Archives of Environmental Protection
Vol. 49 no. 3 pp. 87–99

PL ISSN 2083-4772
DOI 10.24425/aep.2023.147331

Atrazine toxicity in marine algae *Chlorella vulgaris*, in *E. coli lux* and *gfp* biosensor tests

Marzena Matejczyk[1]*, Paweł Kondzior[1], Piotr Ofman[2], Edyta Juszczuk-Kubiak[5], Renata Świsłocka[1], Grażyna Łaska[4], Józefa Wiater[3], Włodzimierz Lewandowski[1]

[1]Bialystok University of Technology, Faculty of Civil Engineering and Environmental Sciences, Department of Chemistry, Biology and Biotechnology, Bialystok, Poland
[2]Bialystok University of Technology, Department of Environmental Engineering Technology, Bialystok, Poland
[3]Bialystok University of Technology, Department of Agricultural and Food Engineering and Environmental Management, Bialystok, Poland
[4]Department of Agri-Food Engineering and Environmental Management, Bialystok University of Technology, Bialystok, Poland
[5]Institute of Agricultural and Food Biotechnology-State Research Institute, Laboratory of Biotechnology and Molecular Engineering, Warsaw, Poland

*Corresponding author's e-mail: m.matejczyk@pb.edu.pl

Keywords: Ecotoxicology, atrazine, *Chlorella vulgaris*, bioluminescent biosensors, water contamination

Abstract: Atrazine (ATR) is a widely used chlorinated herbicide from the s-triazine group. Due to the widespread use of ATR, it leaks into the environment and is detected in drinking water, exceeding the WHO-acceptable concentration of atrazine in drinking water, which is 2 µg/L. The aim of our study was to determine toxicity, protein degradation and genotoxicity of ATR at concentrations of 10; 1; 0.1; 0.01 mg/L on *Chlorella vulgaris* and with the application of *E. coli* bioluminescent biosensor strains. We measured the content of chlorophyll *a*, *b*, carotenoids in *Chlorella vulgaris* and the inhibition of this algae culture growth. *E. coli* RFM443 strains with gene constructs *grpE:luxCDABE*, *lac:luxCDABE*, *recA:luxCDABE* and *E. coli* strain MM294 *trc:luxCDABE* were used to determine toxicity, degradation of cellular proteins and genotoxicity. On the base of the obtained results, we concluded that ATR in the tested concentrations shows a toxic effect in relation to *Chlorella vulgaris*. ATR is toxic and genotoxic in *E. coli* RFM443 strains with *grpE*, *lac*, *recA* promoters and causes degradation of cellular proteins. Moreover, we have detected ATR toxicity toward the GFP protein in *E. coli* strain MM294-GFP. Taking into account the toxicity and genotoxicity of ATR documented in our research and in the experiments of other authors, we conclude that the presence of this herbicide in surface waters and drinking water is a serious threat to living organisms.

Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine, ATR) due to high efficiency, low cost, and application flexibility is one of the most widely used pesticide that is sold in more than 100 countries worldwide with annual consumption of about 70,000–90,000 tons. ATR is a chlorinated herbicide from the s-triazine group that blocks photosynthesis and is used to protect many crops, including corn, sorghum, tea, sugarcane, and various fruit crops. ATR is a carcinogen that can disrupt the endocrine system of frogs at concentrations detected in the environment (Kamaz et al. 2020, Fareed et al. 2021, Hu et al. 2021). It exerts mutagenic, genotoxic, defective cell division, erroneous lipid synthesis and hormonal imbalance in fish, amphibians, and aquatic reptiles (Sun et al. 2020). To date, it has been documented that human exposure to ATR can result in the development of cancers, such as lung, breast, pancreatic, prostate, and ovarian cancer, as well as leukemia (Chen et al. 2021). Atrazine is detected in various agri-food products, such as corn, apples and animal-based products, e.g., meat, milk and eggs. ATR neurotoxic potential in the dopaminergic system, glutamatergic neurons and astrocytes in the human neural differentiation model based on human embryonic stem cells (hESC) was demonstrated (Shan et al. 2021). Research conducted in recent years has also presented that atrazine can pass through the placental barrier and the blood-brain barrier. So far, atrazine has been detected in living organisms, including humans, in the brain, placenta, milk and breast milk, urine, blood, and hair (Shan et al. 2021).

