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Investigation of Degradation of Pesticide Lontrel in Aqueous Solutions

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1. Introduction

Development of new efficient methods for purifying water from industrial pollutants resistant to biodegradation is a challenge because of the current shortage of freshwater reserves in the world (Legrini et al., 1993; Skurlatov et al., 1994). It is known that application of pesticides (Ozelenenie, 1984; Bykorez, 1985; Burgelya & Myrlyan, 1985; Patel et al., 1991; Wan et al., 1994; Arantegui et al., 1995; Fliedner, 1997; Arkhipova et al., 1997), in particular, Lindane (Fliedner, 1997) and 2,4-D (Arkhipova et al., 1997), results in their long-term accumulation in soils and water reservoirs. Furthermore, pesticides' residual presence in foodstuffs and industrial crops causes serious diseases, including hereditary disorders (Eikhler, 1993; Calvert et al., 2004; Whyatt et al., 2004).

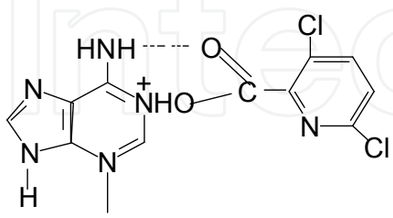
Numerous studies point to long-term preservation of many pesticides in natural waters: ground, river, or sea (Skurlatov et al., 1994; Yudanova, 1989; Yablokov, 1990). Organic chemicals, including the insecticide Lindane, were found in the fat of gray whales caught in the Arctic Ocean (Poliakova et al., 2005).

Lontrel (another commercial name is Clopyralid) is considered to be the herbicide of a wide range of action and, first, for the defense against weeds in grain crops and can be used in a mixture with 2M-4X or 2,4-D; $LD_{50} \approx 5000$mg/kg (Mel`nikov, 1987). Literature data on the mechanism of action of Lontrel are rather insignificant. There are indications (Hall et al., 1985) that Lontrel exhibits auxine-like activity. The most detailed studies are presented in our works. Metal complexes of Lontrel were not studied at all, except of our research group.

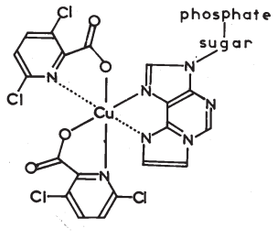
Formerly (Aliev et al., 1988; Saratovskikh et al., 1989b) we showed that 3,6-Dichloropicolinic acid (DCPA), the active principle of the herbicide Lontrel, readily formed complexes with metals, major environmental pollutants, and these complexes were stable under natural conditions. They are capable of participating in further complex formation with bioactive ligands due to the filling of the coordination sphere of the metal.

We have proved for the first time that pesticides themselves and their metal complexes interact with mono-, di-, and polynucleotides (Saratovskikh et al., 1988; 1989a). In all cases, two- or three-component complex systems are formed. It was shown that the pesticide complex with adenosine triphosphoric acid is formed due to the protonation of the N-7

nitrogen atom of the adenine heterocycle, and the nitrogen atom of the terminal NH_2 group can simultaneously be bound to the pesticide molecule due to the formation of a hydrogen bond. The formation of the pesticide complexes with ATP results in an energy deficient in the tissues of organisms. The effect of pesticides and their metal complexes induces the energy deficient of the cell, namely, inhibition of energy metabolism due to the formation of a complex with adenosine triphosphoric acid.



Structure of the [ATF-DCPA] complex.



Structure of complex of ε-ATF with metal complex of DCPA - [ε-ATF-CuL_2].

We pioneered to show that Lontrel and its metal complexes simultaneously inhibit the activity of several enzymatic systems in organisms. These are oxidizing enzymatic systems, for example, NADH-oxidoreductase. In this case, the enzymatic activity is inhibited, first, due to the complex formation with dinucleotide (coenzyme) NADH. Second, this occurs due to the formation of the non-productive complex [enzyme-DCPA] in the active center of the enzyme. For DCPA comparison of inhibition of NADH-oxidoreductase $K_i = 10^4$ M, and competitive type of inhibition take place. The antireductase activity increases in the order: metal ion < DCPA < metal complex (Saratovskikh et al., 2005; 2007c).

We shown than DCPA and its metal complexes are bound into complex compounds with DNA and RNA (Saratovskikh et al., 1989a) and nativity of the DNA double helix is violated because of complex formation. The direct mutagenic effect was shown by us for the TA98 *Salmonella typhimurium* strain, mutations of the reading frame shift type are induced and promutagenity was revealed (Saratovskikh et al., 2007b).

Therefore, it is important to investigate its exposure to a microbial community of activated sludge (AS) and to sunlight, i.e., conditions mimicking those occurring in natural surface water bodies.

The possibility of using UV radiation for the decomposition of various chemical compounds was shown by several authors (Legrini et al., 1993; Guittonneau et al., 1988; Sundstrom et al., 1989; Castrantas & Gibilisco, 1990). Ultraviolet purification of water is superseding conservative chlorine treatment (Skurlatov et al., 1994; Skurlatov & Shtamm, 1997a, 2002). At present, over 1000 ultraviolet-purification devices of different capacities are in operation in 35 countries (Skurlatov et al., 1996; Kruithof et al., 1992). They finely replace the old method of treatment of drinking and waste waters based on the treatment with chlorine. A comparative estimation of the American specialists (Purus Inc., 1992) of the cost of UV quanta, ozone, and reagents used in processes of water preparation and water treatment is as follows: Cl_2 = 0,16$ per 1 mol; O_3 = 0,1$; photons 185, 254 nm (low-pressure Hg lamps, yield 40%) = 0,025$. The bactericide light quantum turned out to be the cheapest reagent (Skurlatov & Shtamm, 1997b). Sometimes the UV treatment is combined with the addition of

oxygen or hydrogen peroxide. As shown (Shinkarenko & Aleskovskii, 1982), ozone formed upon the photochemical oxidation of oxygen dissociates, in turn, to the electron-excited oxygen atom and oxygen molecule in the singlet state. Having a high oxidizability, they attack a molecule of the contaminant and oxidize it (partially or completely). The UV treatment of waste waters produces no dibenzofurans, which are precursors of dioxins as it takes place for the treatment with ozone only (Beltran et al., 1993). The ultraviolet treatment of waste waters is well compatible with methods of biological purification from contaminants (Golubovskaya, 1978). Therefore, study of the products of UV-mediated DCPA degradation is of practical importance.

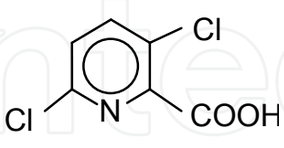
The objective of the present study was to investigate the possibility of DCPA degradation by biochemical and photochemical methods (taken alone or in combination with each other), in order to develop practical methods of degrading pesticides, the most challenging industrial pollutants.

Analysis of the composition of the reaction mixture and final products is a complicated task, which demands up-to-date highly sensitive methods, but it is of environmental importance. This study concerns the kinetics of DCPA degradation under natural conditions (deep-well, river, or sea water) and analysis of its products.

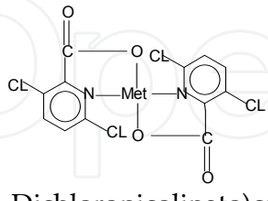
2. Materials and methods

2.1 Used substances, concentrations and replicates

3,6-Dichloropicolinic acid (DCPA) was studied, which is the main active principle of Lontrel and a copper–DCPA complex containing two DCPA molecules per copper atom - CuL_2. Experiments were performed with $1.22 \cdot 10^{-3}$ M DCPA solutions in distilled water, artificially made sea water, and river water from the Klyazma River. Solutions of CuL_2 with the concentration $0.4 \cdot 10^{-3}$ M. The artificial seawater was made from sea salt, containing the following anions (g/l): Na^+ (10.76), K^+ (0.39), Ca^{2+} (0.41), Mg^{2+} (1.30), SO_4^{2-} (2.70), Cl^- (19.35), Br^-(0.06), and CO_3^{2-} (0.07). The concentration of sea salt in the working solution was 35.5 g/l (Artobolevskii, 1977). River water was sampled near Biokombinat Village, Moscow Region, in the middle Klyazma River at the depth 1.5–2 m.



