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# Microbiological and biosensor tests of dichlorodiphenyltrichloroethane toxicity as a water micropollutant

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## ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) is an organochloride pesticide with a global impact on agriculture. Despite the international ban on this pesticide in 2001, DDT is still illegally used for malaria control in some parts of the world, such as India and South Africa. The presence of DDT and its metabolites in waters, soil, and sediments continue to adversely affect living organisms. Understanding the toxic effects of DDT is crucial for global environmental health. The goal of this study was to investigate the toxicity of DDT in *E. coli* ATCC-25922, *Sarcina spp*., ATCC-35659, *Enterobacter homaechei* LBM ATCC-700323, *Staphylococcus aureus* ATCC-25923 and *Candida albicans* ATCC-1023. To confirm oxidative stress as a mechanism of toxicity, *sodA* promoter induction in the *Escherichia coli sodA:luxCDABE* biosensor strain and ROS (Radical Oxygen Species) synthesis in *E. coli* ATCC-25922 strain, across various DDT concentrations (10, 1, 0.1, and 0.01 mg/L) were measured. The results showed that DDT in the range of applied concentrations shows a toxic effect on bacteria/fungi. Analysis of *sodA* promoter induction and ROS synthesis values in *E. coli* strains showed an increase in these indicators following exposure to the tested DDT concentrations. The results confirmed the validity of the hypothesis that the molecular mechanism of DDT toxicity is by induction of oxidative stress. Therefore, the need to develop more effective methods of removing DDT from wastewater and water and reducing the transport of this pesticide into the environment is justified. Moreover, recent evidence has increasingly confirmed positive correlations between human exposure to DDT and the development of cancers.

**Keywords:** DDT, toxicity, microbial biosensors, water pollution

## INTRODUCTION

Currently, one of the top international and national priorities is water pollution control. Major environmental pollutants include pesticides, some of which are listed as priority pollutants of natural water resources (European Union, 2016). Once in the environment, pesticides and their metabolites can cause a range of disturbances in food chains and microbial activity. It has been

scientifically proven that, as even at low doses, pesticides exhibit toxicity and have carcinogenic potential (Lescano et al., 2022). Several legal regulations have been introduced in European Union countries regarding properly handling plant protection products and limiting their transport to the environment. The main piece of legislation is Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official acts performed to ensure the application of food and feed law, animal health and welfare rules, plant health and plant protection products. In the countries of the European Union, the most important document protecting the quality of drinking water is the Directive Council Directive 98/83/EC (1998). This Directive sets out the following requirements that water intended for human consumption should meet: must be free of microorganisms, parasites, and harmful chemicals. In Poland, the main document adapting Polish law to the requirements of the EU Directive is the Dz. U. p. 2294 (2017) and Dz. U. p. 1311 (2019) (Cybulski et al., 2021).

Dichlorodiphenyltrichloroethane (DDT) is an organochloride pesticide (Fig. 1.). DDT was synthesized in 1874, and it was used to control insects including pink bollworm, codling moth, Colorado potato beetle, and European corn borer from 1945 (Liu and Sing, 2022). Due to its lipophilic nature and significant resistance to biodegradation, DDT is classified as a persistent organic pollutant (POP) (Chueycham et al., 2021; Liu and Sing, 2022). It was estimated that the half-life of DDT in the field would be from 2 to 15 years. In aquatic environments, the half-live of DDT extends up to 150 years (Chueycham et al., 2021). DDT concentrations detected worldwide in surface waters range from 1 ng/L (USA) and 250 ng/L (China) to even a value of 5794 mg/L in the Bhopol River (India) (Mansouri et al., 2016).

The primary biodegradation metabolites of DDT include toxic 4-chlorophenol-acetate and highly persistent compounds such as 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE), 1,1-dichloro-2,2-bis(p-chlorophenyl) ethane (DDD), and 4,4 -dichlorobenzophenone (DBP). DDE and DDD are highly toxic to many aquatic organisms, and in many cases, it has been shown that these metabolites have higher toxicity compared to the parent pesticide, DDT (Mansouri et al., 2016). DDT and its metabolites are still detected in waters, soil, sediments and living organisms. For example, within China's Zhejiang Province, significant exceedances of the soil environmental quality standard for DDT were detected in farmland soil samples with values as high as 1.208 mg/kg (Ebsa et al., 2024). Moreover, DDT has been detected in human milk, blood, sweat, and urine (Malusá et al., 2020; Chueycham et al., 2021). Data from measurements of DDT content in Antarctic waters showed the presence of this pesticide in concentrations ranging from 0.07 to 0.25 pg/L (Malagon et al., 2023). Due to the ability to bioaccumulate and the adverse impact of DDT on living organisms, this pesticide was banned for agricultural uses worldwide by the 2001 Stockholm Convention on Persistent Organic Pollutants. Despite this, DDT is still illegally used for malaria control in some parts of the world, such as India and South Africa (Liu and Sing, 2022). According to a WHO report in 2014, at least 10 countries, including Botswana, the Democratic Republic of Congo, Gambia, India, Mozambique, Namibia, South Africa, Swaziland, Zambia, and Zimbabwe, used DDT as an insecticide for indoor residual spraying (IRS) (WHO 2014a, 2014b, 2015). Moreover, DDT is used in dicofol production in China, another widely used pesticide. The final product may contain up to 10% DDT (Tao et al., 2007). In the last years in Mexico waters, high levels of total DDTs, even up to concentrations of 811 ng/ml, were detected (Ochoa-Rivero et al., 2017).

DDT and its metabolites have the ability to bioaccumulate in the tissues of both plant and animal organisms, gradually integrating into the food chain. As the final link in this chain, humans are exposed to these pesticides through the consumption of contaminated plant and animal products. Tests on living organisms such as oysters, fish, mammals, and birds have so far demonstrated the neurotoxic effects of DDT and those that disrupt the functioning of the endocrine (potential EDCs) and reproductive systems in living organisms (Chueycham et al., 2021). It has also been established that DDT damages DNA in blood cells and stimulates the development of pancreatic and

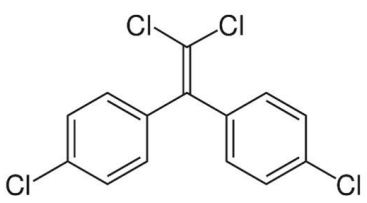


**Figure 1.** Chemical structure of DDT

breast cancers (Rizqi et al., 2023). Scientific studies have found that DDT exposure was associated with a statistically significant 5-fold increased risk of breast cancer among young women (Cohn et al., 2007; Chueycham et al., 2021). In addition, a positive association was also detected between rectal cancer and colorectal cancer (CRC) risk and exposure to DDT and its metabolite DDE. DDT was still detected in food worldwide in 2018 (Matich et al., 2021). Although the toxicity of DDT, on aquatic organisms and laboratory animals as well as human epidemiological studies has been documented so far, the molecular mechanism of action of DDT is not clear (Agency for Toxic Substances and Disease Registry (US) - ATSDR, 2022). Although there are several studies which suggest that the oxidative stress can be a key factor in DDT toxicity, the molecular mechanism should still be extensively studied (Jin et al., 2014; Harada et al., 2019; Russo et al., 2019).

