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THE NATURAL WAYS OF DEGRADATION OF PESTICIDES BASED ON 2,4-DICHLOROPHENOXYACETIC ACID

To increase crop yields by destroying weeds the separate group of pesticides exist, that is called herbicides. Among the herbicides based on chlorophenoxyacetic acids products of sodium and ammonium salts, as well as 2,4-dichlorophenoxyacetic acid (2,4-D), are widely used. However, the production and use of pesticides affects the state of the environment and pose a potential risk for public health. In this paper the need to clarify the mechanisms of transformation and degradation of 2,4-D derivatives under the action of physical, chemical and biological environmental factors was showed. The preparations based on 2,4-D, registered in the Republic of Belarus is reviewed. The mechanisms of action of these xenobiotics are described, analysis of their toxicity for the environment components is performed. Particular attention is paid to the microbial degradation of pesticides, possible ways of their degradation under the action of microorganisms enzyme systems are described, the factors, that affect the biotransformation process are analyzed. It has been shown that the bacterial degradation of 2,4-D is carried with the formation of 2,4-dichlorophenol by enzyme 2,4-dichlorophenoxyacetate- α -ketoglutaratedioxygenase, and further the intermediate degrades with 3,5-dichlorocatechol formation using 2,4-dichlorophenolhydroxylase in two ways: ortho-splitting or peripheral meta-splitting.

Key words: pesticides, 2,4-D, bacteria-destructors, degradation, transformation, enzymes.

Introduction. The destruction of weeds, pests, pesticides is used in order to increase crop yields, which are one of the major sources of environmental pollution, especially in agriculture. The production and use of pesticides affect the state of the environment and pose a potential risk to public health. Significant economic damage to agriculture causes weeds to fight where there is a separate group of pesticides (i.e. herbicidal compositions).

Main part. Herbicides are based on chlorophenoxyacetic acids. They have been extensively used in the destruction of weeds. Among this group of herbicides drugs are sodium and ammonium salts, as well as 2,4-D are widely used [1].

2,4-D (2,4-dichlorophenoxyacetic acid) is organochlorine compound used as a herbicide and plant growth regulator. It is the active ingredient of drugs such as “Dikopur”, “Aminka”, “Levirate”, “Esteron”, “Elant”, “Prima”, “Diamaks”, “Floraks” and of more than 1,500 other herbicides. It was discovered in 1945. 2,4-D is by far one of the most widely used selective herbicides in the world. Some currently registered forms of 2,4-D are: 2,4-D acid; 2,4-D sodium salt; 2,4-D diethylamine; 2,4-D dimethylamine salt; 2,4-D isopropyl ester; 2,4-D butoxyethyl ester; 2,4-D ethylhexyl ester; 2,4-D isopropyl ether. Dimethylamine salt and ethylhexyl ester have approximately 90–95% of the total global consumption. [2]

2,4-D is recommended for the control of broad-leaf weeds in growing cereal crops, lawns and pastures processing [3]. At a rate of 0.4–2.0 kg/ha using a 2,4-D can be destroyed almost all kinds of dicotyledonous weeds (creeping thistle, cow

parsnip common, cornflower, etc.). Such weeds as black mustard, white and field, shepherd's purse, white pigweed, wild radish, quinoa spreading, *Thlaspi arvense*, nettle, hairy vetch are the most sensitive to it. The use of 2,4-D as well as plant growth are regulators in the processing of sugar beet seeds and tomatoes and soaking cuttings of cherry, apple, blackcurrant [4–6].

2,4-D is a plant growth regulator. It simulates the natural plant growth hormone (i.e. auxin). The exact mechanism of action of 2,4-D is not fully understood, and it is possible that it causes a variety of effects, which together are fatal. Low concentrations of 2,4-D can stimulate the synthesis of RNA, DNA and protein, leading to uncontrolled cell growth and division, and ultimately destruction of vascular tissue. On the other hand, high concentrations of 2,4-D can inhibit cell division and growth. Plant death usually occurs within three to five weeks after application. Plants die when their vascular transport systems are blocked and deleted abnormally rapid-growing volume [2, 7].

2,4-D is classified by World Health Organization as a hormonal herbicide toxicity level II (on a scale of I-IV, where I is the most toxic). Xenobiotics can be absorbed through the skin or enter the lungs during breathing. 2,4-D is considered to be a carcinogenic agent affecting the liver, heart and central nervous system that leads to seizures [7, 8].