3,6-Dichloropicolinic acid – DCPA



bis-(3,6-Dichloropicolinato)cuper(II)
CuL_2

All experiments were carried out three times. The measurement errors did not exceed 5-10%.

2.2 The biochemical oxidation of DCPA

Biochemical oxidation of DCPA was studied in 1 l laboratory aeration tanks under conditions of constant aeration and natural sunlight. Concentrated AS was sampled from an

aeration tank of the Treatment plants in Chernogolovka, Moscow oblast. Five samples were studied, each containing 2 ml of AS. The samples were diluted in laboratory tanks to 1 l with a peptone medium. Sample 1 was taken as a control, not exposed to the mutagen (*N*-methyl-*N*-nitrosourea, nitrosomethylurea, NMU). NMU was added to samples 2, 3, and 4 to a concentration of 0.07% (Rapoport, 2010); samples 2 and 4 were exposed to NMU for 6 h, while sample 3 was exposed to NMU for 18 h. Samples 2–4 were treated with NMU once more for the same amount of time as in the initial treatment. Sample 4 was then treated the again after 28 days of the observation, while samples 2 and 3 were treated again after 44 days. Samples 1–3 were supplemented with DCPA at a starting concentration of $1.22 \cdot 10^{-3}$ M (0.23 g/l). Sample 4 was supplemented with a $Cu(L)_2$. This complex was synthesized according to (Aliev et al., 1988). We showed previously that this complex was a stronger herbicide than DCPA itself (Saratovskikh et al., 1990). It forms stable compounds with DNA (Saratovskikh et al., 1989a), mononucleotides, and dinucleotides (Saratovskikh et al., 1988). Such complexes can readily form in runoff from farmlands and in some industrial enterprises' wastewaters containing Lontrel and copper compounds. Sample 5 was supplemented with wastewater of a pilot Lontrel mixture, which contained approximately ~10^{-3} M of the herbicide. Samples for the tests were taken four or five times per day for the first 3 days, once a day for the next 10 days, and then once every few days. The observation was performed for one year. The range of microbial species in the AS was determined according to (Belyaeva & Gyupter, 1969; Liperovskaya, 1977).

2.3 The photochemical oxidation of DCPA

Photochemical oxidation of DCPA under UV irradiation was performed in a quartz reaction vessel with internal diameter 2.2 cm and volume 25 ml at 4 cm from the ray emitter (in various sets of experiments, irradiation was performed with DRSh-1000, DRB-8, or BRA-15 lamps; 250–600 nm; BRA-15 lamp 1.3 mW/cm^2; Institute of Problems of Chemical Physics, Russia). The starting DCPA concentration was $5 \cdot 10^{-4}$ M. Oxygen, ozone, air, or argon were sparged (bubbled through the solution) with a capillary tube. Samples for measuring concentrations were taken at 10-min intervals. DCPA concentrations were measured with a Specord UV-VIS spectrometer (Karl Zeiss, Jena, Germany) according to light absorption at 283 nm.

2.4 Toxicological testings

The samples were subjected to biotests with two sentinel species: the infusorium *Tetrahymena pyriformis* and the luminescent bacterium *Beneckea harveyi*.

2.4.1 The biotest on *Tetrahymena pyriformis*

Tetrahymena pyriformis were cultivated in a peptone or carbohydrate–salt–yeast extract media (Yoshioka et al., 1985). Five milliliters of each sample were placed into three test tubes and one or two drops of a 3- to 4-day-old *Tetrahymena* culture were inoculated. Settled aquarium water was used as a control. The division period for *T. pyriformis* ranges within 4–6 h. The test lasted for 15 min. A change in the behavior or morphology of infusorian cells in the samples or their death was indicative of a strong toxic effect. Fifteen minutes after the mixing, three samples (one drop per sample) were taken from each test tube and fixed with iodine. Infusorian cells were counted under a microscope. In this method, toxicity coefficients *K* were calculated as follows:

$$K = [(Ac - Aex)/Ac]\ 100\%, \quad (1)$$

where *A*c is the number of cells counted by microscopic examination in the control sample and *A*ex is the number of cells in an experimental sample. A sample considered toxic at $K > 50\%$. The statistical significance of values was determined by the Student's -test.

2.4.2 Biotest on *Beneckea harveyi*

In a chronic test, the toxicity of a sample was judged from the suppression of cell propagation after 24 h of incubation. A reduction in cell propagation by 50% or more was indicative of toxicity. A lyophilized preparation of *B. harveyi* was stored in a freezing chamber. Immediately before the experiments, the bacteria were suspended in 3% NaCl. For testing toxicity, 0.3–0.5 ml of the suspension was added to 0.5 ml of a water sample. The control experiment was performed with a 0.85% NaCl solution. Measurements were performed with a BLM-8801 luminometer (SKTB Nauka, USSR) with voltmeter detection. Toxicity was estimated from the decrease in bioluminescence of a sample relative to the control. The sample was considered toxic if its bioluminescence decreased by 50% or more. The level of bacterial luminescence is determined by the intensity of intracellular metabolism involving the enzyme luciferase. A decrease in luminescence may be related to either inhibition of the enzyme itself or to an effect of toxic substances on other links in the metabolic chain. The toxicity coefficient was calculated as:

$$T = [(Ic - Iex)/Ic]\ 100\%, \quad (2)$$

where *I*c is the bioluminescence intensity in the control and *I*ex is the bioluminescence intensity in the sample tested. A sample is considered nontoxic at $T \leq 19\%$, toxic at $19 < T \leq 50\%$, and strongly toxic at $T > 50\%$.

2.5 Infrared spectra

Infrared spectra were recorded at 400–2200 cm^{-1} with a Specord 75IR spectrometer (Karl Zeiss, Jena, Germany) in KBr pellets (250 mg of KBr + 1.2 mg of a sample). Absorbance bands were identified according to established methods (Nakanisi, 1965; Sverdlov et al., 1970; Nakomoto, 1991).

2.6 Gas chromatography/mass spectrometry (GC/MS) analyses

Gas chromatomass spectrometry was performed with a Pegasus 4D chromatomass-spectrometer (LECO, Russia) under the following conditions: ionization energy 70 eV; 30 m RTX-5MS capillary silicon column; temperature program 40°C (5 min), 8°C/min, 300°C (10 min); scan range 28–450 Da. Qualitative identification was performed by reference to the WILEY mass spectrum library, including 270 000 compounds. Perdeuterated naphthalene was used as an internal reference for quantitative assay.

2.7 The elemental analysis

Analysis of photooxidation products was performed in solutions in distilled water after 13 and 38 h of UV irradiation with air bubbling. Aqueous solutions, tagged as "13" and "38," were extracted with dichloromethane 3 times for 10 min each. The extracts were combined,

dried with anhydrous sodium sulfate, and concentrated in vacuum to 1 ml. Elemental analysis was performed after DCPA degradation. Samples were evaporated at 50–80°C, and C, N, Cl, and H were assayed in the completely dried residue (Klimova, 1975).

The contents of C, H and dry residue were determined by the modified Pregl method (Pregl, 1934) based on the ratio of molecular masses of the elements. A weighed sample of 3-5 mg was burned in a current of neat oxygen at t = 1040^0C. The amount of formed CO_2 and H_2O was determined by weighing the corresponding absorbers filled with ascarite (NaOH) and anhydrone (anhydrous Mg perchlorate – $Mg(ClO_4)_2$).

The content of N was determined by the Pregl-Dumas method (Steuermark, 1961). A weighed sample of 3-5 mg was burned in a current of CO_2 in the presence of CuO and then reduced by metallic copper. The volume of evolved gaseous nitrogen was determined with a gas meter.