Due to possible toxic and carcinogenic effects of pesticides on the environment, there is a constant need to study the toxicity and environmental impact of pesticides, which are being used in increasing quantities around the world (Ahmed et al., 2016; Lescano et al., 2022). Therefore, in this study the toxicity tests were carried out with selected microorganisms such as *E. coli* ATCC-25922, *Sarcina spp*., ATCC-35659, *E. homaechei*, LBM ATCC-700323, *S. aureus* ATCC-25923 and *C. albicans* ATCC-10231, among them are fungi and bacteria, both Gram-positive and Gram-negative. These toxicity tests will allow the assessment of a potential impact of that pesticide on microorganisms present in the natural environment, activated sludge and included in the natural microflora of the human body. DDT entering wastewater treatment plants (WWTPs) can negatively affect and disrupt the biodiversity of activated sludge containing the complex microbial consortia, leading to a reduction in the biodegradation efficiency of micropollutants, which then enters surface waters with treated wastewaters, posing a threat to the quality of drinking water (Ahmed et al., 2016). On the other hand, DDT present in contaminated food and drinking water, once it enters the gastrointestinal tract of humans, can disrupt the optimal composition of the gastrointestinal microbiome, and this can lead to serious health effects. Moreover, in the present study the *sodA* promoter induction in the *E. coli* SM342 *sodA:luxCDABE* and ROS level in the *E. coli* ATCC-25922 strain were determined to confirm oxidative stress as the possible DDT toxicity mechanism. The mentioned microorganisms are often chosen as model organisms in toxicity and oxidative stress tests (Matejczyk et al., 2023; Tasselli et al., 2023).

## METHODOLOGY

### Chemical compounds

DDT was purchased from Sigma-Aldrich (Sigma-Aldrich, UK) and used without further purification. The chemical compound was dissolved in dimethyl sulfoxide (DMSO). To limit the effects of DDT on microbial cells, its solutions were diluted in 0.86% NaCl in a ratio of 1:9 (100 µL DMSO and 900 µL 0.86% NaCl). The tested concentrations of DDT were 0.01, 0.1, 1, 10 mg/L. The selected DDT concentrations were within the range of DDT concentrations detected in the environment (on the basis of literature review) as well as the higher concentrations were also examined to study whether the molecular mechanism of DDT toxicity is concentration dependent.

### DDT antimicrobial activity

#### *The microorganisms used*

For DDT antimicrobial activity estimation, the strains of *E. coli* ATCC-25922, *Sarcina spp*., ATCC-35659, *E. homaechei* LBM ATCC-700323, *S. aureus* ATCC-25923, and *C. albicans* ATCC-1023, representing bacteria and fungi, were used. All microorganisms were purchased from ATCC (American Type Culture Collection, USA).

#### *Method for the determination of the antimicrobial activity of DDT*

The antimicrobial potency of DDT was measured based on the growth inhibition effect of tested microorganism cultures. Overnight cultures of the microorganisms mentioned above were established at 37°C on Luria Bertani broth (LB) that contained casein peptone 10 g/L; yeast extract 5 g/L and NaCl 10 g/L, pH 7.0. The next day, the cultures were renewed, diluted with fresh LB broth and incubated in a shaking water bath (130 rpm) at 37°C to the logarithmic growth phase ($OD_{600}$=0.2). Next, in 96-well plates to 100 µl of microorganism cultures, the 100 µl of appropriate concentrations of DDT were added. After mixing, samples were incubated at 37° C for 24 and 48 h.

DDT was not added to the control sample. The microorganism culture growth inhibition effect after DDT treatment was determined based on Optical Density (OD) values measured at 600 nm and using a GloMax® microplate reader (Promega, MA, USA). The results were presented as a percentage of inhibition/stimulation of the growth of the microorganism culture compared to the control sample. Experiments were conducted in triplicate.

### *Enterobacter hormaechei LBM ATCC and Candida albicans ATCC-1023 cells viability assay*

Viability assay with the use of BacTiter-Glo™ Microbial Cell Viability Assay Kit (Promega, Madison, WI, USA) was performed for *Enterobacter hormaechei* LBM ATCC and *Candida albicans* ATCC-1023 that showed high sensitivity in antimicrobial activity tests. In this test, the principle of determining the viability of microorganisms in broth culture is based on the ATP content in viable cells. The level of ATP in viable cells is directly proportional to the intensity of the luminescent signal. BacTiter-Glo™ Microbial Cell Viability Assay Kit is a standard method for the toxicity estimation of the selected chemicals to the microorganisms (Promega instructions). Both strains of *E. hormaechei* and *C. albicans* were grown in Müeller Hinton II broth (MH II) at 37°C for 24 hours. Next, the microorganism cultures were diluted in fresh MH II broth and incubated at 37°C with shaking (130 rpm) to the logarithmic growth phase ($OD_{600}$=0.2). After that, in 96-well plates to 100 µl of microorganism cultures, the 100 µl of appropriate concentrations of DDT were added. Samples were mixed and incubated for 24 h at 37° C. The control sample did not contain any DDT. The viability inhibition effect for both cultures of *E. hormaechei* and *C. albicans* after DDT incubation was assessed based on luminescence values measured using a GloMax® microplate reader (Promega, MA, USA). Moreover, for each sample the bacterial concentration was measured spectrophotometrically as OD value at 600 nm. The results were presented as a percentage of viability inhibition/stimulation of the microorganism culture compared to the control sample. Experiments were conducted in triplicate.

## Oxidative stress determination

To confirm oxidative stress as the mechanism of DDT toxicity, the induction of the *sodA* promoter in *E. coli* SM342/*pBRlux-trp:sodA:luxCDABE* and the level of ROS generation in the *E. coli* ATCC-25922 strain was examined.

### *SodA promoter induction*

Increased levels of ROS in living cells lead to damage to DNA, proteins, lipids, and cell dysfunction. Cells are equipped with some antioxidant enzymes whose task is to reduce the level of dangerous ROS. One of the key enzyme in oxygen defense systems is superoxide dismutase (SOD) which carries out the dismutation of superoxide anion into oxygen and $H_2O_2$. In further stages, the resulting reaction products are converted into water with the participation of catalase or peroxidase (Merkamm and Guyonvarch, 2001). In this study, to determine the potential of generating oxidative stress by DDT, a microbiological luminescent *E. coli* SM342/*pBRlux-trp:sodA:luxCDABE* biosensor with a plasmid gene construct containing the *sodA* promoter was used. In this method, the principle of luminescent assay is based on exposure to superoxide stress activating the *sodA* promoter, resulting in luminescence emission. The level of superoxide stress and luminescence signal is proportional to the intensity of *sodA* promoter induction in *E. coli sodA:luxCDABE*. In the literature, *E. coli* strains containing the *sodA:luxCDABE* plasmid gene construct are often described as valuable tools for evaluating the oxidative stress generation of many chemical compounds, including antibiotics (Melamed et al., 2012; Moraskie et al., 2021; Zhu et al., 2022; Rojas-Villacorta et al., 2022).

*E. coli* SM342/*pBRlux-trp:sodA:luxCDABE* strain was grown overnight in M9 medium at 37°C. After that, the bacteria culture was diluted in fresh LB and regrown at room temperature with shaking (130 rpm) to the early log phase ($OD_{600}$=0.2). The oxidative stress level was monitored as the intensity of *sodA* promoter induction and luminescence signal emission as a response to DDT exposure at 10, 1, 0.1, and 0.01 mg/L concentrations. In the next step, in 96-well plates to 100 µl of *E. coli sodA:luxCDABE* culture, the 100 µl of appropriate concentrations of DDT were added. After mixing, samples were incubated at 37° C for 2 and 24 h. The control sample did not contain DDT. Nalidixic acid (NA) was used as a positive control. The luminescence intensity was measured for each sample using a GloMax® microplate reader (Promega, MA, USA) at time zero and after 2 and 24 h of incubation. The luminescence of each

sample was normalized to its bacterial concentration measured spectrophotometrically as OD value at 600 nm. The results are shown in terms of the percent of induction/inhibition of *sodA* promoter and luminescence signal compared to the control. The experiment was carried out in three independent series.