After application of the herbicide it can be easily transferred into the groundwater due to its high solubility (600 mg/l at 25°C). Even after a long period of non-use of a significant amount of 2,4-D and its main degradation product of 2,4-dichlorophenol (2,4-DCP) it can be found

in surface water and groundwater. 2,4-D may accumulate in the fish, even subjected to such a concentration as low as 0.00005%. Thus, it is necessary to consider the process of degradation of the herbicide, which will determine the factors influencing its decomposition [7].

The decomposition of herbicides simultaneously occurs in several ways: the decomposition by UV light, chemical hydrolysis, biodegradation (Fig. 1).

In soils 2,4-D is decomposed mainly by microorganisms. The fate of 2,4-D is largely dependent on pH. At pH above 7 2,4-D form. It is rapidly converted to the anion which is more susceptible to photodegradation and microbial metabolism, and less likely to adsorption to soil particles. At pH < 4 inhibited microbial degradation of 2,4-D and retains its molecular form and is resistant to degradation. Most forms of 2,4-D are volatile [8].

Evaporation. Most products of 2,4-D are extremely volatile. The most volatile of 2,4-D are methyl and isopropyl esters, which have been banned in the United States, but some inhalants esters of 2,4-D are still available. Alkali metal salts and amines are much less volatile and can be used where it is impossible to use the esters of the damage that they can cause non-target plants. Additionally, 2,4-D volatility increases with increasing temperature, the increase and reduction of soil moisture content of alumina and organic substances in soil [8].

Adsorption. 2,4-D salts are soluble in water and practically not bind to the soil. Esters may also be more actively adsorbed in the soil. In the field, generally the esters are hydrolyzed products, passing in the acid form, especially in an alkaline environment and, therefore, practically not adsorbed on soil particles. Organic matter content and soil pH

are the main factors that determine the adsorption of 2,4-D in the soil. Adsorption increases with the content of organic matter and reduction of the soil pH. Inorganic clay may also bind 2,4-D. Furthermore, with increasing herbicide concentration in the soil decreased the percentage of adsorbed herbicide. Perhaps it is due to the filling of soil particles and their saturation with herbicide [8].

Photodegradation. 2,4-D is rapidly destroyed in the sun in the laboratory, but photodegradation was observed in the field. The period of the photodegradation in laboratory conditions is 50 minutes to salts of 2,4-D for 5 min and 2,4-D esters. Similar results were obtained in other laboratories, which led to the conclusion that the field does not have sufficient levels of ultraviolet radiation from sunlight. In addition, the rate of 2,4-D in soil degradation remain relatively constant during the presence of sunlight and without it [8].

Chemical degradation. 2,4-D is relatively stable in the environment and exposed to chemical degradation difficult compared with other herbicides. However, the hydrolysis of esters to 2,4-D acid and alcohols can easily occur in an alkaline medium. Additionally, 2,4-D salts dissociate to form a salt and an acid residues [8].

Decomposition of 2,4-D in mammals. 2,4-D falls within the mammal by inhalation, with food or through the skin. 2,4-D is metabolized in mammals but is rapidly excreted via the kidneys in the urine as the parent compound. The half-life of 2,4-D in the human organism is 17,7 hours. 2,4-D did not accumulate in any tissue, except 2,4-D are esters which are hydrolyzed to the acid before adsorption. Lymph acid exists predominantly in the ionized form, is not metabolized to form any intermediate products and difficult to penetrate the lipid membranes of tissues without active transport systems [9].