The annual high level of ATR agricultural use leads to the wide introduction of this herbicide into the environment. ATR is very stable and resistant to biodegradation in environmental matrices (Kamaz et al. 2020, Sun et al. 2020, Zhao et al. 2023). Atrazine has a prolonged half-life in soil from 4 to

even 57 weeks, while in water it is about 100 days (Sun et al. 2020). Because of the long half-life and runoff eluviation, ATR is washed by rain to deeper layers of the soil and has a longer duration in the surface and underground water (Shan et al. 2021). Surface and ground waters are mainly used for drinking and irrigation purposes (Fareed et al. 2021). In wastewater treatment plant effluents in the US, concentrations of ATR ranging from 49 to 870 ng/L have been detected (Kamaz et al. 2020). In several countries, such as China, the USA and Brazil the detected concentrations of atrazine in surface and ground waters ranged up to 0.992 mg/L (Sun et al. 2020). The highest recorded concentrations of atrazine in surface waters in the United States were as high as 691 µg/L (Qian et al. 2008). Whereas, in tap water, a high level of up to 3.04 µg/L of ATR and its metabolites was found in Northeastern China (Shan et al. 2021). This is a concentration exceeding the WHO-acceptable concentration of atrazine in drinking water, which is 2 µg/L (Shan et al. 2021). Atrazine dissolved in water sources is harmful to aquatic organisms and human health (Sun et al. 2020, Fareed et al. 2021).

Algae are one of the main components of marine phytoplankton, which are often used as bioindicators of stability of aquatic ecosystems (Su et al. 2022). Algae are among the aquatic organisms most susceptible to atrazine pollution in water (Qian et al. 2008). *Chlorella vulgaris* is one of the most frequently used model organisms in ecotoxicological studies (Su et al. 2022). Therefore, in this study, we selected *Chlorella vulgaris* to investigate the toxic effect of atrazine. The toxicity of atrazine was determined based on its effect on growth and the content of photosynthetic pigments such as chlorophyll *a*, *b* and carotenoids. Autotrophic organisms, thanks to the process of photosynthesis, synthesize organic compounds (saccharides) from simple inorganic compounds (water and carbon dioxide) using a system of photosynthetic pigments that allow energy storage in the form of chemical bonds (Kopcewicz et al. 2020). This is an important process that allows algae to grow. The photosynthetic system consists of two systems, photosystem I (PSI) and photosystem II (PSII), which are sensitive to stress factors. PSII is often used in assessing the toxicity of the test compound on microalgae (Węgrzyn and Mazur 2020, Lu et al. 2023).

Due to the constantly increasing number of scientific reports confirming the harmful effects of ATR on living organisms the aim of our research was to estimate toxicity, protein degradation and genotoxicity of ATR at concentrations of 10; 1; 0.1; 0.01 mg/L on *Chlorella vulgaris* and with the application of *E. coli* bioluminescent biosensor strains. Both model organisms used in the research occur in their respective environmental matrices, such as surface waters and wastewaters. Determining the toxicity and genotoxicity of ATR against *Chlorella vulgaris* and *E. coli* will allow the assessment of a potential impact of ATR on environmental organisms. In the wastewater treatment plants (WWTPs), ATR may have a toxic effect on activated sludge microorganisms and disturb its proper functioning, which may lead to the reduction of the efficiency of biodegradation of pollutants in wastewaters.