### *ROS generation*

An increased level of ROS in the cell is an indicator of oxidative stress, which has a destructive effect on the structural elements of the cell and main molecules such as DNA, proteins, and lipids. In these studies, the intracellular ROS generation potential in an *E. coli* ATCC-25922 strain incubated with DDT was determined using 7′-dichlorofluoresceindiacetate (DCFH-DA) (Sigma-Aldrich, UK). *E. coli* culture was incubated overnight in LB broth at 37°C. The next day, the bacteria culture was diluted in fresh LB broth and then incubated at 37°C with shaking (130 rpm) to reach the log phase ($OD_{600}$ =0.2). After that, the E. coli culture was treated with DDT at concentrations of 10, 1, 0.1, and 0.01 mg/L. Next, DCFH-DA was added to each sample at a final concentration of 5 μM and incubated at 37°C for 50 min. In the control sample, DDT was not included. In this method, the analyzed parameter is the DCF fluorescence intensity measured using a GloMax® microplate reader (Promega, MA, USA) at the excitation wavelength of 485 nm and the emission wavelength of 535 nm. The level of ROS generation is directly proportional to the fluorescence intensity of the DDT-treated sample. Moreover, the $OD_{600}$ values of bacteria cultures were monitored spectrophotometrically for each sample. The results of the ROS generation in *E. coli* culture incubated with DDT were presented as a percent (%) of ROS increase compared to the non-treated control culture. The experiment was done in triplicates.

### Statistical analysis

The Tukey test for equilibrium samples was used to verify the effect of the DDT concentrations on the bacterial and fungal species under consideration. Differences in the individual parameters and species of bacteria and fungi between the control and the sample were verified after a given observation period. The statistical analyses resulted in a statistical significance level of α= 0.05. This level resulted from the methodological assumptions of the laboratory tests. The statistical analysis showed the differences between the control and test samples after a set observation time and between the samples after individual exposure times. In addition, before selecting a statistical test to verify the occurrence of the least significant differences, the data set was subjected to the Bartlett test to verify homogeneity of variance and the Shapiro-Wilk test to determine the normality of the distribution of the variables adopted for analysis.

## RESULTS

### DDT antimicrobial activity

The results showed potential for the antimicrobial action of DDT against *E. coli* ATCC-25922 and *Sarcina spp*. ATCC-35659, *E. homaechei* LBM ATCC-700323, *S. aureus* ATCC-25923 and *C. albicans* ATCC-10231 cultures (Figures 2–6).

Among the tested the microorganisms, *Sarcina spp*. and *E. coli*, especially at the highest DDT concentrations at 10 and 1 mg/L, showed the most significant sensitivity after 24 h and 48 h of incubation, respectively, up to 45. 82% (24 h) and 44. 23% (48 h) for *Sarcina spp*. and up to 43.59% (24 h) and 36. 98% (48 h) for *E. coli* (Fig., 2, 3). Also, 24 h exposure to DDT at a concentration of 1 mg/L induced more than 42% growth inhibition of *Sarcina spp*. cultures. In contrast, exposure to *Sarcina spp*. cultures to the lowest DDT tested concentration (0.01 mg/L) induced more than 4% stimulation of culture growth. Compared to *Sarcina spp*. and *E. coli* strains, DDT showed lower antimicrobial activity against the *Enterobacter homaechei* strain (Fig. 3), where both after 24 h and 48 h only at the highest tested concentration of 10 mg/L more than 13% (24 h) and more than 16% (48 h) inhibition of bacterial culture growth was detected. For the other DDT concentrations (1, 0.1, and 0.01 mg/L) after 24 h and 48 h of incubation of *E. homaechei* culture, there was a stimulation of culture growth ranging from 0.39% (48 h, 0.01 mg/L) to over 19% (48 h, 1 mg/L). The *Staphylococcus aureus* strain also had lower DDT sensitivity than *E. coli* and *Sarcina spp*. The pesticide induced more than 14% (24 h) and more than 21% (48 h) growth inhibition of bacterial cultures after incubation with *S. aureus* (Fig. 4) cultures at the highest DDT concentration tested

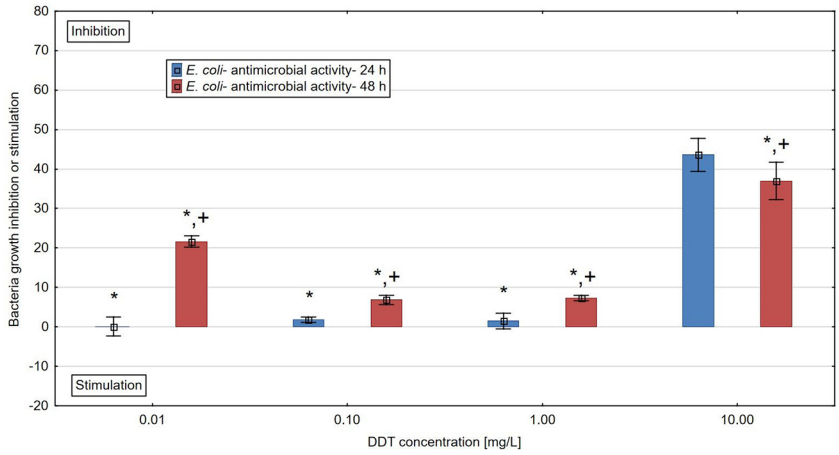


**Figure 2.** The antimicrobial activity of DDT against *E. coli* ATCC-25922.

**Note:** *- statistically significant difference at α= 0.05 between control sample, +- statistically significant difference between 24 and 48 h for a given concentration of DDT. Error bars indicate the standard deviation (SD) of the mean of three independent replicates.

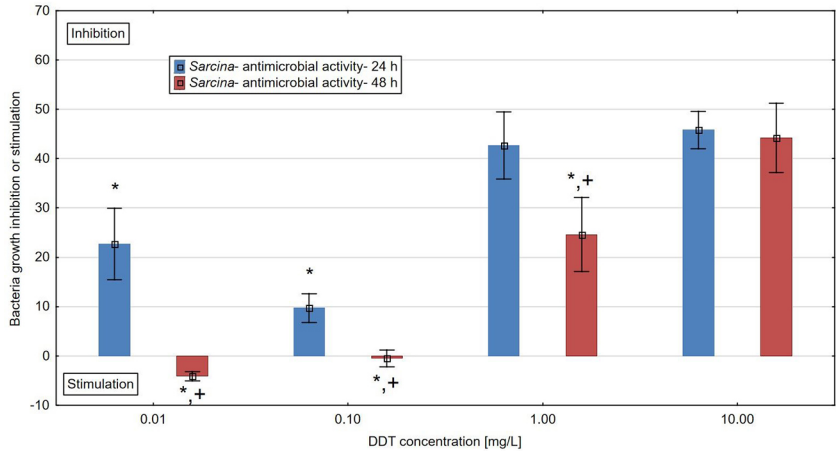


**Figure 3.** The antimicrobial activity of DDT against *Sarcina spp*., ATCC-35659.

**Note:** *- statistically significant difference at α= 0.05 between control sample, +- statistically significant difference between 24 and 48 h for a given concentration of DDT. Error bars indicate the standard deviation (SD) of the mean of three independent replicates.

