**CHAPTER** 67

**Recent advances in soil glyphosate contamination and removal**

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#### **ABSTRACT**

The soil has been constantly exposed to a variety of contaminants and their mixtures in various concentrations, organic/inorganic chemical

exposure, which mainly includes pesticides and their mixtures of monomers), which contaminate the soil to a greater extent and can also be transported within the soil and transfer the mixed contaminants, integrating into the food chain and passing through different trophic levels, causing health effects of organisms exposed to them. Pesticides are chemical compounds used to eliminate pests. They are chemical or biological agents, which weaken, incapacitate and kill pests. The emerging contaminant glyphosate (N- (phosphonomethyl)glycine) is widely used in agriculture in several countries. It is considered the most widely used herbicide in the world, as well as being one of the most harmful to human health. This compound is used to control harmful or invasive weeds. Glyphosate is the best-selling active ingredient in Brazil, with 195,056 tons sold in 2018. Brazil stands out for the massive agricultural use of glyphosate. An increasing number of countries have begun to restrict/ban the use of glyphosate-based products, such as Germany, Austria, Bulgaria, Colombia, and Costa Rica, among others, based on evidence already available on the direct incidence of glyphosate at various levels of the global ecosystem, as well as favoring the emergence of serious problems in human health. In this sense, the present study emphasizes the importance of knowing the main properties of pesticides and the study in search of efficient technological advances for the recovery of soils impacted by the use of glyphosate.

**Keywords:** Agriculture, Pesticide, Environment, Biotechnology, Environmental recovery.

### **1 INTRODUCTION**

The agricultural sector has undergone an intense process of industrialization and the intense use of organic and inorganic compounds in agriculture has consequently contaminated the soil (Bokade et al., 2023). The rampant use of organic compounds as pesticides has led to a multitude of havoc for human health, biodiversity, and the ecosystem. The pesticides were manufactured for agricultural use with various purposes such as fungicides, insecticides, nematicides, herbicides, molluscicides, rodenticides, plant growth regulators, and others. The widespread use of pesticides contaminates the soil with a persistent chemical load. About 80-90% of applied pesticides remain unused on land as

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waste and kill non-target vegetation, and organisms, severely affecting the well-being of humanity and the agricultural ecosystem (Sun et al., 2018). The concern raised by pesticide pollution in the soil raises an urgent need for a recovery process.

It is important to evaluate all the parameters that enable the recovery of soils that constantly receive a high chemical load from pesticides. The physicochemical conditions of these soils must be considered, as well as all fauna and flora present in the local ecosystem. Several techniques for the recovery of soils contaminated by glyphosate have been studied by researchers around the world to provide a more sustainable environment and, consequently, more quality of life for humans.

Pesticides are chemical compounds used to eliminate pests. They are chemical or biological agents, which weaken, incapacitate and kill pests (Raffa; Chiampo, 2022). The rampant use of these substances has generated major negative impacts on the soil. It is considered the most widely used herbicide in the world, as well as being one of the most harmful to human health (Ramula et al., 2022).

In this sense, the present study addresses the forms of soil contamination by pesticides, as well as the most relevant characteristics of this product with a focus on the organophosphate pesticide glyphosate. In addition, recent studies focused on the recovery of soils contaminated by glyphosate are presented in this work.

## **2 SOIL CONTAMINATION**

The level of environmental pollution is related to climatic conditions, landforms, development and industrialization. The development of an industrial area and the consequent demand for higher living standards have led to the degradation and disintegration of natural environments around the world. The consequences of excessive human interference in the environment may be irreversible and may constitute a serious threat to biological life, including human existence, in the future (Wolejko et al., 2022).

Soil has been constantly exposed to a variety of contaminants and their mixtures in various concentrations (e.g., micro/nano plastics, heavy metal accumulation (Run et al., 2021), organic/inorganic chemical exposure, which mainly includes pesticides and their monomer mixtures), which contaminate the soil to a greater extent and can also be transported within the soil and transfer the mixed contaminants, integrating into the food chain and going through different trophic levels, causing health effects of organisms exposed to them (Ahmad et al., 2022).

