as specific sorbents of pesticides. *Journal of Soils and Sediments*, 18, 2692–2702. doi: <https://doi.org/10.1007/s11368-018-1976-5>
- El-Beltagi, H. S., Al-Otaibi, H. H., Parmar, A., Ramadan, K. M. A., da Silva Lobato, A. K., & El-Mogy, M. M. (2023). Application of potassium humate and salicylic acid to mitigate salinity stress of common bean. *Life*, 13(2), 448. doi: <https://doi.org/10.3390/life13020448>
- Hamaoui, B., Fernández-Pascual, M., Carmona, M., & Barrueco, C. (2001). Effects of inoculation with *Azospirillum brasilense* on chickpeas (*Cicer arietinum*) and faba beans (*Vicia faba*) under different growth conditions. *Journal of Horticultural Research*, 10(1), 55–61.
- Ihsan, A., & Hussein, N. N. (2005). Isolation and characterization of salt-tolerant strains of *Rhizobium leguminosarum* bv. *viciae*. *Journal of Applied Sciences and Environmental Management*, 9(3), 77–79.
- Liu, P. W. G., Cao, L., Huang, X., Yu, Z., & Wu, Q. (2011). Bioremediation of petroleum hydrocarbon-contaminated soil: Effects of strategies and microbial community shift. *International Biodeterioration &*

- Biodegradation*, 65(6), 757–763. doi: <https://doi.org/10.1016/j.ibiod.2011.09.002>
- Nedoroda, V. (2021). Analysis of petroleum biodegradation by a bacterial consortium of *Bacillus amyloliquefaciens* ssp. *plantarum* and *Bacillus subtilis*. *Journal of Ecological Engineering*, 22(3), 48–55. doi: <https://doi.org/10.12911/22998993/143017>
- Nedoroda, V., Kibarov, O., & Trokhymenko, G. (2024). Analysis of the feasibility of using fertilizers based on fulvic acids in bioremediation of contaminated soil. *Soil Science Annual*, 75(4), 195814. doi: <https://doi.org/10.37501/soilsa/195814>
- Pan, Y., Wang, Z., Yu, J., Chen, X., & Zhu, H. (2022). Root exudates and rhizosphere soil bacterial relationships of *Nitraria tangutorum* are linked to k-strategist bacterial community under salt stress. *Frontiers in Plant Science*, 13, 997292. doi: <https://doi.org/10.3389/fpls.2022.997292>
- Stanojević, A. B., Kojić, M., Lazarovits, G., Mihajlović, T., & Jevtić, M. (2023). Evaluation of the ex-situ bioremediation of petroleum hydrocarbons contaminated soil. *Bioremediation Journal*, 27(3), 161–170. doi: <https://doi.org/10.1080/10889868.2023.2283580>
- Synelnikov, S. M., Vinnikov, I. M., Onyshchenko, V. M., & Tkachenko, V. F. (2019). Improvement of environmental safety of agricultural systems as a result of encapsulated mineral fertilizer implementation. *Environmental Problems*, 4(4), 222–229. doi: <https://doi.org/10.23939/ep2019.04.222>
- Zhang, L., Liu, X., Li, L., & Yan, M. (2012). Integrated investigations on the adsorption mechanisms of fulvic and humic acids on three clay minerals. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 413, 313–318. doi: <https://doi.org/10.1016/j.colsurfa.2012.05.003>