ATR toxicity in *Chlorella vulgaris* was determined based on the content of chlorophyll *a*, *b*, carotenoids and the inhibition of this algae culture growth. In the present work three different recombinant bioluminescent *E. coli* strains containing the promoters *recA* (DNA damage), *grpE* (protein damage) and *lac* (constitutive expression) fused to the bacterial operon *luxCDABE* from *P. luminescens* were used to describe the mechanism of toxicity of ATR. Moreover, for toxicity estimation, we applied *E. coli* MM294 strain with *gfp* (green fluorescent protein) reporter gene under the control of a strong *trc* promoter (constitutive expression). For the last quarter of a century, microbial biosensors containing *gfp* and *lux* reporter genes have been used in the study of the toxicity of metals in polluted soils, polychlorinated biphenyls (PCBs) in wastewater, organic pollutants in treated wastewater, chromium, arsenic, lead, pesticides, and petroleum hydrocarbons in waters (Moraskie et al. 2021, Zhu et al. 2022). Bacterial biosensors with the *lux* gene were also used for the detection and monitoring of toxic compounds in waters, including drinking water (Woutersen et al. 2011). *E. coli* RFM443 *grpE:luxCDABE* and *E. coli* RFM443 *lac:luxCDABE* strains were successfully applied for soil toxicity assessment (Bae et al. 2020). So far, microorganisms, including *E. coli* strains containing the *recA:luxCDABE* plasmid gene construct, have been used to determine the genotoxicity of many chemical compounds. These compounds include antibiotics (Melamed et al. 2012), phenol, chromium (Cr^{6+}), lead (Pb^{2+}) and the potential effects of UV radiation exposure on bacterial cells (Song et al. 2014, Jiang et al. 2017). In environmental monitoring, *E. coli* strains *recA:luxCDABE* were used to determine the genotoxicity of crude oil in surface water samples and the genotoxicity of municipal wastewater (Zhu et al. 2022, Rojas-Villacorta et al. 2022). In the work of Zappi et al. (2021), two microbial *E. coli* biosensors containing the *gfp* and *lux* genes were used to determine the toxic effects of nitrification inhibitors (allylthiourea, phenol, and copper) in industrial and municipal wastewater. *Gfp* bacterial biosensors were involved in the cytotoxicity detection of 17β-estradiol and aromatic hydrocarbons in wastewater as well as formaldehyde and heavy metals in surface waters (Ali et al. 2021, Moraskie et al. 2021, Zhu et al. 2022, Rojas-Villacorta et al. 2022).

Materials and methods

Chemicals preparation

ATR was purchased from Sigma-Aldrich. For sample preparation ATR was dissolved in dimethyl sulfoxide (DMSO). To reduce the impact of DMSO on bacterial cells, atrazine solutions were diluted in 0.86% NaCl in a ratio of 1:9 (100 µL DMSO and 900 µL 0.86% NaCl). ATR was added to the *E. coli* cultures for a final concentration of 0.01; 0.1; 1 and 10 mg/L. According to the literature, ATR in the environmental matrices is detected even at concentration of mg/L (Sun et al. 2020, Shan et al. 2021). In our research, the range of atrazine concentrations tested included the herbicide concentration detected in wastewaters, surface and ground waters (0.1 and 0.01 mg/L) and higher concentrations. Such a range of tested ATR concentrations allowed for the validation of the bioluminescent biosensors used in the work for both higher and lower herbicide concentrations.

Chlorella vulgaris used in the study

Algae of the species *Chlorella vulgaris* BA02, obtained from the Culture Collection of Baltic Algae (CCBA) at the Institute of Oceanography, Faculty of Oceanography and Geography,

University of Gdańsk in Gdynia, were used in the study. Fig. 1 shows a photo of the *Chlorella vulgaris* BA02 algae taken under the Olympus BX61 microscope.

E. coli strains used in the study

For investigation of toxic and protein damage potency, we applied *E. coli* RFM443 strains with a transcriptional fusion of *grpE* and *lac* promoters with *luxCDABE* reporter gene and *E. coli* MM294-GFP strain with *trc* promoter fused to *gfp* gene. In previous work, the authors reported the usefulness of *E. coli* strains with plasmid constructs with reporter genes *luxCDABE* and *gfp* in toxicity studies (Bae et al. 2020, Rojas-Villacorta et al. 2022). For genotoxicity measurement, we used *E. coli* RFM443 *recA:luxCDABE* with *recA* promoter. *E. coli* strains with plasmid genetic constructs with the *recA* – genotoxin sensitive promoter are commonly used for genotoxicity analysis of chemicals (Melamed et al. 2012, Zhu et al. 2022, Rojas-Villacorta et al. 2022). The *E. coli* strains used in the research come from the laboratory collection of microorganisms of Prof. Shimshon Belkin, Hebrew University of Jerusalem, Israel and Prof. Imrich Barák, Slovak Academy of Sciences, Bratislava. *E. coli* strains used in this study are presented in Table 1.