The transport of these pollutants occurs through earthworms, ants, rats, microbes and other organisms that bury themselves in the soil. Some contaminants are washed horizontally, leaching into nearby surface waters, or are blown by the wind and dispersed into the air (Venier et al., 2019). Several other soil contaminants can be carried by erosion or volatilization and can move deep into the soil and settle; this is due to physical, chemical, and biological degradation processes such as leaching, diffusion, photolysis, tectonic plate movements, and microbial resilience (Seiber; Cahill, 2022). These pollutants undergo major changes in their structure, which can cause them to dissipate or interact with other pollutants, thus forming a new combination called emerging pollutants with enhanced toxicokinetics and toxicodynamics (Bertrand et al., 2015). They can further penetrate the soil from where they can reach animals, humans and mainly plants, bioaccumulating in the terrestrial biota (Barali et al., 2020; Chu et al., 2021).

## **3 PESTICIDES – BRIEF HISTORY**

Pesticides are chemical compounds used to eliminate pests. They are chemical or biological agents, which weaken, incapacitate and kill pests (Raffa; Chiampo, 2022).

During the nineteenth and twentieth centuries, plant extracts, namely pyrethrins, were used as insecticides, fungicides, and herbicides. The increased use of pesticides happened with synthetic chemistry during the 1930s. During this period, inorganic chemicals, such as arsenic and sulfur compounds, were applied to protect crops. The arsenic poison was senic, and sulfur compounds were applied for crop protection. Arsenic venom was fatal to insects, while sulfur was used as a fungicide. At the beginning of World War II, numerous pesticides were synthesized, mainly organic chemicals such as dichlorodiphenyltrichloroethane (DDT), aldrin, and dieldrin were used as insecticides, while 2-methyl-4-chlorophenoxiace (Matthews, 2018).

After 1945, there was a rapid development of the agrochemical field, characterized by the introduction of many insecticides, fungicides, herbicides and other chemicals, to control pests and ensure yields from agricultural production. In addition, pesticides are applied in aquaculture, horticulture and various domestic applications in general. They are also used to control vector-borne diseases (e.g., malaria and dengue) (Van Den Berg et al., 2012). From 1990 to 2018, quantities of pesticides used by every country in the world were recorded, especially in Asia and America. The world average quantity increased from 1.55 kg·ha−1 in 1990 to 2.63 kg·ha−1 in 2018. Observing the types, fungicides and bactericides are used more than the others (Raffa; Chiampo, 2022).

## **4 GENERAL CLASSIFICATION PESTICIDES**

The classifications of pesticides are widely known in 4 main groups: as to their origin, toxicity, target organism and chemical classification. We will cover each class in the following sessions.

## 4.1 ORIGIN OF PESTICIDES

Pesticides can be obtained from raw material of artificial origin (agrochemicals), in addition to also being chemically synthesized. There are also biopesticides, the source of which usually occurs through microorganisms. Table 1 presents recent studies involving chemically synthesized pesticides and biopesticides of technological interest.



Table 1 - recent studies regarding chemically synthesized pesticides and biopesticides.

Source: (Own authorship)

## 4.2 TOXICITY

In Brazil, the agency that regulates the use and classifies the toxicity of these products is the National Health Surveillance Agency – ANVISA. In 2019, an ordinance was published that updated these criteria (Figure 1).

The categories determining the toxicity of pesticides are classified by colour:

I – Category 1: Extremely Toxic Product – red band;

II – Category 2: Highly Toxic Product – red band;

III – Category 3: Moderately Toxic Product – yellow band;

IV – Category 4: Low Toxic Product – blue belt;

V – Category 5: Product Unlikely to Cause Acute Harm – blue belt; and

VI- Not Classified – Product Not Classified – green belt.

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Figure 1 - Classification of pesticides according to their toxicity (Source: Self-authored)

#### 4.3 TARGET ORGANISM

Insecticides - chemical and biological compounds that attack and kill insects. These compounds are used in agriculture, horticulture, forestry, and gardening, but are also used to control vectors, such as mosquitoes and ticks, that are involved in the spread of human and animal diseases, such as dengue (Haddi et al., 2017) and malaria (Kleinschmidt et al., 2018; Raffa; Chiampo, 2020)

Herbicides - are used to control and remove unwanted plants and weeds. These compounds are mainly applied to agricultural soils, before or during cultivation to maximize crop productivity. Herbicides are also used in forest management and suburban and urban areas. The modes of action depend on the chemical composition and usually involve a plant enzyme or a biological system. In this way, the regular growth and development of plants are impaired or interrupted, causing eventual death of the plant (Sherwani et al., 2015).