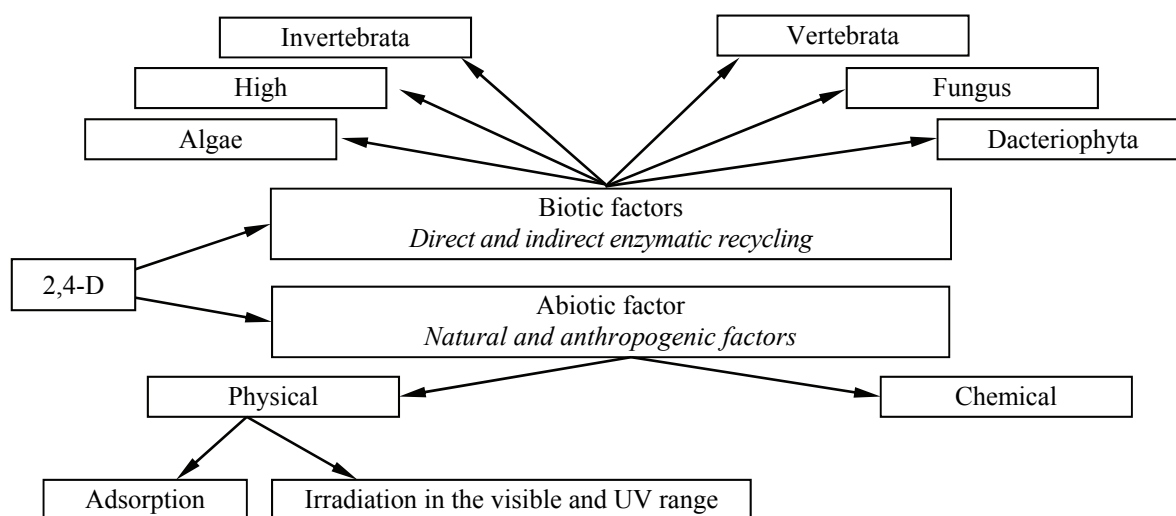


Fig. 1. Factors involved in the decomposition of 2,4-dichlorophenoxyacetic acid

Degradation in plants. One of the important advantages of 2,4-D to other organochlorine compounds is used in agriculture as pesticides. It is transformed into plants to non-toxic derivatives. Almost immediately after the detection of the herbicidal properties of the 2,4-D has been found that in plants it undergoes decarboxylation to release into the atmosphere of carbon dioxide. Carbon dioxide may also be included in the metabolism in plants [10]. It is found that the oxidative decarboxylation is quite complex and can take place in various patterns (Fig. 2).

However, the direction of 2,4-D transformations lead to the formation of carbon dioxide and dichlorophenol, which is further degraded by the degradation of the benzene ring in the molecule [11]. Also decarboxylation of 2,4-D can be subjected to plants and other transformations, namely the esterification, followed by ring hydroxylation binding carbohydrate (glycosylation), amino acid conjugation, complexation with proteins. All transformations lead to the formation of inactive metabolites, which are part of 2,4-D. It loses the ability to exercise their herbicidal properties, i.e. it leads to detoxification [11].

Microbial degradation. In soils 2,4-D is decomposed by microorganisms primarily (even after

36 days in sterile soil retained 70% of the initial dose of 2,4-D). The magnitude of the population of microorganisms, the concentration of 2,4-D and the ratio of these two factors determine the rate of degradation of 2,4-D.

Conditions that increase the number of microbial populations in the soil (i.e. warm and humid climate), contribute to the degradation of 2,4-D. Additionally, 2,4-D decomposition occurs rapidly in the soil that had previously been treated with 2,4-D, presumably because the L-2,4-destroying bacteria accumulated in the soil after the first application of the herbicide [8, 12].

2,4-dichlorophenol is the first product in a way intermediate 2,4-dichlorophenol acid. We have been allocated several bacteria and fungi that 2,4-dichlorophenol is used as the sole carbon and energy source, these include: *Achromobacter* sp., *Aeromonas* sp., *Bacillus insolitus*, *Pseudomonas* sp., *Pseudomonas* sp, DP-4, *Rhodococcus opacus* 1G, *Rhodococcus erythropolis*, *Pseudomonas* sp. NCIB9340, *Burkholderia cepacia*, *Sphingomonas paucimobilis*, *Streptomyces viridosporus*, *Flavimonas oryzihabitans*, *Chryseomonas luteola*, *Aspergillus penicilloides*, *Mortierella isabelina*, *Chrysosporium pannorum*, *Mucor genevensis*.