The content of Cl was determined according to the Schöniger flask method (Schöniger, 1955). The analyzed substance (2-4 mg) was wrapped in filter paper placed in a Pt grid and hanged up to the cork of the flask. The flask was filled with oxygen. 2N KOH (1 ml), 0.5 мл H_2O_2 (0.5 ml), and bidistilled H_2O (5 ml) were placed on the flask bottom. The end of the paper (in which the weighed sample is wrapped) was ignited, and the flask was rapidly corked. Absorption was carried out for 0.5-1 hour. The contents was titrated with 0.01N $Hg(NO_3)_2$ in the presence of diphenylcarbazone to lilac-violet color. The content of Cl was determined by the titrant volume.

3. Results and discussion

Prior to the experiment, nine lower species were identified in the AS sample under study: algae, amoebae, sessile infusoria, and flagellates (Table 1). Addition of the pollutant reduced the number of species to seven. One group, blue-green algae, became predominant, apparently being the most resistant. Unicellular algae have also been reported as resistant to the herbicides Diuron and *o*-Phenanthroline (Laval-Martin et al., 1977).

Treatment with the mutagen caused a change in the species composition of AS and an increase in the range of the species. This increase was related to the fact that the populations of some species were too small to be detected before addition of NMU or DCPA. The presence of a certain pollutant may provide conditions for accelerated growth of those species that consume the pollutant as a preferable nutrient, thus allowing their identification. This phenomenon provides grounds for the purification of wastewaters from chemicals (Golubovskaya, 1978). In our experiment, the biocenosis that formed after NMU treatment included two phyla of lower plants (bacteria and algae) and five invertebrate classes (ciliates, sarcods, mastigiophores, nematodes, and rotifers). The number of species identified in AS sample 3, treated with NMU for 18 h, was greater than in sample 2, treated for 6 h (20 and 15 species, respectively). The population of rotifers notably increased, and sulfur bacteria and testaceous amoebae were identified (Table 1). This community successfully resisted the anthropogenic load. The toxicity of the sample treated for 18 h was lower than that after 6-hour treatment (Fig. 1). Long-term exposure (several months) reduced the range of species.

Organism	Control (without NMU treatment)	NMU treatment, 6 h	NMU treatment, 18 h	Repeated treatment, 6 h	Repeated treatment, 18 h
Ulothrix sp.	+	+	mass	+	+
Scenedesmus obliguus	+	+	+	+	
Chlorella vulgaris		+			
Flagellata sp.			+	mass	
Oicomonas socialis				+	+
Bodo globosus		+	+	+	+
Zooglea ramigera					occasional
Euglena viridis					+
Filamentous bacteria					occasional
Bacillus					+
Beggiatoa minima			+		+
Jromia neglecta sp.			+	+	
Arcella vulgaris			+		
Centropixis acullata	+		+		
Pamphagus hyalinus	+	+			+
Euglypha laevis				+	
Amoeba sp.				+	+
Aspidisca costata			+		+
Aspidisca lynceus		+	+		
Lacrimaria sp.		+		+	
Litonotus anser		+		+	
Chilodonella uncinata		+			
Vorticella alba		+	+		
Thuricola similes	+				
Telotpox		+	+		
Rotaria rotatoria	+	mass	+	+	+
Colurella sp.		+	+	+	+
Rotaria neptunia			+		+
Lelane Monostyla			+	+	+
Brachionus angularis		+			
Cephalodella gibba			+	+	+
Notommata sp.			+	+	+
Cephalodella forticula			+		+
Chaetonotus brevispinosus	+		+		

Table 1. Hydrobiological analysis of activated sludge.

The presence of DCPA, its copper complex, or industrial waste (samples 1–5) at concentrations used in the experiment exerted acute and chronic effects on the infusorium culture. Figure 1 shows that the toxicity of sample 1 remained high (~80%) after two months

of monitoring. The toxicity of sample 2, treated with NMU for 6 h, remained high (~90%) for 36 days. Then, it decreased abruptly, and, after 56 days, the sample was virtually nontoxic. By the beginning of the third month, its toxicity reached 70%. As mentioned above, the decrease in toxicity after an 18-h treatment (as compared to the 6-h exposure to NMU) may be related to a change in the proportions of species in AS as a result of the mutagenic effect. The history of toxicity of sample 4 suggests that the CuL_2 had no pronounced toxic effect on the AS for 20 days after the onset of exposure to NMU. This result may be related to a decrease in the reactivity of DCPA in the copper complex. After 20 days, the complex appears to have been degraded and the toxicity of the sample abruptly increased. The history of toxicity of sample 5, containing industrial waste, was similar to that of sample 4. This finding suggests the presence of various complexes between DCPA and metals, whose decay after 20 days increases the toxicity of the sample dramatically.

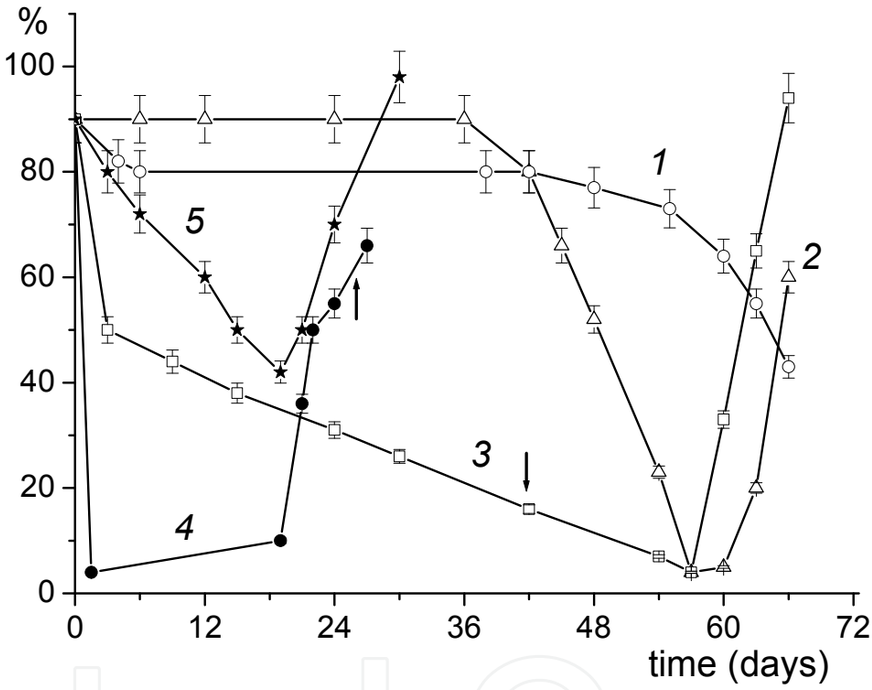


Fig. 1. Changes in the toxicity (with *B. harveyi*) of AS samples containing (*1, 2, 3*) DCPA, (*4*) the CuL_2 complex, and (*5*) industrial waste water: (*1*) control sample with intact AS, (*2*) AS treated with NMU for 6 h, and (*3*) AS treated with NMU for 18 h. Starting concentrations: DCPA, $1.22 \cdot 10^{-3}$ M; industrial wastewater, $\approx 1 \cdot 10^{-3}$ M; the CuL_2 complex, $0.4 \cdot 10^{-3}$ M. Temperature 25°C. Arrows indicate repeated NMU treatment.

As seen in Fig. 2, DCPA concentrations in samples 4 and 5 remained constant throughout the experiment. Most likely, it is only the proportions of various CuL_2 that changed, thereby altering the toxicity of these samples (Fig. 1). In samples 2–4, AS was treated with NMU to obtain mutations most resistant to the toxic substance under study. After the first 20 days (Fig. 2), DCPA concentrations changed neither in the control sample nor in samples exposed to the mutagen. After 18–20 days, DCPA concentrations in samples 1–3 began to decrease. The decrease in DCPA concentrations in samples 2 and 3 occurred faster than in the control sample. A steady state was established approximately 40 days after the beginning of the

experiment. Another treatment with NMU was undertaken to further increase the oxidative potential of AS; however, this attempt did not cause a significant degradation of the substances under study. After 66 days, the content of DCPA decreased by 25%, while that in the sample not treated with NMU decreased by 20%.