Rodenticide - act to kill rodents, such as rats, mice, squirrels and nutria; All of these rodents can cause damage to crops, transmit diseases, and cause ecological damage. Rodent infestations arise in a wide variety of situations: in agricultural soils, in and around buildings, in sewers, in dumps and/or in open areas. Most rodenticides act as anticoagulants that interfere with blood clotting and cause death due to excessive bleeding. Rodenticide products are block or paste-shaped baits (Koivisto et al., 2018).

Fungicide - are compounds that kill parasitic fungi or their spores. They allow the control of fungal infestations, especially the food supply. Fungicides are widely used in the agricultural industry (Raffa; Chiampo, 2021). Fungicides are responsible for interfering with several biochemical processes present in the cytoplasm and mitochondria, in addition to performing severe inhibition of various enzymes and proteins, such as lipid metabolism, fungal respiration and the production of adenosine triphosphate (ATP) (Thind; Hollomon, 2018).

## 4.4 CHEMICAL CLASSIFICATION

In this classification, four major groups of pesticides are identified: organochlorines, organophosphates, carbamates, and pyrethroids. The information regarding the chemical classifications of pesticides is fundamental for the determination of the mode of application, as well as the necessary precautions in the handling of the product. Table 2 presents the description of the characteristics of each group mentioned.

<b>GROUP</b>	<b>CHEMICAL</b> <b>COMPOSITION</b>	<b>CHARACTERISTICS</b>	<b>REFERENCES</b>
Organochlo	Nonpolar and	Soluble lipid, toxic to animal	He et al., 2017;
rines (DDT,	lipophilic atoms	varieties and persistent in the	Arrebola et al.,
aldrin,	including carbon,	long term	2015
lindane,	chlorine, hydrogen		
chlordane)	atoms		
Organofosf	Aliphatic, cyclic	Soluble in organic solvents	Mdeni et al., 2022
orado	and heterocyclic	also and water. Less	
(Malathion,	and have central	persistent than chlorinated	
diazinon,	phosphorus atom	and hydrocarbons	
paration)	in the molecule		
Pyrethroids	Alkaloids obtained	Less persistent than other	Ensley, 2007
(pyretrin)	from petals of	pesticides, therefore safer to	
	plant species,	be used as a household	
	namely	insecticide	
	Chysanthemun		
	cinerariefolium		
Carbamates	Chemical structure	Relatively low persistence	Struger et al.,
(carbaryl)	based on the		2016; Aref et al.,
	alkaloid of plant		2012
	species named		
	Physostigma		
	venenosum		

Table 2 - classification and chemical characteristics of pesticides

(Source: Self-authored)

## **5 GLYPHOSATE**

The emerging contaminant glyphosate (N-(phosphonomethyl)glycine) is widely used in agriculture in several countries (Pereira et al., 2021). It is considered the most widely used herbicide in the world, as well as being one of the most harmful to human health. This compound is used to control harmful or invasive weeds (aChen; Liu 2007; Ramula et al., 2022).

Despite claims that it degrades rapidly in ecosystems (Baylis, 2000; Duke; Powles, 2008), glyphosate residues may persist, especially in northern soils, long after application (Laitinen et al., 2009; Helander et al., 2012, 2018). These residues can affect plant quality by modifying hormonal pathways and plant physiology, with possible consequences for plant-herbivore interactions (Fuchs et al., 2021; Ramula et al., 2022). The plant absorbs glyphosate through its leaves and new stems, being transported throughout the plant's enzymatic system, inhibiting monoacids (phenylalanine, tryptophan, and tyrosine) and preventing the formation of lignin, alkaloids, favonoids, and benzoic acids (Aitbali et al. 2018; Aristilde et al. 2017).