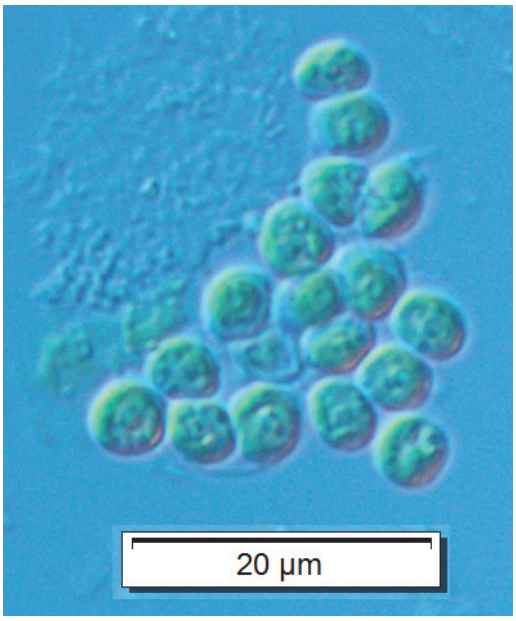

Fig. 1. *Chlorella vulgaris* BA02 (photo taken by the authors) (400×)

ATR toxicity determination in Chlorella vulgaris

Research stands and the culture medium

ATCC (American Type Culture Collection) Medium 616 – BG 11 medium (Blue Green Algae) was selected as the culture medium. Medium in one liter of distilled water contains 1.5 g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4 \cdot 7H_2O$, 0.036 g $CaCl_2 \cdot 2H_2O$, 6.0 mg citric acid, 6.0 mg ferric ammonium citrate, 1.0 mg EDTA, 0.02 g Na_2CO_3, 1.0 mL Trace Metal Mix A5 (which consists of: 2.86 g H_3BO_3, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.222 g $ZnSO_4 \cdot 7H_2O$, 0.039 g $Na_2MoO_4 \cdot 2H_2O$, 0.079 g $CuSO_4 \cdot 5H_2O$, 49.4 mg $Co(NO_3)_2 \cdot 6H_2O$, per 1000 mL distilled Water) (source: https://www.atcc.org/, access date January 20, 2023). Algae culture was carried out in bioreactors, in which the volume of the culture medium was 100 mL. The algae count in the substrate was adjusted to the same optical density of $OD_{680} \approx 0.375$ which corresponds to the initial cell concentration of $2.5 \cdot 10^6$ cell/mL. Atrazine was then added to the medium at the appropriate concentration. The control sample contained the test algae culture suspended in DMSO (dimethyl sulfoxide) solvent in the appropriate proportions in which the atrazine solutions had been prepared, but no atrazine. The medium was aerated with atmospheric air which passed through a Midisart 2000, pore size 0.45 µm from Sartorius. The bioreactors were shaken on a PSU-10i shaker from Biosan. During the experiments, algae were illuminated by LED (light-emitting diode) panels with warm white light with an intensity of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 10 hours, followed by a period of 14 hours without lighting. The algae were incubated at room temperature (25°C).