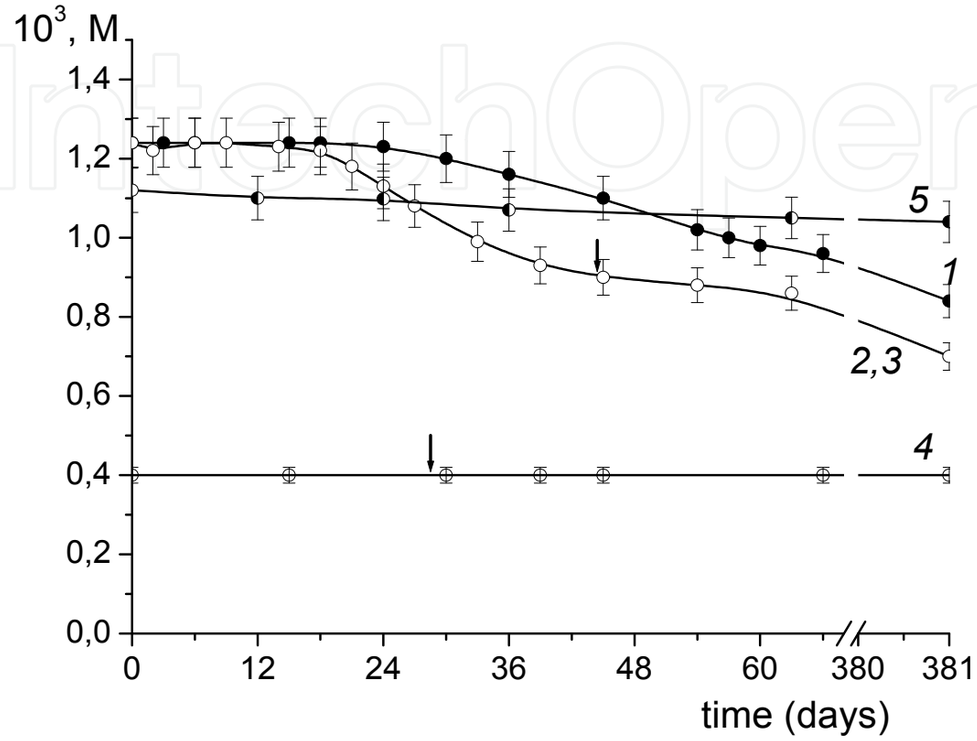


Fig. 2. Kinetic curves of the degradation of (*1*, *2*, *3*) DCPA, (*4*) the CuL_2 complex, and (*5*) industrial wastewater. Designations follow Fig. 1.

After one year of treatment of the pollutants with a solution of AS treated with the mutagen, DCPA concentration had decreased by 45% in total, i.e., by less than half; in the control (nonmutagenized) AS sample, the DCPA concentration had decreased by 30% in total. During the same one-year term, the concentration of toxic substances in sample 5, containing wastewater of the herbicide mixture, had decreased only by 7% in total.

Thus, our data indicate that DCPA the main active principle of herbicide Lontrel belongs to bioresistant organochlorine herbicides. Wastewater treatment in industrial treatment plants lasts only for 8 ÷ 11 h. During this treatment, Lontrel can form compounds with copper or other industrial pollutants. It is natural to assume that both Lontrel and its metal derivatives remain intact in treatment plants. Hence, the toxic substances can penetrate surface or subsurface water bodies and accumulate there, posing a hazard for microflora, plants, and fish. Because of Lontrel bioresistance, its application in agriculture for controlling weeds can exert selection pressure and give rise to mutant weeds, resistant to the herbicide. This effect has been shown for Simazine and Atrazine, which are resistant to biodegradation in soil (Fedtke, 1985). Bioresistance was the reason for prohibition of DDT, although it was nontoxic at recommended concentrations (Burgelya & Myrlyan, 1985).

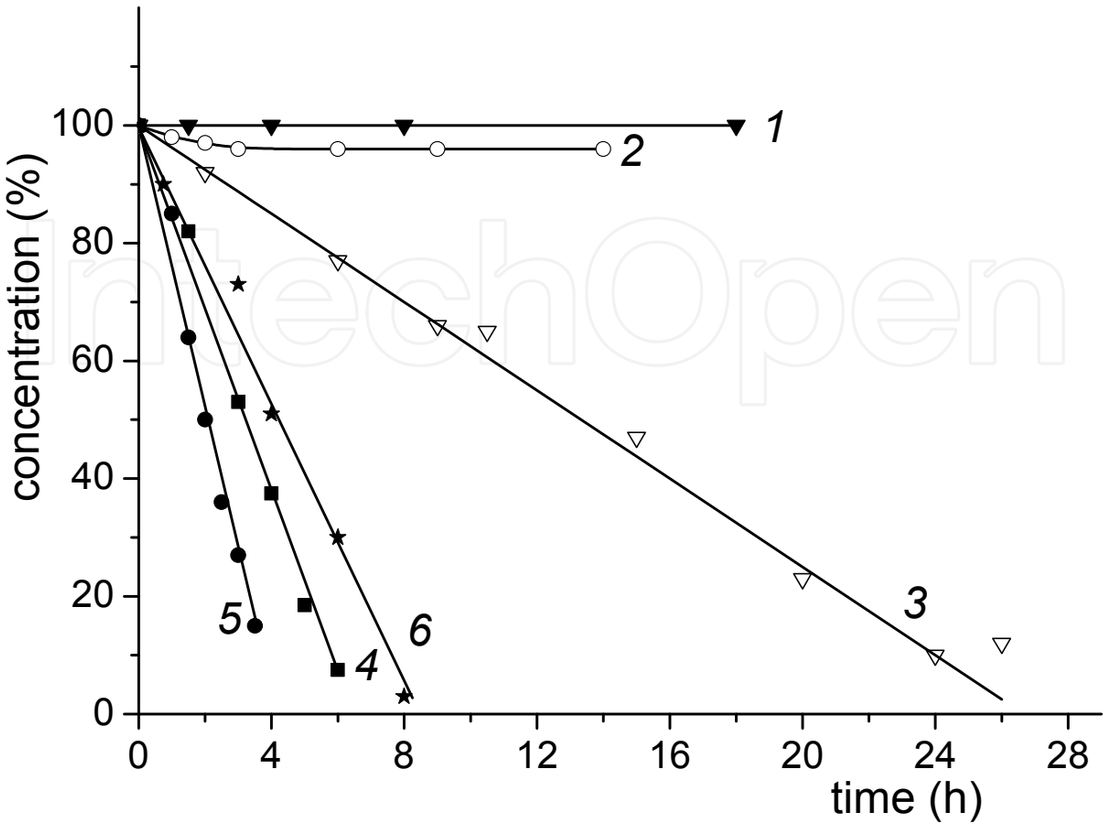


Fig. 3. Changes in DCPA concentrations (as percentages of the starting concentration), as determined by the time of UV irradiation: (*1*) without bubbling; (*2*) argon, DRB-8 lamp; (*3*) air, DRB-8 lamp; (*4*) ozone, DRB-8 lamp; (*5*) air, DRSh-1000 lamp; and (*6*) oxygen, hydrogen peroxide, BRA-15 lamp.