The herbicide glyphosate (CAS Registry Numbers® 40465- 66-5, 1071-83-6) has a molar mass of 169.05 g/mol, molecular formula  $C_{3H8NOSP}$  and relatively small and polar chemical structure (Figure 2). It also has a basic character that comes from the electrons of nitrogen (N) and oxygen (O) in its composition, being able to make chemical bonds by coordination with metals (M+) forming thermodynamically stable complexes such as glyphosate-Ca2+, glyphosate-Zn2+, glyphosate-Ni+, glyphosate-Fe 3+ and glyphosate-Mg2+. The stability of complexes formed with glyphosate decreases in the order Fe3+ > Al3+> Cu2+ > Zn2+ > Fe2+ > Mn2+ > Ca2+> Mg2+ (Valle et al., 2019).





(Source: Self-authored)

The amine (NH-R), phosphate (PO3-R) and carboxyl (COOH) groups present in the glyphosate molecule in aqueous solution may undergo different protonation and deprotonation conditions due to variations in  $H^+$  and OH- ions, presenting equilibrium distributions of population species, where their amounts depend mainly on the pH of the solution (Hosseini; Toosi 2019; Mohsen Nourouzi et al., 2014; Serra-Clusellas et al., 2019; Valle et al., 2019).

#### **6 RISKS OF GLYPHOSATE**

Glyphosate is the most debated herbicide (Camargo et al. 2020; De Castilhos Ghisi, 2020) and has been the target of great environmental concern (Cao et al. 2019; Duke 2021de ; Meftaul et al., 2020; De Castilhos Ghisi et al., 2020 ) because of its properties (such as high solubility in water: 12 g/L at 25<sup>o</sup>C, octanol-water partition coefficient at pH 7: 20<sup>o</sup>C: 6.31  $\times$  10–4, vapor pressure at 20<sup>o</sup>C: 0.0131 mPa), (Amarante Junior 2002; Lewis et al. 2016) and its ability to spread contamination around the world, given its high commercialization and diverse use (Hébert et al., 2019). Corroborating this caution, the company Bayer, which bought Monsanto in 2018, faces more than 42,000 lawsuits in which the use of glyphosate is related to the incidence of several types of cancer. A recent lawsuit was settled for more than \$2 billion with the plaintiff couple claiming to have acquired cancer after years of using the herbicide Roundup® (Cohen, 2019; Reuters, 2019)

Therefore, it is necessary to distinguish the possible deleterious effects of the extensive use of glyphosate on the environment, in different regions of the world, which is important to understand them more objectively, as environmental damage tends to increase with the greater use of glyphosate the product (Hébert et al. 2019).

The different environmental effects of glyphosate use in the environment are directly related to some factors, such as: water properties, soil temperature, as well as the way the composite is sprayed (Maria et al., 2018). In addition, it is mainly spread by adsorption and biodegradation (Toni et al., 2006; Kästner et al., 2014).

Thus, long-term use of glyphosate can interfere with ecosystems and negatively impact the dynamics of terrestrial microorganisms (Evans et al., 2010; Hayat et al., 2010). This environmental contamination generates impacts on macrofauna, such as rats and rabbits, cardiac electrophysiological changes (Gress et al., 2015), in addition to the loss of biomass in earthworms (Gaupp-Berghausen et al., 2015) and the reduction of mycorrhizal fungi population (Zaller et al., 2015; Helander et al., 2018).

### 6.1 HARMFUL IMPACTS ON SOIL HEALTH

Pesticide molecules can be adsorbed physically (Van der Waals forces) or chemically (electrostatic interactions) on soil particles. The process can be described with adsorption isotherms (Alfonso et al., 2017) The adsorption constant is evaluated as it provides information about the mobility of the solute. If pesticides have a low affinity for adsorption, they tend to spread more easily in the environment. Several soil parameters influence the adsorption process, namely soil organic matter content, clay content, clay mineralogy and pH (Raffa; Chiampo, 2021) .

Successive applications of glyphosate on crops result in greater impact on soil microbiota when compared to a single application, since the half-life of the herbicide can be prolonged. In addition, the accumulation of the molecule in the soil can hinder microbial action, reducing the rate of biodegradation (Lancaster et al., 2010; Henrique Saes Zobiole et al., 2010).