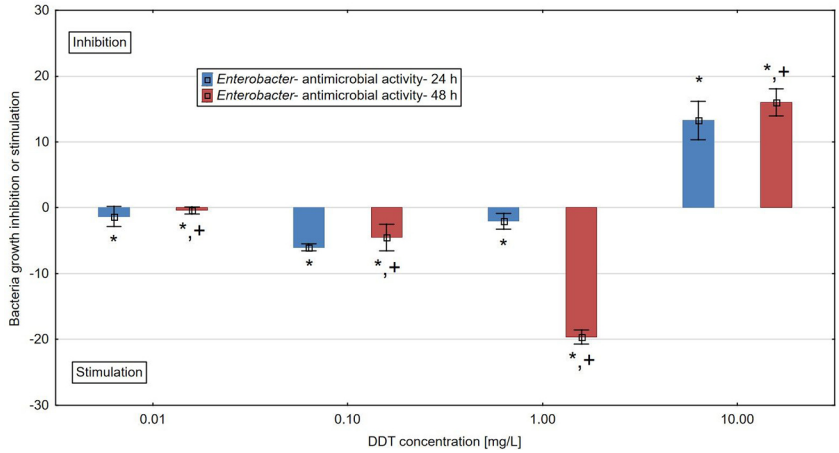


**Figure 4.** The antimicrobial activity of DDT against *E. homaechei* LBM ATCC-700323.

**Note:** *- statistically significant difference at α= 0.05 between control sample, +- statistically significant difference between 24 and 48 h for a given concentration of DDT. Error bars indicate the standard deviation (SD) of the mean of three independent replicates.

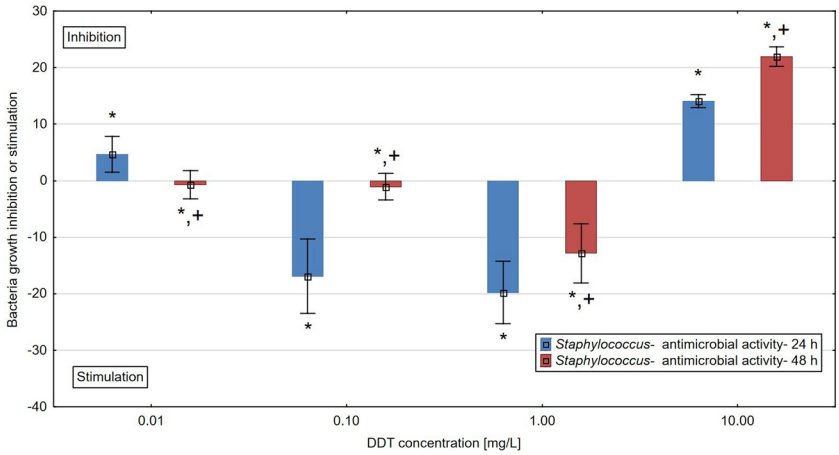


**Figure 5.** The antimicrobial activity of DDT against *S. aureus* ATCC-25923.

**Note:** *- statistically significant difference at α= 0.05 between control sample, +- statistically significant difference between 24 and 48 h for a given concentration of DDT. Error bars indicate the standard deviation (SD) of the mean of three independent replicates.

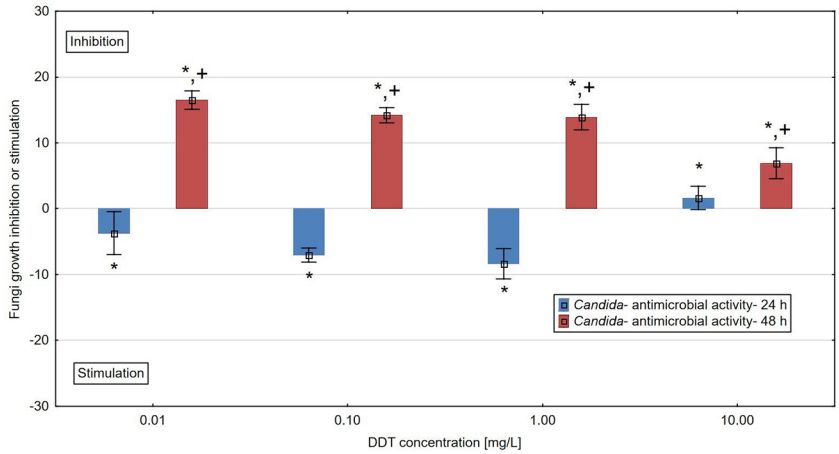


**Figure 6.** The antimicrobial activity of DDT against *C. albicans* ATCC-1023.

**Note:** *- statistically significant difference at α= 0.05 between control sample, +- statistically significant difference between 24 and 48 h for a given concentration of DDT. Error bars indicate the standard deviation (SD) of the mean of three independent replicates.

(10 mg/L). Lower DDT concentrations (1 and 0.1 mg/L) stimulated bacterial culture growth up to more than 19% after 24 h (1 mg/L) and up to more than 12% after 48 h (1 mg/L). At a concentration of 0.01 mg/L DDT and after 24 h, a slight inhibition of *S. aureus* culture growth to over 4% was detected. The study showed that after 24 h of exposure to DDT, *Candida albicans* ATCC-10231 cells appeared to be the least sensitive to the pesticide (Fig. 6). At the concentrations of 1, 0.1, and 0.01 mg/L, DDT induced growth stimulation of *C. albicans* cultures with a peak value of over 8% for 1 mg/L. After 48 h, for *C. albicans,* the most inhibitory culture growth effect up to almost 18% was detected at the concentration of 0.01 mg/L DDT.

## Statistically significant differences in DDT antimicrobial activity

It was observed that, for most of the tested DDT concentrations and considered bacterial or fungal species, statistically significant differences occurred between the control sample (Fig. 2–6). This regularity was present for almost all adopted variants of the experiment. A deviation from the indicated regularity was observed in the following cases: for *Sarcina spp*., ATCC-35659 at a DDT concentration of 1.00 mg/L, where no statistically significant differences were observed between the control sample and the test sample after 24 h, and in the case of a DDT concentration of 10.00 mg/L, for *E. coli*

ATCC-25922 after 24 h of observation to the control sample, and for *C. albicans* ATCC-1023 when observed after 24 and 48 h.

### *E. homaechei* and *C. albicans* cells viability assay

Studies conducted with BacTiter-Glo™ Microbial Cell Viability Assay showed that DDT in the range of all concentrations affected the viability of *E. homaechei* and *C. albicans* cultures (Fig. 7). In the case of *E. homaechei*, DDT stimulated an increase in the number of viable bacterial cells to more than 26% and 20% after 24 h incubation at the concentrations of 1 and 0.1 mg/L, respectively. In contrast, for concentrations of 10 and 0.01 mg/L, bacterial cell viability was reduced to more than 4% for the 10 mg/L concentration and more than 3% for the 0.01 mg/L concentration. In viability assay, comparable to *E. homaechei,* a different sensitivity to DDT was found for *C. albicans*, where 24 h incubation of the fungal culture with the pesticide-induced in all tested DDT concentrations of 10, 1, 0.1 and 0.01 mg/L an inhibition of culture viability ranging from over 9% (1 mg/L) to over 22% (0.01 mg/L).

#### *Statistically significant differences in E. homaechei and C. albicans cells viability assay*

For studies dedicated to analyzing the effect of selected DDT concentrations on inhibition or stimulation of bacterial or fungal viability (Fig. 7), *E. homaechei* LBM ATCC-700323 and *C. albicans* ATCC-1023 were tested as representative microorganisms for the concerned groups. During the research work for *E. homaechei*, viability stimulation was observed for a DDT at concentrations of 0.01 mg/L and 10.00 mg/L. Viability inhibition was observed for concentrations of 0.10 and 1.00 mg/L. In the case of *C. albicans*, viability stimulation was observed for the individual DDT concentrations. In addition, it should be noted that statistically significant differences were observed for all considered samples compared to the control sample, which relates directly to a clear viability stimulation or inhibition of *E. homaechei* and *C. albicans* for the considered DDT concentrations.