Measurement methods

The pigment contents of chlorophyll *a*, *b* and carotenoids were measured spectrophotometrically using a PerkinElmer Lambda Bio+ spectrophotometer on the base of the method described by Xiong et al. (2016). For this purpose, a sample of 2 mL of the algae culture was collected and centrifuged at 4500 rpm for 10 minutes. The supernatant was removed, and 2 mL of 90% methanol was added to the centrifuged mass of algae. Then the tubes were secured with a stopper, and the contents of the sample were mixed and placed in a thermoblock at 60ºC for 10 minutes. After this time, the sample was centrifuged again for 10 minutes at 4500 rpm, and the obtained supernatant was subjected to spectrophotometric analysis at wavelengths of 470, 652, and 665 nm. The content of pigments was calculated according to formulas 1–3 (Xiong et al. 2016):

Table 1. *E. coli* biosensor strains used in this study

Strain	Gene promoter acting as sensing element	Reporter gene	Type of stress sensed	Reference
E. coli RFM443	*GrpE*	*Lux*	Protein damage and general toxicity	Bae et al., 2020
E. coli RFM443	*LacZ*	*Lux*	General toxicity	Bae et al., 2020
E. coli RFM443	*RecA*	*Lux*	Genotoxicity	Melamed et al., 2012, Matejczyk et al., 2020a,2020b,2020c
E. coli MM294	*trc*	*Gfp*	General toxicity	Baumstark-Khan et al., 1999, 2007, Rabbow et al., 2002, Matejczyk et al., 2022

$$C_a = 16.82A_{665} - 9.28A_{652}\ [\frac{mg}{L}] \quad (1)$$

$$C_b = 36.92A_{652} - 16.54A_{665}\ [\frac{mg}{L}] \quad (2)$$

$$C_k = \frac{(1000A_{470} - 1.91C_a - 95.15C_b)}{225}\ [\frac{mg}{L}] \quad (3)$$

where: C_a – chlorophyll *a* content, C_b – chlorophyll *b* content, C_k – carotenoids content, A_n – measured absorbance at wavelength *n*.

Optical density was measured at a wavelength of 680 nm. The contents of photosynthetic pigments and optical density were determined twice in three independent experiments. The inhibition values of photosynthetic pigments and optical density were calculated based on the following formula 4:

$$I_n = -\left(100 - \frac{A_p}{B_k} \times 100\right)\ [\%] \quad (4)$$

where: I_n – the inhibition values of photosynthetic pigments or optical density; A_p – content of the tested parameter in the sample with atrazine; B_k – content of the tested parameter in the control sample.

Toxicity and protein damage estimation with bioluminescent E. coli strains

Two bacterial biosensor strains *Escherichia coli* RFM443 with plasmid transcriptional fusion of *grpE* and *lac* promoters with *luxCDABE* gene were used for ATR toxicity measurement. *GrpE* is an inducible promoter that is sensitive to general toxicity and protein damage (Bae et al. 2020). *GrpE* gene codes a protein that is important for the regulation of protein folding machinery, as well as the heat shock response. The second plasmid in *E. coli* RFM443 *lac:luxCDABE* generates luminescence constitutively. In this strain when the cells are exposed to toxic chemicals or stresses the luminescence decreases. The level of luminescence inhibition is proportional to the toxic effect of ATR. In these biosensor strains the action of the enzyme luciferase leads to bioluminescence production (Bae et al. 2020). Bacteria strains were cultured overnight in Luria Bertani (LB) medium with 100 µg/mL of ampicillin at 37°C. After thatthe bacteria cultures were diluted in fresh LB and regrown at 37^{0}C with shaking (150 rpm) to the early log phase (OD_{600}≈0.2). The measurements were carried out in 96-well white plates with an optical bottom (Grainer Bio One, Germany). To each well of the plate, we added 100 µl of appropriate concentrations of ATR and 100 µl of *E. coli* RFM443 *grpE:luxCDABE* and *E. coli* RFM443 *lac:luxCDABE* cultures (OD_{600}≈0,2). The plates were incubated at 37°C for 2 and 24 hours. Luminescence was measured using a GloMax® microplate reader (Promega) at time zero, after 2 and 24 h of incubation. The luminescence values were normalized to the bacterial concentration measured spectrophotometrically as the OD value at 600 nm (OD_{600}). For each sample the luminescence intensity was calculated according to the formula: $L = IL_A/OD_{600}$, where: L – luminescence, IL_A – luminescence intensity read from the apparatus, OD_{600} – optical density of the sample. *GrpE* and *lac* promoter responses in tested samples are presented as the percentage (%) of promoters' induction or inhibition, compared to the control. The experiment was carried out in three independent series.