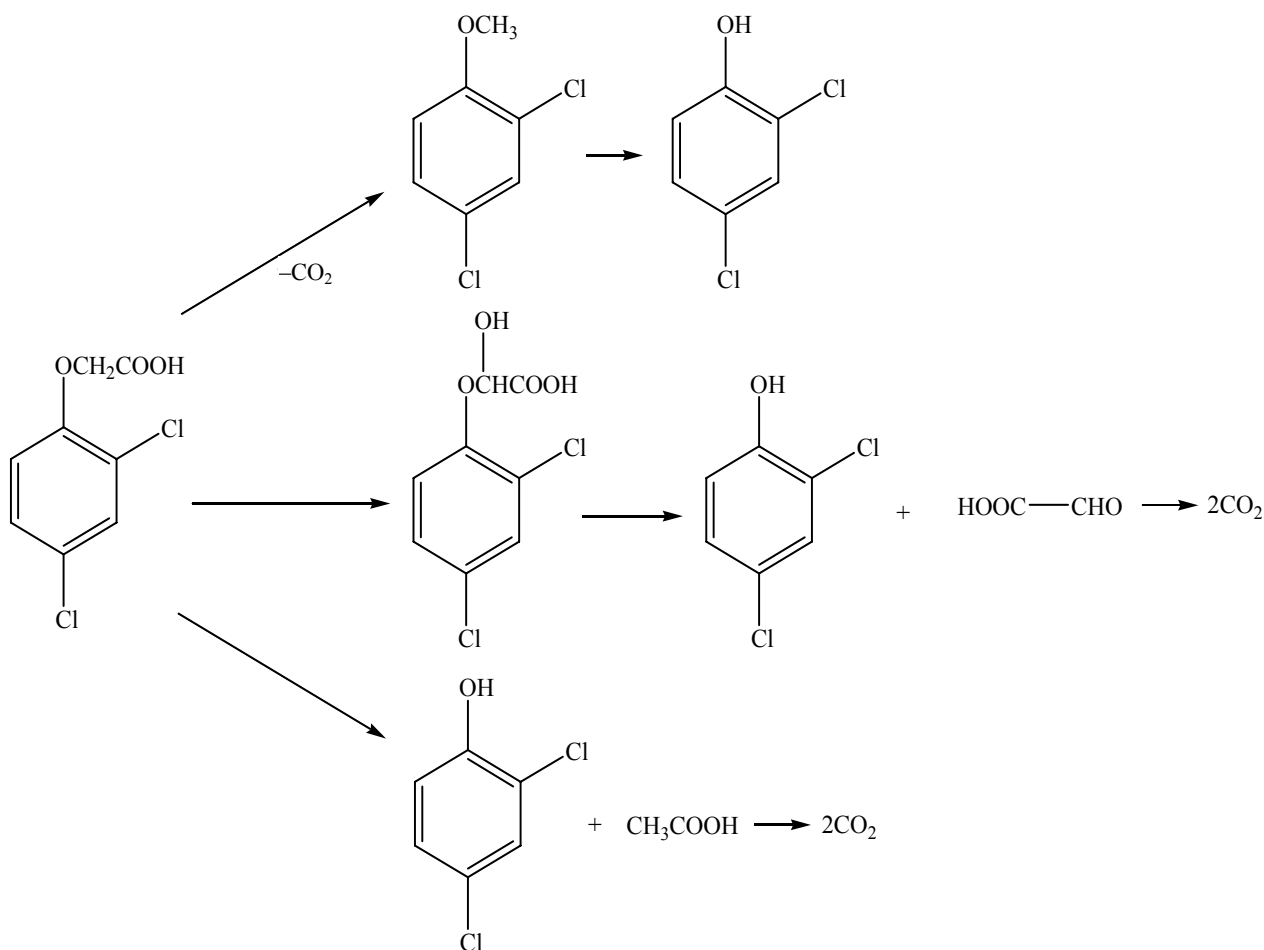


Fig. 2. The process of oxidative decarboxylation of 2,4-D in plants

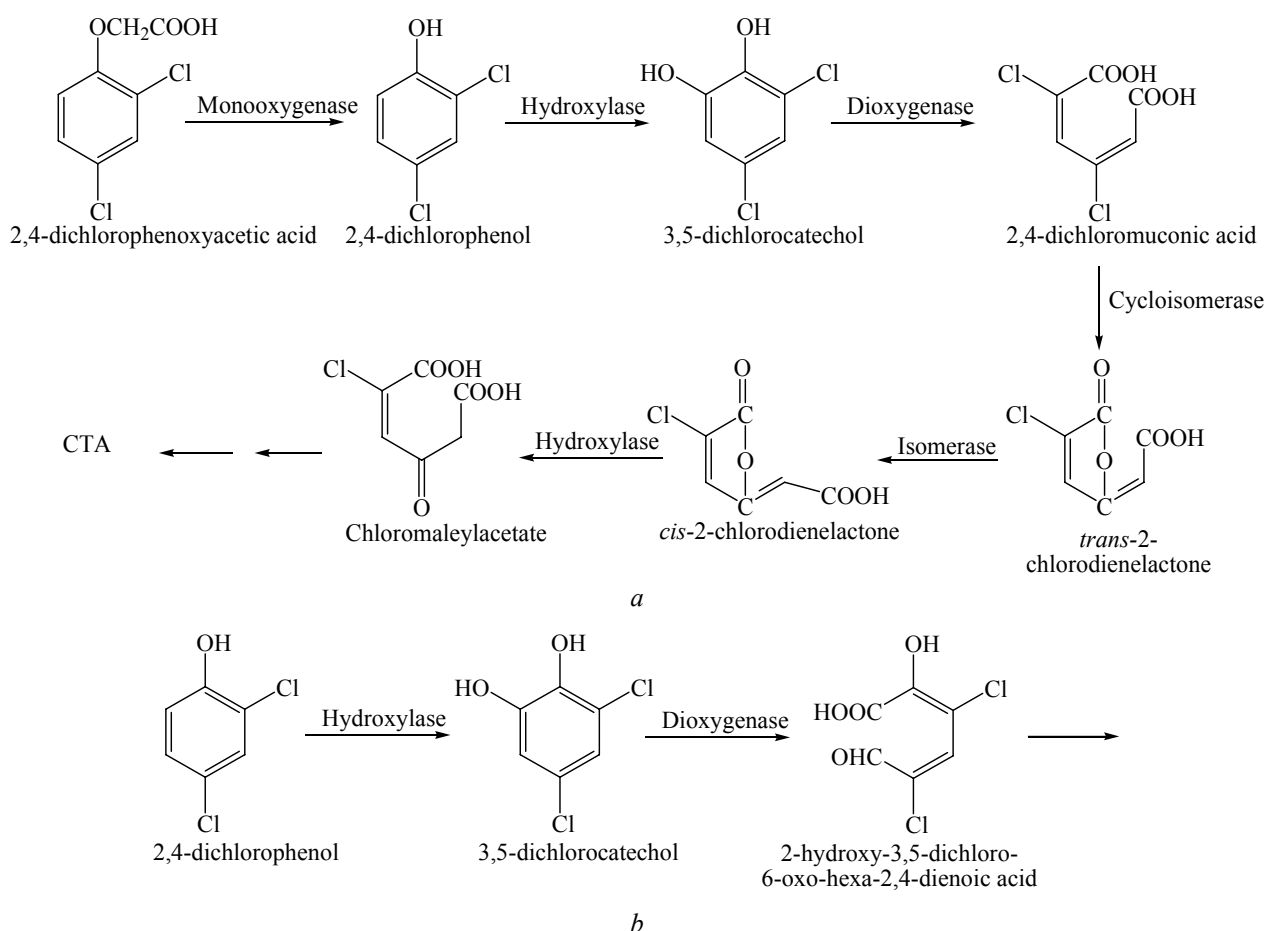


Fig. 3. Bacterial degradation pathway of 2,4-dichlorophenol:
a – ortho-cleavage; *b* – peripheral meta-cleavage

The bacterial degradation of 2,4-D is initiated by the formation of 2,4-dichlorophenol by the enzyme 2,4-dichlorophenoxyacetate/alpha-ketoglutarate dioxygenases. 2,4-dichlorophenol is further degraded to form 3,5-dichlorocatechol using 2,4-dichlorophenol hydroxylase. In the third step, 3,5-dichlorocatechol is ortho-cleaved to 2,4-dichloromuconic acid by 3,5-dichlorocatechol dioxygenase.

In the next step isomerase catalyzes the conversion of 2,4-dichloromuconic acid to *trans*-2-chlorodienelactone via the removal of one chloro group, which is further converted to *cis*-2-chlorodienelactone by an isomerase that is subsequently degraded via formation of chloromaleylacetate by a hydroxylase. The chloromaleylacetate is further degraded to maleylacetate by removal of the chloro group and then to 3-oxodipic acid by a maleylacetate reductase. Cells of *Cupriavidus necator* JMP222 (a derivative of *C. necator* JMP134 that had lost plasmid pJP4) degraded 2,4-DCP via a distal meta-cleavage pathway. In that

process, 2,4-dichlorophenol is first oxidized to 3,5-dichlorocatechol, which is subsequently degraded via a distal *meta*-cleavage pathway through the formation of 2-hydroxy-3,5-dichloro-6-oxo-hexa-2,4-dienoic acid (Fig. 3) [13–17].

Conclusion. Thus, 2,4-dichloroacetic acid and its derivatives when applied to the soil undergo transformation under the influence of chemical, physical and biological factors. Mechanisms and degradation, primarily dependent on the structure of the herbicide, the type of soil, humidity, quantity of sunshine. A key role in the degradation of said xenobiotic microbiota plays soil [17]. It is the availability of soil microorganisms-destroyers primarily depends on the degree of the negative effects of 2,4-D on the environment and human health. Isolation of pure cultures of microorganisms using 2,4-D as a carbon and energy source requires the study of degradation mechanisms and identifying all intermediate intermediates.

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