### Oxidative stress determination

#### *SodA promoter induction*

Studies on the oxidative stress generation potential of DDT showed that the pesticide, at all concentrations, 10, 1, 0.1, and 0.01 mg/L after 2 and 24 h incubation induced the *sodA* promoter in the *E. coli* SM342 *sodA:luxCDABE* strain (Fig. 8). The strongest effect of DDT on the *sodA* promoter and *luxCDABE* gene expression was detected after 2 h incubation of *E. coli* SM342 *sodA:luxCDABE* cultures with DDT compared to 24 h incubation. The most significant increase in *sodA* promoter induction to almost 190% was detected at a concentration of 1 mg/L DDT and after 2 h of incubation. For the other tested concentrations, *sodA* promoter induction values ranged from more than 118% (0.01 mg/L) to 130% (10 mg/L). After 24 h incubation of *E. coli* SM342 *sodA:luxCDABE* cultures with DDT, a decrease in s*odA* promoter activity and *luxCDABE* gene

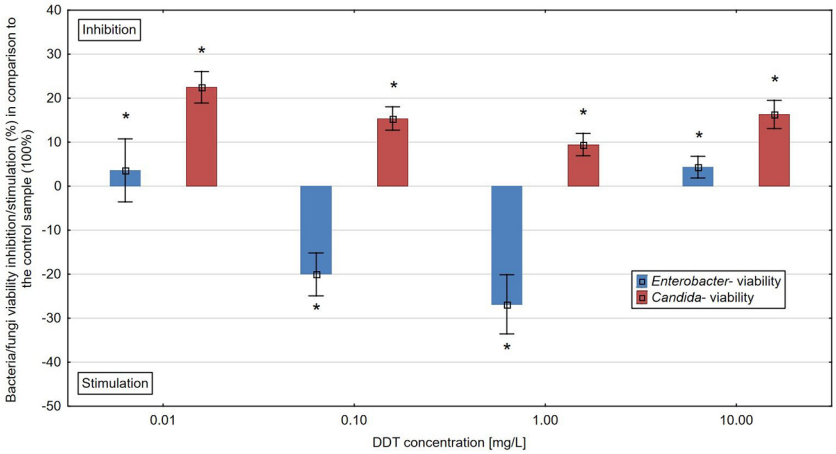


**Figure 7.** The *E. homaechei* LBM ATCC-700323 and *C. albicans* ATCC-1023 vability assay after exposure to DDT

**Note:** *- statistically significant difference at α= 0.05 between control sample. Error bars indicate the standard deviation (SD) of the mean of three independent replicates.

expression was observed compared to 2 h. For an incubation time of 24 h, the highest level of stimulation of the *sodA* promoter, up to 128%, was detected for a concentration of 1 mg/L DDT. In contrast, DDT at the concentration of 10, 0.1, and 0.01 mg/L induced the *sodA* promoter in a range of values from 104% (0.01 mg/L DDT) to 125% (10 mg/L DDT).

### *Statistically significant differences for sodA promoter induction*

For the induction of the *sodA* promoter in *E. coli sodA:luxCDABE* (Fig. 8), statistically significant differences were observed for a DDT dose of 1.00 mg/L. Then, the observed exposure results indicated statistically significant differences observed in the control sample after 2 and 24 h of observation and between 2 and 24 h of exposure. For a DDT concentration of 10.00 mg/L, a difference in the induction value of the *sodA* promoter was observed in the control sample only after 2 h. For DDT concentrations of 0.01 and 0.10 mg/L, no statistically significant differences in *sodA* promoter induction values were observed.

### ROS synthesis

In the range of studied DDT concentration, 10, 1, 0.1, and 0.01 mg/L, a stimulating effect of ROS synthesis was detected in *E. coli* ATCC-25922 cultures exposed to this pesticide (Fig. 9). Concentrations of 10 and 1 mg/L induced a significant increase in the level of ROS generation (up to 136.1% and 119.2%, respectively) in bacteria. The lower DDT doses (0.1 and 0.01 mg/L) had much less effect on ROS synthesis levels. DDT at 0.1 mg/L induced stimulation of ROS synthesis to 110.1%. On the other hand, the lowest of the tested pesticide concentration of 0.01 mg/L slightly (102.7%) affected the values of the ROS parameter.

### *Significant differences in ROS synthesis*

For ROS synthesis in *E. coli* ATCC-25922 (Fig. 9), statistically significant differences were observed between the control sample for DDT concentrations of 1.00 and 10.00 mg/L. However, in the case of DDT concentrations of 0.01 and 0.10 mg/L, no statistically significant differences were observed in ROS values for the control sample.

## DISCUSSION

Our results are consistent with the data from the work of previous authors in which the antimicrobial activity of DDT against bacteria, fungi, and algae was detected (Lal and Saxena, 1982; Liu et al., 2021). In the available literature, most studies are also concerned with the effects of DDT on soil microorganisms. The main ways microorganisms are exposed to DDT in the environment are the application of this pesticide to the soil, the spread of spraying, and infiltration into the soil. In addition to bacteria and fungi, microalgae are present in the soil, which can be an essential component of the soil microflora, making up to

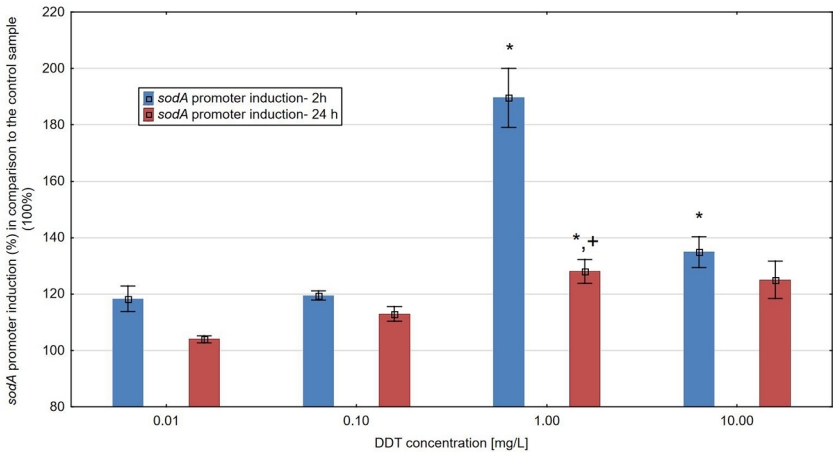


**Figure 8.** The *sodA* promoter induction in *E. coli sodA:luxCDABE* after exposure to DDT.

**Note:** *- statistically significant difference at α= 0.05 between control sample, +- statistically significant difference between 24 and 48 h for a given concentration of DDT. Error bars indicate the standard deviation (SD) of the mean of three independent replicates.