The high resistance of Lontrel to biodegradation under natural sunlight prompted us to perform an experiment on photochemical oxidation of the herbicide with the use of hardUV radiation, which is the only part of the solar UV spectrum (wavelength range, 400–360 nm) that reaches the surface of Earth, shorter waves being trapped by the atmosphere. Photochemical activity can be manifested by radiation within the absorption spectrum of a compound. In our case, for DCPA, the active wavelength is λ = 283 nm. Therefore, we used mercury lamps with emission lines within 254–579 nm. The energy of a quantum with a wavelength of 283 nm exceeds the energy of the C=N bond (~260 kJ/mol) by a factor of about 1.5, an amount of energy that may be sufficient for cleavage of the very stable pyridine ring, which is not oxidizable by AS microorganisms. The efficiency of UV irradiation in inducing redox degradation of various contaminants has been shown previously (Skurlatov & Shtamm, 1997b; Kruithof et al., 1992). It has been reported that Atrazine is degraded by UV irradiation alone within 20 min and by ozone alone within almost 3 h (Prado & Esplugas, 1999). Application of UV radiation, including its combination with the use of hydrogen peroxide, to the degradation of phenols has been reported (Legrini et al., 1993; Guittonneau et al., 1988; Sundstrom et al., 1989; Castrantas & Gibilisco, 1990).

No positive results were obtained in the first set of experiments, in which DCPA solutions were treated with UV radiation alone (Fig. 3, curve *1*). After 8 h of irradiation, optical density did not change; thus, the herbicide was not degraded at all.

To eliminate stagnation zones and to provide uniform illumination of the reaction-mixture layers distant from the UV lamp, the solution was bubbled with the inert gas argon. Figure 3, curve *2*, shows that the concentration of the starting substance decreased by 5% after 3 h of the reaction; after that, however, the reaction completely stopped.

To increase the oxidative effect of hard UV radiation, the reaction mixture was bubbled with air instead of argon. The cooperative action of UV, atmospheric oxygen, and mixing almost completely degraded the herbicide after 24–25 h of the reaction (Fig. 3, curve *3*). Simultaneous treatment with UV, oxygen, and hydrogen peroxide reduced the degradation time to 8 h (Fig. 3, curve *6*). Irradiation under the conditions of ozone bubbling accelerated DCPA oxidation four- to fivefold (as compared to air bubbling, other factors being the same). The herbicide was oxidized almost completely after 6–6.5 h of the photochemical reaction (Fig. 3, curve *4*).

Thus, bubbling of the reaction mixture with argon, i.e., mechanical mixing with inert gas, slightly accelerates DCPA degradation, but the use of air is much more efficient. In our experiments, agitation with air or oxygen flow (1) increased the reaction surface and (2) supplied the solution to the reaction zone. In addition, ozone is one of the products of photochemical oxidation of oxygen; when affected by UV irradiation, the ozone molecule, in turn, dissociates to an electronexcited oxygen atom and an oxygen molecule in the singlet state. These chemical species, having high oxidizing potentials, attack herbicide molecules and degrade them, either partially or completely (Shinkarenko & Aleskovskii, 1982).

Experiments were performed with DRB-8 and DRSh-1000 lamps. Complete DCPA degradation with a DRB-8 lamp and bubbling with air occurs within 24 h, whereas degradation with the use of a DRSh-1000 lamp takes 3–3.5 h, that is, a seven- to eightfold smaller amount of time. Thus, the rate of DCPA oxidation depends significantly on the power of the UV-radiation source.

The solution obtained by complete UV-induced degradation of DCPA was tested for toxicity according to changes in the enzymatic activity of luminescent bacteria. In the control experiment, the toxicity of DCPA was detected at concentrations of 10^{-7}–10^{-3} M in the absence of UV radiation.

The data presented in Table 2 indicate that no toxic effect was detected in the samples after either 5-min (effect on the cell membrane) or 30-min (effect on cellular metabolism) exposure throughout the whole range of the initial concentrations studied. Irradiated samples (containing products of complete herbicide oxidation) were toxic after 5-min exposure for all starting DCPA concentrations (Table 3). After 30-min exposure, toxicity was detected only in the sample with a starting concentration of 10^{-3} M, whereas samples with the starting concentrations of 10^{-5} and 10^{-7} M did not show any toxic effect. It may be inferred that a product of photochemical degradation of DCPA is inherently toxic to luminescent bacteria.

DCPA concentration, M	Toxicity coefficient (*T*), %											
	before irradiation				after irradiation				after irradiation and treated with AS for 24 h			
	exposure, min											
	5		30		5		30		5		30	
10^{-7}	0.90	non-toxic	11.80	non-toxic	44.0	toxic	1.80	non-toxic	4.00	non-toxic	3.90	non-toxic
10^{-5}	8.10		33.20		18.5		4.10		4.00		3.80	
10^{-3}	11.0		88.60		25.7		33.2		3.70		3.70	

Table 2. Toxicity coefficients of samples before and after UV irradiation as determined from the change of the luciferase activity of luminescent bacteria.

However, this substance, having a simpler structure than the pyridine ring, should be easily metabolized by AS microorganisms, as demonstrated by the experiment described below. After irradiation under the conditions of air bubbling for 24 h, samples with DCPA were placed into an aerated tank with AS for 24 h and the toxicity of the resulting solution was tested. According to the data obtained with the luminescent bacterium *B. harveyi*, the association of AS microorganisms successfully neutralized the contaminant formed after UV irradiation (Table 2). The solutions were nontoxic at all starting concentrations, even the highest one (10^{-3} M). The absence of toxicity in the samples was also shown with the infusorium *T. pyriformis* for all starting concentrations (Table 3).

DCPA concentration, M	Toxicity coefficient (*K*), %			
	exposure to AS for 24 h		exposure to AS for 48 h	
10^{-7}	0.81	nontoxic	15.8	nontoxic
10^{-5}	13.7	nontoxic	13.1	nontoxic
10^{-3}	10.5	nontoxic	16.9	nontoxic

Table 3. Toxicity coefficients of samples before and after UV irradiation and treatment with AS for 24 and 48 h as determined using the infusorium culture.

We studied the composition of products of the photochemical degradation of DCPA. The electronic spectra of the aqueous DCPA solutions show that the intensity of the band at 283 nm gradually decreased throughout the time of the experiment (Fig. 4). No additional bands or shift of the absorption maximum were observed in this region. Probably, the products of DCPA degradation had no intense bands in the UV or visible regions, or their concentrations were insignificant. The pH value of the solution decreased from 4.25 to 3.81.

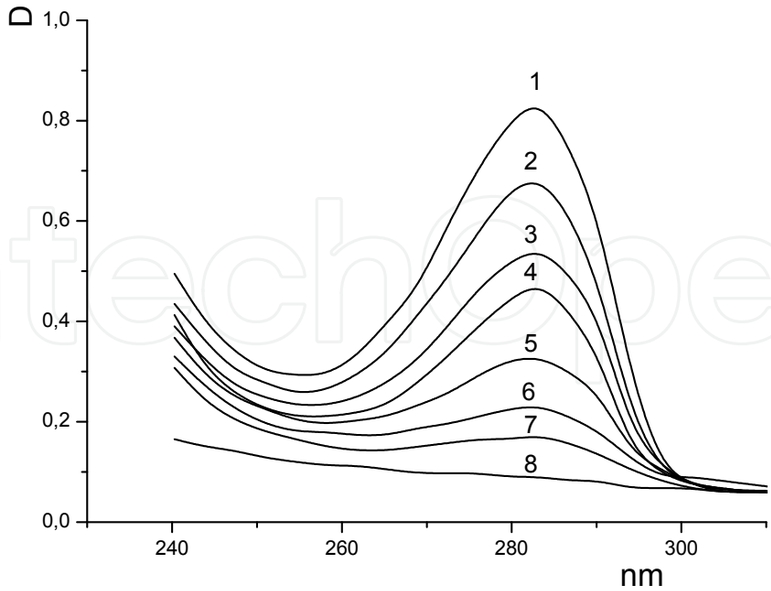


Fig. 4. Change of the DCPA electronic spectrum during irradiation. (*1*) Starting solution: $5 \cdot 10^{-4}$ M DCPA; (*2–8*), solution after UV irradiation for 6, 11, 13, 18.5, 23, 24, and 38 h, respectively.