According to studies published by Sebiomo et al. (2012) and Miranda et al. (2017), impacts on soil fertility and the binding of glyphosate to ions adsorbed to soil causes a mineral chelating effect. The inhibition of the enzyme 5-enolpiru vilshiquimate-3-phosphate synthase (EPSPS), encoded in the plant nucleus with catalytic action on the chloroplast, interferes with the production of essential amino acids for plant defense and microbial composition, such as phenylalanine, tyrosine and tryptophan. This generates an imbalance in carbon input and, consequently, results in a high concentration of phytotoxic compounds that act on metabolic pathways, such as shikimati-3-phosphate (Kruse et al., 2000; Pink; Vidal 2010; Krüger et al., 2013; Arango et al., 2014; Duke 2018).

## **7 USE OF GLYPHOSATE IN BRAZIL AND WORLDWIDE**

Glyphosate is the best-selling active ingredient in Brazil, with 195,056 tons marketed in 2018 (Institute, 2019). In 2018 it was reclassified by ANVISA (Brazilian Health Surveillance Agency, 2018) as class III, dangerous to the environment. In Brazil, the maximum limit concentration of 65 μg· L −1 in surface waters was determined by the National Council of the Environment-Conama by resolutions No. 357/2005 and No. 20/1986. According to Ordinance No. 518/2004 of the Brazilian Ministry of Health, the maximum permissible concentration of glyphosate in drinking water intended for human consumption is 500 μg· L−1 (BRAZIL, 2005; Lopes-Ferreira et al., 2022).

Brazil stands out for its massive agricultural use of glyphosate (De Moraes, 2019; Demichelli et al., 2020; Mendes et al., 2020; Mendonça et al., 2020; Melo et al., 2020), which leads to the contamination of several environmental matrices by this compound. As an example, Fernandes et al. (2019) proved the presence of glyphosate and its metabolite AMPA in epilithic bioflmes in a watershed in southern Brazil, varying according to local use and climatic conditions. Because it is a good bioindicator of environmental contamination, the contaminating capacity of this herbicide has already been identified, constituting a public health problem (Marques et al., 2021).

A growing number of countries have begun to restrict/ban the use of glyphosate-based products, such as Austria, Bulgaria, Colombia, Costa Rica, Denmark, El Salvador, Greece, Madeira, Malta, Oman, Sri Lanka, Vietnam (Dyer 2014; Usda 2017; Phys 2019; Vietnam 2019; Malkanthi et al. 2019), based on evidence already available on the direct incidence of glyphosate at various levels of the global ecosystem, in addition to favoring the emergence of serious problems in human health (Marques et al., 2021), as described in Table 3.



Table 3 - incidence of glyphosate in different areas of the global ecosystem.



(Source: Self-authored)

In addition, weed resistance to the effects of glyphosate has also been mentioned (Cerdeira et al. 2006; Pan-Europe 2018), requiring extra doses of other herbicides and fungicides to achieve efficient pest elimination (Pan-Europe 2018).

## **8 TECHNIQUES FOR REMOVING GLYPHOSATE FROM SOIL**

The glyphosate molecule is relatively stable when compared to other compounds in its class, persisting in water and soil for months. In some cases, this compound is adsorbed by rocks that aid in its decomposition (Arroyave et al., 2016; Jiang et al., 2018; Xu et al., 2019). Several techniques are studied around the world to remove this contaminant adsorbed in the soil to minimize the harmful effects of this substance. However, not all forms of removal are effective, or can cause even greater damage to the soil, compromising the proper functioning of the local ecosystem. The present study evaluated the recent studies committed to the recovery of soils by the contaminant glyphosate.

## 8.1 REMOVAL BY PHYSICO-CHEMICAL METHODS

Naghdi et al., 2023 evaluated the adsorption of glyphosate from water using hierarchically porous metal-organic structures. The relationships between contact duration, glyphosate concentration and adsorbent dosage were investigated, as well as the impact of these parameters on the efficacy of glyphosate removal. The introduction of additional mesopores increased adsorption capacities by almost 3 times, with record values exceeding 440.9 mg g-1, which ranks these MOFs (metal-organic structures) among the best-reported adsorbents. The study shows good results of glyphosate adsorption in contaminated samples, however no study was conducted on the toxicity of the products used.