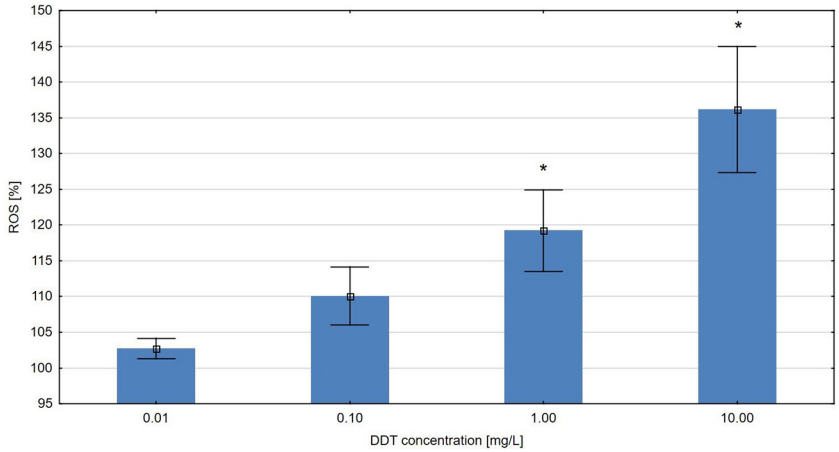


**Figure 9.** ROS synthesis (%) in *E. coli* ATCC-25922 after exposure to DDT

**Note:** *- statistically significant difference at α= 0.05 between control sample. Error bars indicate the standard deviation (SD) of the mean of three independent replicates.

27% of the total microbial biomass in the soil (Megharaj et al., 2000; Mansouri et al., 2017).

The earlier study on the effects of DDT on soil microorganisms was comprehensive, including tests on soil cultures of bacteria, fungi, green algae, and cyanobacteria (Mansouri et al., 2017). The results showed a decrease in the number of bacteria and algae with an increase in DDT dose up to 27 mg of DDT residue per kg of soil. In the case of fungi from the species *Aspergillus flavus* and *Aspergillus parasiticus*, culture growth was stimulated under the influence of DDT at a concentration of 2 ppm. The researchers also observed changes in algae and cyanobacteria species composition in DDT-contaminated soils, with sensitive species being eliminated in soils with moderate and high contamination levels. This indicates that DDT also affected the biodiversity of soil microflora. Additionally, the differential sensitivity of soil microorganisms to DDT was found, a conclusion that aligns with the results obtained in our study and those of others (Mansouri et al., 2017; Russo et al., 2019; Ebsa et al., 2024).

Studies on DDT's cellular mechanism of action have shown that the pesticide affects the function of cell membranes, enzymes, and nucleic acids. Experiments conducted on *E. coli* revealed that the action of DDT caused changes in the proportions of polar groups of major phospholipids and the composition of fatty acids (Lal and Saxena, 1982, Encarnacao et al., 2019, Tasselli et al., 2023). Polar phospholipids are the main building blocks of cell membranes. Studies on *Casearia fasciculata* found that the presence of DDT at a concentration of 425 ppm caused inhibition of [$^3$H]thymidine and [$^3$H]uridine uptake, resulting in an inhibitory effect on DNA and RNA production. This effect on nucleic acid synthesis has been attributed to the interaction of DDT with complex regulatory processes involved in the transport of precursors necessary for nucleic acid production (Lal and Saxena, 1982; Kumar et al., 2018; Encarnacao et al., 2019). Disruption of the proper function of cell membranes and genetic material in the cells of the microorganisms probably underlies the toxicity of DDT, as we demonstrated in the antimicrobial activity and viability assays presented in this work.

Oxidative stress is caused by increased levels of ROS synthesis in living cells. The most significant forms of ROS in oxidative stress are $O_2$, hydrogen peroxide, and hydroxyl radical. ROS are highly reactive molecules that cause oxidative damage to critical cellular biomolecules such as DNA, RNA, proteins, and lipids. To date, the induction of ROS generation and oxidative stress in bacteria exposed to metals, acids, salts, redox compounds, pesticides, antibiotics, disinfectants, and nanoplastics has been demonstrated in the scientific literature (Imlay, 2019; Buchser et al., 2023). The results of this study demonstrated an increase in ROS synthesis across the tested DDT concentrations (10, 1, 0.1, and 0.01 mg/L), suggesting that oxidative stress is a key mechanism of DDT toxicity. Following 2 h of exposure to 1 mg/L DDT, the *E. coli sodA:luxCDABE* strain exhibited the most significant activation of the *sodA* promoter, showing an increase of approximately 190% compared to the control. The

intensity of *sodA* promoter excitation in *E. coli sodA:luxCDABE* is directly proportional to the level of superoxide stress (Melamed et al., 2012; Moraskie et al., 2021; Zhu et al., 2022; Rojas-Villacorta et al., 2022). A previous study by Melamed et al. (2012) showed an increase in *sodA* promoter induction in *E. coli sodA:luxCDABE* in the superoxide stress response of bacterial cells to selected antibiotics.

In recent years, more papers have appeared in the scientific literature reporting the significant potential of ROS generation and oxidative stress in tests on bacteria and animal and human cell lines treated with pesticides (Sazykin and Sazykina, 2023). Unfortunately, there are no studies on the correlation between bacterial exposure to DDT and the potential for oxidative stress generation, so we can only contrast the results obtained in our study with those obtained in studies with other pesticides.

To date it has been shown that atrazine (ATR), a widely used herbicide worldwide, have the ability to induce ROS synthesis and oxidative stress in soil bacterial cells. The conducted experiments proved that after exposure of bacteria to ATR, the increased level of intracellular ROS led to single and double-strand breaks in DNA and a genotoxic effect (Sazykin and Sazykina, 2023). The genotoxic effect of ATR was also observed in the work of Matejczyk et al. (2023) in the *E. coli recA:luxCDABE* strain. Moreover, it has been shown that in *E. coli* K12 and *Bacillus subtilis* B19 strains, atrazine induced an increase in activity of superoxide dismutase (SOD), catalase, glutathione transferase and to increase the amount of glutathione, which led to an enhanced of antioxidant response (Sazykin and Sazykina, 2023).

The potential for oxidative stress induction in *Pseudomonas aeruginosa* and *Pseudomonas fulva* was detected when these bacteria were cultivated in the presence of clomazone and ametrin herbicides. Analysis of oxidative stress parameters, such as the activity of glutathione reductase, glutathione transferase, superoxide dismutase, catalase, and cellular glutathione content showed species-differentiated antioxidant system responses of *P. aeruginosa* and *P. fulva* to the tested herbicides (Peters et al., 2014; Sazykin and Sazykina, 2023).

In our studies we obtained the induction of *sodA* promoter in *E. coli* strain and ROS synthesis, after bacteria exposition to DDT. These results support the hypothesis that DDT in bacterial cells increases ROS and oxidative stress, which induces some disruptions of cellular signal transduction pathways and homeostasis in living cells, leading to cell death. In cellular defense response to elevated levels of ROS, the expression of organized networks of genes encoding HSPs, is switched on. HSPs have a cytoprotective function through their chaperoning activities, ranging from polypeptide folding to conducting repairs and degrading irreparable peptides (Ikwegbue et al., 2018; Szller and Bill-Lula, 2021). ROS in cells induces DNA fragmentation and a consequent genotoxic effect. One of the molecular mechanisms of action of the HSP70 family has been shown to reduce this effect significantly. Moreover, HSPs under conditions of cellular oxidative stress have been shown to support the antioxidant system against the destructive effects of ROS (Wu et al., 2015).