Kinetic curves of photochemical DCPA degradation with oxygen bubbling are shown in Fig. 5. The figure reveals a difference among the shapes of the kinetic curves in distilled, sea, and river water. Curve *1* is linear: DCPA concentration decreases at a constant rate, reaching zero after 22 h. Curve *3* can be approximated by the log equation:

$$y=100\exp(-x/16), \quad (3)$$

whereas curve *2* can be approximated by the sum of 2 log curves:

$$y=30\exp(-x/3)+70\exp(-x/50). \quad (4)$$

The rates of DCPA degradation in both river and sea water were higher than in distilled water for the first 10 h.

However, the rate of DCPA degradation decreased significantly after irradiation for 7 h in seawater and 10 h in river water, being lower than in sample 1. After 45 h of observation, the DCPA concentrations in both samples were significantly different. The rate of DCPA photooxidation in river water proved to be higher than in seawater. According to our previous studies, this difference can be related to the fact that the herbicide forms UV-resistant complexes with metals occurring in natural media (Aliev et al., 1988; Saratovskikh et al., 1989b). At the beginning, 2 processes occur in the solution: DCPA degradation and binding to metals. As seawater is richer in metals, the concentrations of the metal complexes are higher, and the rate of DCPA degradation is lower than in river water (curve *3*).

Higher (Fig. 3), reported the kinetics of DCPA degradation by UV with air bubbling. According to electronic spectra, DCPA degraded by 50% after 13 h and by 90%, after 24 h. Complete DCPA degradation was achieved after 38 h irradiation.

Comparison of the IR spectra of the starting and UV-irradiated DCPA revealed substantial changes (Fig. 6, curves *1* and *2*). The intense absorbance band (AB) ν(C=O) at 1710 cm^{-1} was split into 4 bands: 1780, 1740, 1715, and 1690 cm^{-1}, which is attributable to DCPA degradation and the formation of other compounds. The AB at 1780 cm^{-1} can be related to ν(C=O) in the group $-\underset{\displaystyle X}{\overset{\displaystyle |}{C}}-\overset{\displaystyle O}{\overset{\displaystyle \|}{C}}-$; at 1740 cm^{-1}, to conjugated with an unsaturated C=C bond; at 1715 cm^{-1}, to asymmet-rical vibrations of two (C=O) bonds, and at 1690 cm^{-1}, to vibrations of C=O conjugated with the aromatic ring.

The ABs of fragment $N\overset{C}{\diagdown\!\diagup}C$ at 1560 and 1540 cm^{-1} (ν_{as} and ν_s of bonds C_{ar}.....N) in the starting DCPA shifted to higher frequencies (1620 and 1565 cm^{-1}). Apparently, the lone pair of nitrogen moved from the antibonding orbital to the complex-forming molecular orbital.

The sharp and intense ABs of the valence ν(C=C) and planar deformational δ_{II}(CO) vibrations at 1440, 1410, and 1305 cm^{-1} in the DCPA spectrum fused into a single very wide band with scarcely distinguishable maxima at 1450 and 1380 cm^{-1} in the spectrum of the degradation product. This may be related to the formation of a series of compounds derived from carboxylic groups COO^-, including those entering linear compounds.

Significant changes occurred in the range of low frequencies (800–500 cm^{-1}), including the ABs ν(Car –Cl) and ν(C–Cl). They involved band intensities and frequency shift. Very intense ABs appeared in the product spectrum at 605 and 530 cm^{-1}, probably related to C–Cl and Car–Cl vibrations, which is natural, because chlorine- containing compounds could be accumulated.

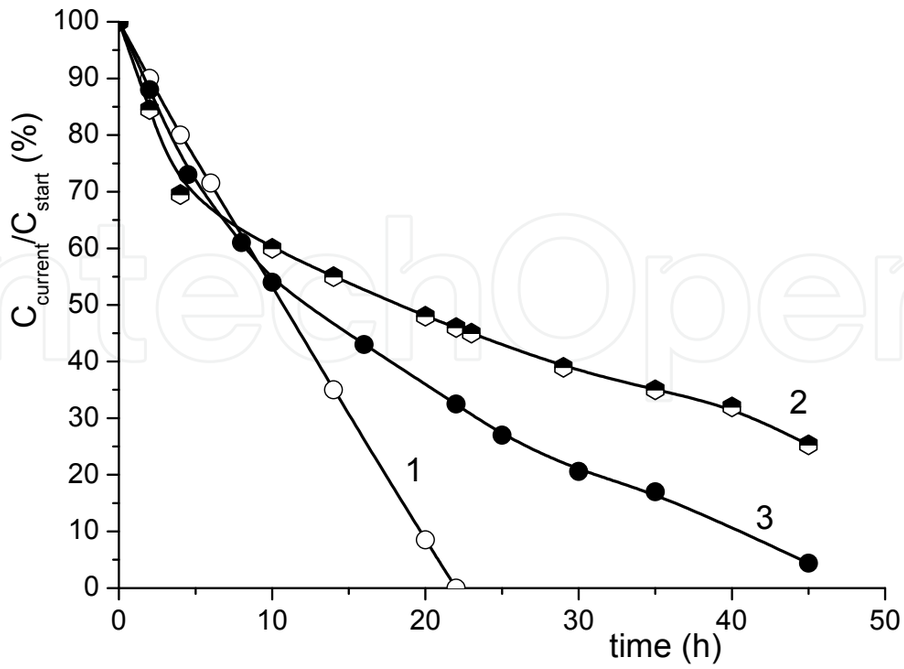


Fig. 5. Kinetics of DCPA concentration under the effect of UV irradiation with oxygen bubbling at 25°C. DCPA concentration $1.42 \cdot 10^{-3}$ M. *1*, distilled water; *2*, artificial seawater; *3*, river water.

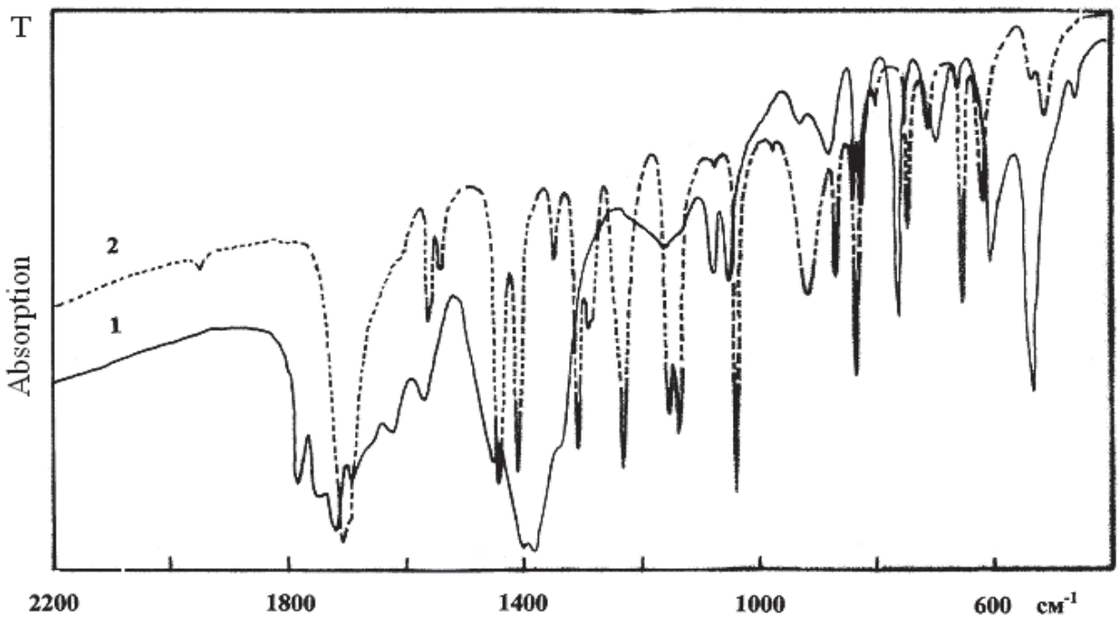


Fig. 6. Infrared spectra of (*1*) the product of DCPA photolysis; (*2*) intact DCPA.