ATR toxicity analysis with gfp gene as reporter

ATR toxicity was also determined using the *E. coli* strain MM294 with the *gfp* reporter gene. This strain contains the plasmid gene construct pKK233-2 in which the *gfp* gene is under the control of the strong *trc* promoter. According to this method, the determination of the toxic effect was based on the quantification of the fluorescence intensity of the GFP protein in the *E. coli* MM294-GFP culture containing the plasmid gene construct with the *gfp* gene, after its 2- and 24-hour incubation with ATR. If the bacterial cells are exposed to a toxic chemical, there is a decrease in fluorescence intensity, which is the result of the toxic effect on the GFP protein. The *E. coli* MM294-GFP culture was incubated in Luria-Bertani (LB) broth with ampicillin at a concentration of 100 µg/mL at 37°C for 24 hours. After that, the bacterial culture was diluted in LB broth with ampicillin (100 µg/mL) and incubated at 37°C with shaking (150 rpm) to the early logarithmic phase (OD≈0,2). Black 96-well plates for fluorescence measurement were used for the study. To each plate well, 100 µl of the appropriate atrazine solutions was added to 100 µL of cultures of *E. coli* MM294-GFP. Samples were incubated at 37°C for 2 and 24 hours. A control sample, an *E. coli* MM294 culture without chemical compounds, was carried out in parallel and under the same conditions. The fluorescence intensity of the GFP protein in *E. coli* MM294 cultures incubated with ATR and in a control culture was measured at an excitation wavelength of 405 nm and an emission wavelength of 505 nm using a GloMax®. To standardize the fluorescence of the *E. coli* MM294-GFP culture response, the optical density (OD) value of bacterial cultures was determined spectrophotometrically at a wavelength of 600 nm. Fluorescence values for each sample were calculated according to the formula: $F=FA/OD_{600}$, where: F – fluorescence, FA – fluorescence intensity read from the apparatus, OD_{600} – optical density of the sample. Fluorescence inhibition (FI) values after 2 and 24 hours of incubation of *E. coli* MM294 with ATR were calculated according to the formula: FI (%) = FCS (%) – FTS (%), where: FCS (%) – fluorescence of the control sample = 100%; FTS (%) – fluorescence of the tested sample (%). The experiment was conducted in three independent series.

ATR genotoxicity determination

Genotoxicity was determined using *E. coli* strain RFM443 with a plasmid gene construct containing the *recA* promoter fused to the *luxCDABE* reporter gene (Melamed et al. 2012). The *recA* promoter is a DNA damage-inducible promoter of the bacterial SOS response regulon. It is involved in several different DNA repair mechanisms, including the elimination of daughter-strand DNA gaps, double helix breaks, as well as a replication error tolerance mechanism called SOS mutagenesis. The transcriptional fusion of the *recA* promoter with the *luxCDABE* reporter gene has the features of an effective genotoxicity biosensor (Melamed et al. 2012, Zappi et al. 2021, Zhu et al. 2022, Rojas-Villacorta et al. 2022).

Biosensors with the *luxCDABE* reporter gene rely on the production of luminescence by the action of the luciferase enzyme (Melamed et al. 2012). The genotoxic effect was determined based on the quantification of the luminescence signal intensity in the *E. coli* RFM443 culture containing the plasmid gene construct with the *luxCDABE* gene, after its 2- and 24-hour incubation with ATR, compared to a control culture not treated with a chemical. The luminescent signal is proportional to the level of *recA* promoter induction and genotoxic potency of ATR. *E. coli* RFM443 *recA:luxCDABE* culture was incubated for 24 h at 37°C with ampicillin at a concentration of 100 µg/mL. Nextly, the culture was diluted in fresh LB broth with ampicillin at a concentration of 100 µg/mL and incubated at 37°C with shaking (150 rpm) to the early logarithmic growth phase (OD_{600}≈0,2). In a 96-well white plate, to 100 µl of *E. coli recA:luxCDABE* culture we added 100 µL of the appropriate concentration of ATR. Samples were incubated for 2 and 24 hours at 37°C. The luminescence intensity measurement was performed using a GloMax® plate reader after 2 and 24 hours of incubation of the bacterial culture with the test chemical. To standardize the luminescent culture response of *E. coli recA:luxCDABE* for all samples, the optical density (OD) value was determined spectrophotometrically at a wavelength of 600 nm. The control sample, the culture of *E. coli* RFM443 *recA:luxCDABE*, did not contain any chemical compounds. The luminescence intensity for each sample was calculated according to the formula: $L = IL_A/OD_{600}$, where: L – luminescence, IL_A – luminescence intensity read from the apparatus, OD_{600} – optical density of the sample. *RecA* promoter induction values after 2 and 24 hours of *E. coli recA:luxCDABE* culture incubation with test compounds are presented as the percentage of promoter' induction, compared to the control. The experiment was carried out in three independent replications.