A significant problem in the protection of the environment and water resources is the high durability and resistance to biodegradation of pesticides, including DDT. Pesticides are not completely removed from wastewater and are transported from WWTPs together with treated wastewater to surface waters, which are often drinking water intakes. At the WWTPs, many metabolic processes are engaged in the microbial degradation of pesticides in activated sludge (AS), involving a variety of enzymes encoded by biodegradation genes (BDGs) and pesticide degradation genes (PDGs). In the work of Fang et al., (2018), the authors demonstrated the linkages of bacterial communities and BDGs/PDGs activity in the AS, and the pesticide wastewater characteristics. Pesticides contained in wastewater may affect biodiversity, metabolic activity and the level of gene expression in activated sludge microorganisms equipped with BDGs/PDGs. In the cells of activated sludge microorganisms exposed to DDT the induction of oxidative stress occurred. Therefore, ROS damages DNA (genotoxic effect), RNA, proteins and cellular lipids, resulting in disorders or inhibition of gene expression, lipid peroxidation, degradation of cellular proteins and inactivation of critical cellular enzymes (Brody, 2015; Ikwegbue et al., 2018; Chueycham et al., 2021; Matich et al., 2021; Rizqi et al., 2023). Finally, oxidative stress induced by DDT affects miRNA activity, which can regulate or block the expression of many genes (Ebrahimi et al., 2020; Arfin et al., 2021; Jelic et al., 2021), including biodegradation genes (BDGs) and pesticide degradation

genes (PDGs), which leads to a decrease in the metabolic activity of AS microorganisms and a decrease in the biodegradation efficiency of pesticides in wastewater (Fang et al., 2018; Lescano et al., 2022).

## CONCLUSIONS

In this study, the toxicity of DDT on various microorganisms was evaluated, revealing significant growth inhibitory effects, particularly on *E. coli* and *Sarcina sp.*, with oxidative stress identified as a likely mechanism of DDT toxicity. DDT exposure increased both the induction of the *sodA* promoter activity and ROS synthesis, with the most pronounced effects observed at higher concentrations of DDT. These findings align with global concerns about the environmental and health risks associated with DDT, particularly its potential role in carcinogenesis. The study underscores the urgent need for improved methods of pesticide removal from wastewater and stricter regulatory compliance to mitigate the harmful effects of DDT on the environment and human health. Promising approaches for achieving this include bio-membrane technologies and advanced oxidation processes (AOPs).

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## REFERENCES

1. Ahmed, M. B., Zhou, J. L., Ngo, H. H., Guo, W., Thomaidis, N.S., & Xu J. (2016). Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: A critical review. *Journal of Hazardous Materials*, 12(3), 1864555. https://doi.org/10.1016/j.jhazmat.2016.04.045
2. Arfin, S., Jha, N. K., Kesari, K. K., Ruokolainem, J., Roychoudhury, S., Rathi, B., & Kumar, D., (2021). Oxidative stress in cancer cell metabolism. *Antioxidants (Basel)*, 10, 642. https://doi.org/10.3390/antiox10050642.
3. Brody H., (2015). Colorectal cancer. *Nature*, 14, 521. https://doi.org/10.1038/521S1a.
4. Buchser, R., Sweet, P., Anantharaman, A., & Contreras, L., (2023). RNAs as sensors of oxidative stress in bacteria. *Annual Review of Chemical and Biomolecular Engineering*, 14:265–81. https://doi.org/10.1146/annurev-chembioeng-101121-070250.
5. Cohn, B.A., Wolff, M.S., Cirillo, P.M., & Sholtz, R.I., (2007). DDT and breast cancer in young women: new data on the significance of age at exposure. *Environment Health Perspective*, 115, (4), 1406–1414. https://doi.org/10.1289/ehp.10260.
6. Cybulski, J., Witczak, A., & Pokorska-Niewiada, K., (2021). The effect of water and sewage treatment on reducing residues of selected organochlorine pesticides in Szczecin (Poland). *Water Air and Soil Pollution*, 232: 310. https://doi.org/10.1007/s11270-021-05261-6.
7. Directive, 1998 Council directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption.
8. Dz. U., item 1311 (Journal of laws), 2019. Regulation Minister for Maritime Economy and Inland Navigation of July 12, 2019 on substances that are particularly harmful to the aquatic environment.
9. Dz. U., item 2294 (Journal of laws), 2017. Regulation of the Minister of Health of 7 December 2017 on the quality of water intended for human consumption. http://prawo.sejm. gov.pl/isap.nsf/ DocDetails.xsp?id=WDU2017000 2294
10. Ebrahimi, S. O., Reiisi, S., & Shareef, S., (2020). miRNAs, oxidative stress, and cancer: A comprehensive and updated review. *Journal of Cell Physiology*, 235, 8812-8825. https://doi.org/10.1002/jcp.29724.
11. Ebsa, G., Gizawa, B., Admassie, M., Degu, T., & Alemu, T., (2024). The role and mechanisms of microbes in dichlorodiphenyltrichloroethane (DDT) and its residues bioremediation. *Biotechnology Reports*, 42(6), e00835. https://doi.org/10.1016/j.btre.2024.e00835.
12. Encarnacao, T., Pais, A.A., Campos, M.G., & Burrows, H.D., (2019). Endocrine disrupting chemicals: impact on human health, wildlife and the environment. *Science Progress*, 102, 3–42. https://doi.org/10.1177/0036850419826802.
13. European Union, 2016. Monitoring-based exercise: second review of the priority substances list under the Water Framework Directive. https://circabc.europa.eu/sd/a/7fe29322-946a-4ead-b3b9-e3b156d0c318/monitoring.
14. Fang, H., Zhang, H., Han, L., Mei, J., Ge, Q., Long, Z., & Yu, Y., (2018). Exploring bacterial communities and biodegradation genes in activated

sludge from pesticide wastewater treatment plants via metagenomic analysis. *Environmental Pollution*, 243, 1206e1216. https://doi.org/10.1016/j.envpol.2018.09.080.