It should be noted that the large widening of ABs in the regions 550 (large-amplitude proton vibration), 1400, and 1700 cm^{-1} can be related to associates with water. An intense and wide AB completely covered the region 3500–2700 cm^{-1}.

For final identification of compounds forming during UV degradation of DCPA, we performed GC/MS analysis of samples after UV irradiation. Ten compounds, including DCPA, were found in the sample taken after 13-h irradiation. The compounds and their ratios to DCPA are presented in Table 4. From these data, we deduced their molar concentrations.

No	Compound	13 h		38 h	
		percentage of DCPA	concentration, $\cdot 10^{-5}$ M	percentage of DCPA	concentration, $\cdot 10^{-6}$ M
I	4-Chloro-1,2-dimethylbenzene	0.05	1.71	0.17	1.16
II	Dichlorobutanol	0.06	2.00	0.26	1.75
III	3-Chlorobenzoyl chloride	0.06	1.65	0.28	1.54
IV	3,6-Dichloropicolinic acid	1.00	25.0	1.00	5.00
V	4-Chlorobenzoyl chloride	0.11	3.00	0.50	2.74
VI	Trichlorobutanol	0.18	4.87	1.32	7.14
VII	2,3,5-Trichloropyridine	0.12	3.16	-	-
VIII	Trichlorobutanol (isomer)	0.02	0.54	0.26	1.41
IX	Hexachlorocyclohexane	0.02	0.34	0.48	1.62
X	2,6-Dichloro-3-nitropyridine	0.11	2.74	-	-

Table 4. Areas of peaks of degradation products in percentage of DCPA after 13 and 38 h of UV irradiation and calculated concentrations (Note: –, not detected).

It is apparent from Table 4 that DCPA (compound IV) was predominant after 13 h of UV irradiation. However, 9 more compounds were identified in the mixture (I–X), which appeared as products of DCPA degradation. In addition to pyridine derivatives (primary degradation products), major components included substituted chlorobenzenes and chlorobutanols. The appearance of the latter can be explained by cleavage of the pyridine ring, as any aromatic ring, by secondary oxidation (Tretyakova et al., 1994). Further irradiation increased the concentrations of these products. Formation of chlorobenzenes can be explained by the Kost–Sagitulin rearrangement (Danagulyan, 2005), converting picoline derivatives to anilines. Subsequent oxidation of the amino group could give rise to the whole series of identified products of this kind.

It is more difficult to correlate the appearance of hexachlorocyclohexane (HCCH – compound IX) with the structure of the initial molecule, exposed to irradiation. It is reasonable to suggest that HCCH was a minor impurity in the starting pesticide.

It should be noted that some degradation products could be missed. In particular, salts of aniline derivatives, polyoxy compounds, or dicarboxylic acids could be too polar to be extracted from water and to get through to the chromatographic column (Lebedev et al., 1996).

After 13-h UV irradiation, the mixture contained $4.87 \cdot 10^{-5}$ M compound VI, 5 times less than DCPA. The amounts of compounds V, X, and VII were 10 times less than that of DCPA: $3.0 \cdot 10^{-5}$; $2.74 \cdot 10^{-5}$, and $3.16 \cdot 10^{-5}$ M, respectively. The amounts of compounds I, II, and III were 20 times less ($1.71 \cdot 10^{-5}$; $2.0 \cdot 10^{-5}$; and $1.65 \cdot 10^{-5}$ M, respectively), and the amounts of

compounds VIII and IX, fifty times less ($0.54 \cdot 10^{-5}$ and $0.34 \cdot 10^{-5}$ M) than the amount of the herbicide to be degraded.

Continuation of UV irradiation changed the pattern. After 38-h irradiation, chloropyridine intermediates VII and X were completely degraded, apart from DCPA itself, whose concentration was $5 \cdot 10^{-6}$ M. The proportions of other degradation products increased considerably: three- to fivefold for compounds I–III and V (concentrations ≤ $1.16 \cdot 10^{-6}$; $1.75 \cdot 10^{-6}$; $1.54 \cdot 10^{-6}$; and $2.74 \cdot 10^{-6}$ M, respectively). The relative proportion of HCCH also increased significantly (IX; ≤ $1.62 \cdot 10^{-6}$ M), by a factor of virtually 25. The same was with trichlorobutanol (VIII ≤ $1.41 \cdot 10^{-6}$ M) and VI (≤ $7.14 \cdot 10^{-6}$ M). Trichlorobutanol (VIII and VI) became the predominant component of the mixture. In the final sample, its concentration increased eightfold in comparison with 13-h irradiation and exceeded the concentration of DCPA.

The data of elemental analysis are shown in Table 5. The detected and predicted amounts were fairly close only for hydrogen. The contents of other elements differed significantly from the predicted values. This may be related to the fact that volatile oxides were released during evaporation; however, the humid sample released water with difficulty and began to melt.

Element	Found, %	Calculated, %
C	12.10-11.05	37.12
H	4.84-4.65	3.14
Cl	11.25-14.62	57.495
N	29.66-29.52	1.91
Ash	5.9-4.37	

Table 5. Data of the elemental analysis of the sample after 38 h of irradiation.

Our results indicate that DCPA is difficult to degrade. Photolysis resulted in cleavage of the pyridine cycle and formation of simpler compounds. However, the insecticide Lindane was identified among the photolysis products. Ultraviolet-mediated DCPA degradation in river and sea water was slower than in distilled water, probably because of formation of metal complexes. The rate of DCPA degradation in seawater at 25°C was lower than in river water. This fact is likely to hold true in natural ecosystems.

There is a firm opinion that the use of herbicides enhances the crop of the fields. However, many literature data (Skurlatov et al., 1994; Yablokov, 1990) indicate, most likely, an opposite fact: the use of "chemical remedies of plant protection" has stopped long ago to favor the crop and now gives an opposite effect (Fig. 7). This is because the microflora and humus matter of the fertile ground layer are annihilated (Golovleva & Fil`kenshtein, 1984; Saratovskikh & Bokova, 2007). The results of our studies showed the mechanisms of these negative processes (Saratovskikh et al., 1989a, 1988, 2005, 2007b, 2008b).

The surface areas of agricultural grounds decrease because of contamination with heavy metals and herbicides. This results in deep changes in the physicochemical, agrochemical, and biological properties of the arable land, an increase in the negative balance of humus (up to 1-3 t/hectare annually), and a decrease in the overall storage of biomass in soils. In

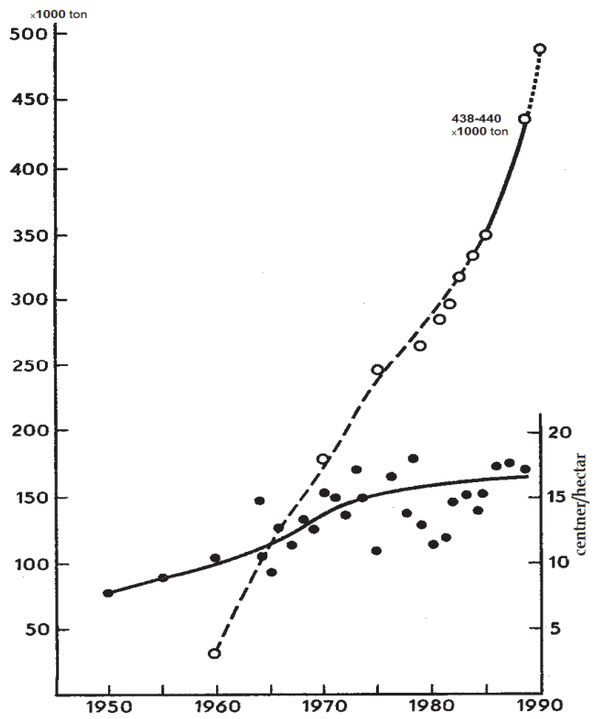


Fig. 7. Pesticides use in USSR and raising the level of crop yield. (Yablokov A.V., 1990). Scale of pesticide practice in USA has increased tenfold from the middle of 1970s to the early 1980s, weed losses increasing from 8 to 12 %.

the nearest 10-15 years the fertility of soils can decrease to a crop capacity of grain crops of 8-10 centner/hectare (Postanovlrenie, 2001).