Potential limitations or challenges faced during the experimental procedures

Organisation for Economic Co-operation and Development (OECD) recommends that the initial concentration of algae in toxicity tests be from $2 \cdot 10^3$ to $5 \cdot 10^5$ cell/mL and the intensity of lighting should be in the range of 60–120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (OECD 2011). However, it is estimated that higher initial concentrations of cells may provide more information about the mechanisms of action of toxic substances on microalgae (Sun et al. 2020). The use of other than recommended light intensities in algae cultures contributes to different effects of the tested compounds (Camuel et al. 2017). For algae of the *Chlorella vulgaris* species, the optimal growth conditions are in the range of light intensity from 232 to 465 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Dębowski 2018). The research using microbial biosensors was carried out in such a way as to avoid the influence of the limitations of the method used on the results obtained. Firstly, microbial biosensor strains were cultured in LB broth and algae in BG 11 medium, which contained the necessary nutrients to ensure the right growth rate of algae and bacteria and to sustain the bioluminescent and fluorescent signal in the bacterial cells. Secondly, readings of the results obtained were conducted after 2 and 4 days of algal culture and after 2 and 24 hours of bacterial culture. In order to prevent the inhibitory effect of metabolic products accumulated in the medium and to avoid loss of nutrients, cultures were not carried out for more than it was planned in the experiments (Melamed et al. 2012).

Statistical analysis

The individual variables adopted for statistical analysis were characterised by a normal distribution according to the Shapiro-Wilk test and heterogeneity of variance according to the Bartlett test. The Tukey test for equal samples was used to compare the statistical significance of the individual measurement results. The level of statistical significance was taken as $\alpha = 0.05$. Statistical calculations were carried out using Statistica 13.1 software running on a Windows 11 platform. The statistical analysis examined the difference in the values of the parameter's chlorophyll *a*, *b*, carotenoids content and algae growth tested in relation to the control sample and between days 2 and 4 of the research work. The second stage of statistical analysis considered the effects of the tested doses of atrazine on the induction or inhibition of the *grpE* promoter in *E. coli* strain RFM443 *grpE:luxCDABE*, the *lac* promoter in *E. coli* RFM443 *lac:luxCDABE*, induction of the *recA* promoter in *E. coli* strain RFM443 *recA:luxCDABE* and inhibition of *GFP* protein fluorescence in *E. coli* strain MM294-GFP compared to the control sample after exposure times of 2 and 24 hours.

Results

ATR toxicity determination in Chlorella vulgaris

Inhibition of chlorophyll a content

Toxicity on *Chlorella vulgaris* algae was determined based on a decrease in the content of chlorophyll *a*, *b*, carotenoids, and inhibition of the growth of algae cultures. After two days of incubation of the algae culture with ATR and after 4 days, inhibition of chlorophyll *a* content was observed (Fig. 2). Our studies have shown that the content of chlorophyll *a* in algae cells decreases with increasing concentration of atrazine in the culture medium. After two days of exposure of algae to this herbicide, the highest level of inhibition of chlorophyll a content was observed at the highest tested concentration of ATR 10 mg/L and it was over 57% in comparison to the control sample. A subsequent 48-hour ATR treatment of algae enhanced the toxic effect of the herbicide on the content of chlorophyll *a*. The highest level of chlorophyll *a* inhibition up to over 70% was observed at the highest concentrations of ATR 10 and 1 mg/L tested.