15. Harada, T., Takeda, M., Kojima, S., & Tomiyama, N., (2016). Toxicity and carcinogenicity of dichlorodiphenyltrichloroethane (DDT). *Toxicological Research*, 1, 21-33. https://doi.org/10.5487/TR.2016.32.1.021.
16. Ikwegbue, P. C., Masamba, P., Oyinloye, B. E., & Kappo, A. P., (2018). Roles of heat shock proteins in apoptosis, oxidative stress, human inflammatory diseases, and cancer. *Pharmaceuticals*, 11, (4), 1-18. https://doi.org/10.3390/ph11010002.
17. Imlay, J. A., (2019). Where in the world do bacteria experience oxidative stress? *Environmental Microbiology*, 21, 521–530. https://doi.org/10.1111/1462-2920.14445.
18. Jelic, M. D., Mandic, A. D., Maricic, S. M., & Srdjenovic, B. U., (2021). Oxidative stress and its role in cancer. *Journal of Cancer Research and Therapy*, 17, (8), 22-28. https://doi.org/10.4103/jcrt.JCRT_862_16.
19. Jin, X., Song, L., Liu, X., Chen, M., & Li, Z., et al., (2014). Protective efficacy of vitamins C and E on p,p9-DDT-induced cytotoxicity via the ROS mediated mitochondrial pathway and NF-kB/FasL pathway. *PLoS ONE* 9: e113257. https://doi.org/10.1371/ journal.pone.0113257.
20. Kumar, R., & Mukherji, S., (2018). Threat posed by persistent organochlorine pesticides and their mobility in the environment. *Current Organic Chemistry*, 22 (10), 954–972. https://doi.org/10.2174/1385272822666171227150106.
21. Lal, R., & Saxena, D. M., (1982). Accumulation, metabolism, and effects of organochlorine insecticides on microorganisms. *Microbiological Reviews*, 46, (7), 95-127. https://doi.org/10.1128/mr.46.1.95-127.1982.
22. Lescano, M., Fussoni, N., Vidal, E., & Zalazar, C., (2022). Biodegradation of pesticide-contaminated wastewaters from a formulation plant employing a pilot scale biobed. *Science of the Total Environment*, 10, 150758. https://doi.org/10.1016/j.scitotenv.2021.150758.
23. Liu, S., Fang, S., Xiang, Z., Chen, X., Song, Y., Chen, C., & Ouyang, G., (2021). Combined effect of microplastics and DDT on microbial growth: A bacteriological and metabolomics investigation in *Escherichia coli*. *Journal of Hazardous Materials*, 407, (4), 124849. https://doi.org/10.1016/j.jhazmat.2020.124849.
24. Malagon, C. G., Gomez-Aburto, V. A., Hirmas-Olivares, A., Luarte, T., Berrojalbiz, N., & Dachs, J., (2023). Dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) levels in air and surface sea waters along the Antarctic Peninsula. *Marine Pollution Bulletin*, 197, 1156-1169. https://doi.org/10.1016/j.marpolbul.2023.115699
25. Malusá, E., Tartanus, M, Danelski, W., Miszczak, A., Szustakowska, E., Kicińska, J., & Furmanczyk, E. M., (2020). Monitoring of DDT in agricultural soils under organic farming in Poland and the risk of crop contamination. *Environmental Management*, 66, (8), 916–929. https://doi.org/10.1007/s00267-020-01347-9.
26. Mansouri, A., Cregut, M., Abbes, C., Durand, M. J., Landoulsi, A., & Thouand, G., (2017). The environmental issues of DDT pollution and bioremediation: a multidisciplinary review. *Applied Biochemistry and Biotechnology*, 181, 309-339. https://doi.org/10.1007/s12010-016-2214-5.
27. Matejczyk, M., Kondzior, P., Ofman, P., Juszczuk-Kubiak, E., Świsłocka, R., Łaska, G., Wiater, J., & Lewandowski W., (2023). Atrazine toxicity in marine algae *Chlorella vulgaris*, in *E. coli lux* and *gfp* biosensor tests. *Archives of Environmental Protection*, 49, 87-99. https://doi.org/10.24425/aep.2023.147331.
28. Matich, E. K., Laryea, J. A., Seely, K. A., Stahr, S., & Su, L. J., (2021). Association between pesticide exposure and colorectal cancer risk and incidence: a systematic review. *Ecotoxicology and Environmental Safety*, 219: 1123-1127. https://doi.org/10.1016/j.ecoenv.2021.112327
29. Megharaj, M., Kantachote, D., Singleton, I., Naidu, R., (2000). Effects of long-term contamination of DDT on soil microflora with special reference to soil algae and algal transformation of DDT. *Environmental Pollution*, 109, (13), 35-42. https://doi.org/10.1016/s0269-7491(99)00231-6.
30. Moraskie, M., Roshid, H., O'Connor, G., Dikici, E., Zingg, J.-M., Deo, S., & Daunert, S., (2021). Microbial whole-cell biosensors: current applications, challenges, and future perspectives. *Biosensors and Bioelectronics*, 191, 113359. https://doi.org/10.1016/j.bios.2021.113359.
31. Ochoa-Rivero, J. M., Reyes-Fierro, A. V., Peralta--Pérez, M., Zavala-Díaz de la Serna, F. J., Ballinas--Casarrubias, L., Salmerón, I., Rubio-Arias, H., & Rocha-Gutiérrez, B. A., (2017). Levels and distribution of pollutants in the waters of an aquatic ecosystem in northern Mexico. *International Journal of Environmental Reserch and Public Health*, 14, (8), 456. https://doi.org/10.3390/ijerph14050456
32. Peters, L. P., Carvalho, G., Martins, P. F., Dourado, M. N., Vilhena, M. B., Pileggi, M., & Azevedo, R. A., (2014). Differential responses of the antioxidant system of ametryn and clomazone tolerant bacteria. *PLoS One*, 9, e112271. https://doi.org/10.1371/journal.pone.0112271.
33. Regulation (EU) 2017/625 of the European Parliament and of the Council Of 15march 2017 On

official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products.

34. Rizqi, H. D., Purnomo, A. S., & Ulfi, A., (2023). The effect of bacteria addition on DDT biodegradation by BROWN-ROT fungus *Gloeophyllum trabeum*. *Heliyon*, 9, (5), e18216. https://doi.org/10.1016/j.heliyon.2023.e18216.
35. Rojas-Villacorta, W., Rojas-Flores, S., De La Cruz-Noriega, M., Chinchay Espino, H., Diaz, F., & Gallozzo Cardenas, M., (2022). Microbial biosensors for wastewater monitoring: mini-review. *Processes*, 10, 2-13. https://doi.org/10.3390/pr10102002.
36. Russo, F., Ceci, A., Pinzari, F., Siciliano, A., Guida, M., Malusà, E., Tartanus, M., Miszczak, A., Maggi, O., & Persiani, A. M., (2019). Bioremediation of dichlorodiphenyltrichloroethane (DDT)-contaminated agricultural soils: potential of two autochthonous saprotrophic fungal strains. *Applied Environmental Microbiology*, 21, e01720-19. https://doi.org/10.1128/AEM.01720-19.
37. Sazykin, I. S., & Sazykina, M. A., (2023). The role of oxidative stress in genome destabilization and adaptive evolution of bacteria. *Gene* 857, (9), 147170. https://doi.org/10.1016/j.gene.2023.147170.
38. Szller, J., & Bill-Lula, I., (2021). Heat shock proteins in oxidative stress and ischemia/reperfusion injury and benefits from physical exercises: A review to the current knowledge. *Oxidative medicine and Cellular Longevity*, 31, 6678457. https://doi.org/10.1155/2021/6678457.
39. Tao, S., Li, B. G., He, X. C., Liu, W. X., & Shi, Z., (2007). Spatial and temporal variations and possible sources of dichlorodiphenyltrichloroethane (DDT) and its metabolites in rivers in Tianjin, China. *Chemosphere*, 68, 10–16. https://doi.org/10.1016/j.chemosphere.2006.12.082.
40. Tasselli, S., Marziali, L., Roscioli, C., & Guzzella, L., (2023). Legacy dichlorodiphenyltrichloroethane (DDT) pollution in a river ecosystem: sediment contamination and bioaccumulation in benthic invertebrates. *Sustainability*, 15, (6), 6493. https://doi.org/10.3390/su15086493.
41. Toxicological Profile for DDT, DDE, and DDD. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US); 2022. https://www.atsdr.cdc.gov/toxprofiles/tp35.pdf.
42. World Health Organization. 2014a. WHO Recommended Insecticides for Indoor Residual Spraying against Malaria Vectors. Updated Nov. 17th, 2014.
43. World Health Organization. 2014b. World Malaria Report. Geneva, Switzerland. http://www.who.int/malaria/publications/world_malaria_report_2014/en/.
44. World Health Organization. 2015. World Malaria Report. Geneva, Switzerland. http://www.who.int/malaria/publications/world-malaria-report-2015/report/en/.
45. Wu, C.-W.; Biggar, K. K.; Zhang, J.; Tessier, S. N.; Pifferi, F.; Perret, M.; & Storey, K. B., (2015). Induction of antioxidant and heat shock protein responses during torpor in the gray mouse lemur, *Microcebus murinus*. *Genomic and Proteomic Bioinformatics*, 13, 119–126. https://doi.org/10.1016/j.gpb.2015.03.004.
46. Zhu, Y., Elcin, E., Jiang, M., Li B., Wang, H., Zhang, X., & Wang, Z., (2022). Use of whole-cell bioreporters to assess bioavailability of contaminants in aquatic systems. *Frontiers in Chemistry*, 10, (3), 1018124. https://doi.org/10.3389/fchem.2022.1018124.