Esmaeilian, Dionysiou and O'shea, 2023 evaluated the incorporation of n magnetic anossorbents composed of magnetite (Fe3O4) nuclei coated with humic acid synthesized in the removal of glyphosate (GLY) by adsorption using theoretical analysis. Humic acid-coated magnetic nanomaterials (HA-MNP) exhibited a high maximum adsorption capacity for GLY at pH 4.2, 30.4 mg g-1. The result of the thermodynamic study indicated that the adsorption is endothermic and spontaneous. The removal efficiency increased with the decrease in the pH of the solution. Chloride ions exhibit no inhibition, while nitrate and phosphate compete with the adsorption of GLY to HA-MNP. Although multiparameter models have been successfully applied to model adsorption processes, such studies often require large sets of experiments and complex mathematical representations to capture the interactive play of different variables. In addition, magnetic humic acid nanoparticles can be reused after regeneration and the removal efficiency of HA-MNPs decreased by less than 35% after four adsorption-desorption cycles.

Li et al., 2023 conducted a study using bimetallic cobalt-nickel sulfide structured with core and shell with double redox cycles to activate peroxymonosulfate for glyphosate removal. In this study, a bimetallic cobalt-nickel sulfide structured in core-shell NiCo2S4/Co9S8/NiS (NCS) was synthesized by a simple two-step hydrothermal method and used as a novel peroxymonosulfate activator (PMS) for glyphosate degradation. The radical extinction experiments revealed that NCS interacted with PMS to generate the SO4•− and •OH, O2•− and 1O2 radicals together to achieve efficient glyphosate degradation. This work provided new insight to produce high-performance multiphase catalysts for glyphosate degradation as well as practical applications.

## 8.2 REMOVAL OF GLYPHOSATE THROUGH BIOLOGICAL METHODS

The decomposition routes of glyphosate are well described in the literature. They involve microorganisms to catalyze this process, generating sarcosine as a byproduct of route 1 (Figure 3a) (Ezaka et al., 2019; La Cecilia; Maggi 2018; Zhan et al. 2018). Route 2 consists of the formation of the compound AMPA (aminomethylphosphonic acid) (Figure 3b) (Ezaka et al. 2019; Jiang et al. 2018; Torretta et al. 2018).



Figure 2 - glyphosate degradation routes, where a) represents the formation of sarcosin, and b) formation of AMPA

(Source: Self-authored)

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Zhang et al., 2022 conducted a study successfully isolating a new bacterial strain, *Chryseobacterium* sp. Y16C, which efficiently degrades glyphosate and its main metabolite, aminomethylphosphonic acid (AMPA). The Y16C strain was found to completely degrade glyphosate at a concentration of 400 mg· L-1 in four days. The kinetic analysis indicated that the biodegradation of glyphosate was concentration-dependent, with maximum specific degradation rate, half-saturation constant and inhibition constant of 0.91459 d-1, 15.79796 mg· L-1 and 290.28133 mg· L-1, respectively. AMPA has been identified as the main degradation product of glyphosate degradation, suggesting that glyphosate was first degraded through cleavage of its C-N bond before subsequent metabolic degradation. The Y16C strain has also been shown to tolerate and degrade AMPA at concentrations up to 800 mg· L-1. In addition, the Y16C strain accelerated the degradation of glyphosate in the soil indirectly, inducing a slight change in the diversity and composition of the soil microbial community. Taken together, our results suggest that the Y16C strain may be a potential microbial agent for bioremediation of glyphosate-contaminated soil.

Mazotti et al., 2023 conducted a recent study considering the use of bacteria that interact with plants, alone or bacteria and plants together, for the removal of the herbicide glyphosate. This study also evaluated that microorganisms that interact with plants with plant growth-promoting characteristics can also increase plant growth and contribute to successful bioremediation strategies.

Bokade et al., 2023 published a review article that critically examines the methodical challenges that address the feasibility of restoring and recovering pesticide-contaminated sites, along with ecotoxicological risk assessments. These processes are: functional or genomic screening, enrichment isolation; mapping of functional pathways, production of surfactant metabolites to increase bioavailability and bioaccessibility, employing genetic engineering strategies for modifications in existing catabolic genes to increase degradation activity. Overall, the study highlights the need to adjust available processes and employ interdisciplinary approaches to make microbe-assisted bioremediation the method of choice for recovering pesticide-contaminated sites.

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