The main soil-forming role belongs to the forest and grassy vegetation (Kusnetsov et al., 2005; Saprikin, 1984). Microorganisms (bacteria, microscopic fungi, and algae) play the most important role in the formation of soil fertility. Bacteria decompose organic residues to simple mineral compounds, perform processes of ammonification and denitrification, oxidize mineral compounds, and participate in nitrification (Jiller, 1988; Zviagintsev, 1987).

The organic component is presented by humus substances, which serve as a nutrient source for microorganisms and pedobionts. Soil fertility is determined by the content of humus substances in it. These substances are chemically and microbiologically stable. Due to the high content of ligand functional groups, they possess high complexation ability and are characterized by hydrophobic interactions. Black earths (chernozems) contain up to 15% humus, whereas the medium-humus soils contain up to 7% humus (Orlov, 1974). The change in the amount and qualitative composition of organic residues coming to the soil results in the situation that microorganisms use humus of the soil until its complete degradation (Mil`to et al., 1984).

We studied the change in the life cycles and population dynamics of soil-inhabitant collemboles of the species *Folsomia candida* and *Xenylla grisea (Hypogastruridae)* under the effect of various herbicides and metal complexes (Saratovskikh & Bokova, 2007). It was shown that under the action of DCPA the terms of appearance of the first sets of *F. Candida*

increase from 10 to 48 days. The number of eggs in the sets decreases by 3 times. The duration of embryonic development elongates from 7 to 13 days. In a month the multiplicity of population decreases from 16 to 0.9 times. The biological activity of CuL_2 is multiply higher than that of DCPA. The action of CuL_2 results in a decrease in the population of adults. This effect is more negative for the population of posterity and an increase in whitebait even when using in low (10^{-7} M) concentrations. Evidently, the contact with herbicides is a reason for the violation of reproductive functions of the organism and decreases the population of the posterity of microarthropodes and also other microorganisms dwelling in the soil.

Pathology forms	Mean quantity on Russia	Environmental trouble area
an allergy to food in early childhood	70.0	400
bronchial (spasmodic) asthma	9.7	24
recurrent bronchitis	6.0	94
vascular dystonia	12.0	144
gastritis and gastroduodenitis	60.0	180
congenital malformation	11.0	140
encephalopathy	30.0	50
decrease intelligence quotient (IQ)>70%	30.0	138

Table. 6. Prevalence of pathology forms for children (per 1000 people) in environmental trouble area and on average over Russia.

The effect of herbicides and their metal complexes on hydrobionts is negative to the same extent (Saratovskikh et al., 2008a). So, DCPA and CuL_2 suppress the reproductive ability of hydrobionts, for example, infusorium *Tetrahymena pyriformis*. The effect is observed in a wide concentration rage from 10^{-1} to 10^{-7} M. Herbicides and their metal complexes decrease the activity of enzymes, for instance, luciferase of bacterium *Beneckea harveyi*. The inhibition of enzymes, for example, HADH-oxyreductase (Saratovskikh et al., 2005), ceases oxidation processes in polycellular organisms and results in the elimination of hydrobiont species and active silt and accumulation of contaminants in water ecosystems.

Larger inhabitants of flora and fauna disappear after representatives of the lowest trophic levels. The process gained the catastrophic character (Koptug, 1992).

Moreover, available published data show that the use of pesticides causes the most part of diseases of a modern human being (Table 6) (Gichev, 2003; Klyushnikov, 2005; Rakitsky et al., 2000) and is followed by lesions of the next generations of warm-blooded beings, including the man. This is the reason of many taken ill with cancroid (Popechitelev & Startseva, 2003). There is one more serious danger of using pesticides. It was indicated above that the pesticides have no selectivity of action. Gene-modified types of plants are developed to enhance the resistance of agricultural plants to the action of specific pesticides (Christoffers et al., 2002; Pyke et al., 2004; Sakagami et al., 2005). Based on the results presented, we may assert that the use of pesticides should drastically be reduced.

Nevertheless, this does not take place; on the contrary (Table 7), the absurdity of the situation is enhanced by the enlargement of application of gene-modified types of plants.

This cannot be explained from the scientific point of view, but economical reasons can be discussed. Diseases and death of people in all countries of the world, huge expenses of governments to (a) payment of sick leaves, (b) building of oncological and other medical centers, (c) payments of various medical insurances, and others, all these matters are profitable only for large chemical companies. Chemical companies produce: (a) pesticides, (b) gene-modified sorts of agricultural plants, and (c) drugs, which become more expensive and whose administration is accompanied by serious secondary effects.

USA	Economic loss from pollution of the air priced at a 20 billion US $ in year
Japan	damage bring of pollution of the environment averaged 5 trillion yen in year
Russia	damage bring of Chernobyl an accident averaged in 10 billion rouble (1990)
FRG	regulate application of pesticides on farms and on plot of land

Table 7. Economic loss from pollution of the environment.

4. Conclusion

Thus, 3,6-Dichloropicolinic acid (DCPA) the main active principle of herbicide Lontrel is poorly degradable by AS microbial association. Natural solar radiation does not affect its oxidation either, thus allowing the herbicide to accumulate in the environment. This results in dramatic changes in the composition of phytoplankton associations and decreases in the range of microbial species. These consequences may cause irreversible changes to the bioproduction of water bodies.

Application of chemical mutagenesis brings about AS with a broader range of species and, in turn, intensifies the oxidation of pollutants. Treatment of AS samples with NMU for 18 h resulted in more efficient detoxification as compared to the 6-hour treatment.

The rate of oxidation of DCPA by the action of UV radiation depends heavily on the source power. This rate increases three to fourfold when the reaction mixture is bubbled with oxygen or ozone as compared to air bubbling. Photochemical degradation of DCPA by UV radiation yields inherently toxic chemicals; however, they are successfully metabolized by the microbial association of the AS.

Ultraviolet irradiation (mimicking the natural sunlight action) did not degrade DCPA completely to environmentally safe products. The rate of DCPA degradation was notably lower when distilled water was replaced by river water and even lower in sea water. Chromatomass spectrometry revealed 9 compounds among the photolysis products, in addition to undergraded DCPA.

The use of “chemical remedies of plant protection” has stopped long ago to favor the crop and now gives an opposite effect. This is because the microflora and humus matter of the fertile ground layer are annihilated.

In the issue, it can be stated that both the pesticides and its decomposition products are high-toxicity substances. Therefore, the application of the pesticides must be reduced to minimum.

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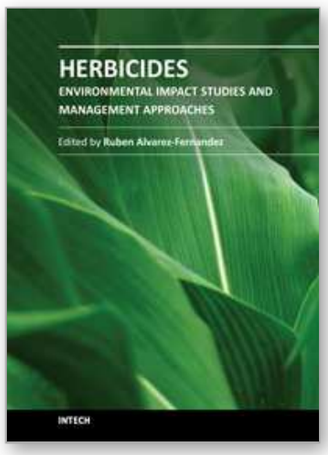

Herbicides - Environmental Impact Studies and Management Approaches

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Weeds severely affect crop quality and yield. Therefore, successful farming relies on their control by coordinated management approaches. Among these, chemical herbicides are of key importance. Their development and commercialization began in the 1940's and they allowed for a qualitative increase in crop yield and quality when it was most needed. This book blends review chapters with scientific studies, creating an overview of some the current trends in the field of herbicides. Included are environmental studies on their toxicity and impact on natural populations, methods to reduce herbicide inputs and therefore overall non-target toxicity, and the use of bioherbicides as natural alternatives.

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