Inhibition of chlorophyll b

ATR also had a similar toxic effect on the content of chlorophyll *b* in *Chlorella vulgaris*. The highest level of inhibition of chlorophyll *b* content was detected after a 4-day incubation of the algae culture with the tested herbicide concentrations of 10; 1; 0.1 and 0.01 mg/L (Fig. 3). For the tested concentrations, the inhibition of chlorophyll *b* ranged from over 9 to over 79%, with the highest values for the highest ATR concentrations tested.

Inhibition of carotenoids

As in the case of chlorophylls *a* and *b*, the toxic effect of ATR on the content of carotenoids was observed. Both 2- and 4-day exposure of algae to the tested herbicide concentration led

to a significant, even 80% inhibition of the content of these pigments in *Chlorella* cells (Fig. 4). In relation to carotenoids, higher inhibition values of their content were observed after a 4-day incubation of algae compared to a 2-day incubation.

Inhibition of Chlorella vulgaris growth

In addition to the toxic effect of ATR on the content of chlorophyll *a* and *b* pigments and carotenoids, this herbicide also inhibited the growth of *Chlorella vulgaris* cultures at all tested concentrations of 10; 1; 0.1 and 0.01 mg/L, both after 2 and 4 days of exposure (Fig. 5). The highest values of inhibition of the growth of algae cultures, up to more than 60%, at the highest herbicide concentrations of 10 and 1 mg/L were obtained after 4 days of incubation.

Toxicity and protein damage estimation with bioluminescent E. coli strains

The toxicity and protein damage potential of ATR was estimated with the use of *E. coli* RFM443 *grpE:luxCDABE* strain. The obtained results indicated that ATR in all tested concentrations of 10, 1, 0.1 and 0.01 mg/L influenced the *grpE* promoter after 2 and 24 hours of incubation (Fig. 6). A stronger effect of

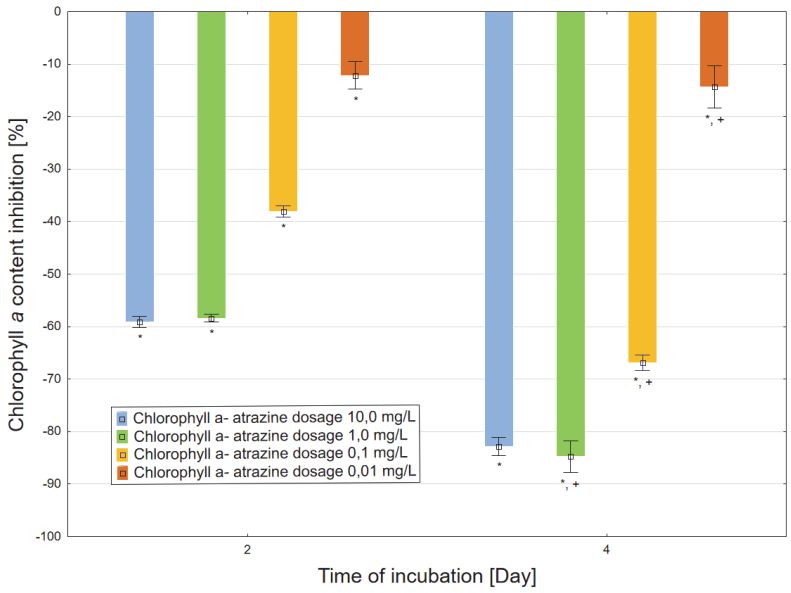

Fig. 2. Effect of studied doses of atrazine on chlorophyll *a* content in *Chlorella vulgaris*, presented as a percent (%) of the inhibition. *- statistically significant difference at α= 0.05 between control sample, + - statistically significant difference at α= 0.05 between sample after 2 days. Error bars indicate the standard error of the mean of three independent replicates.

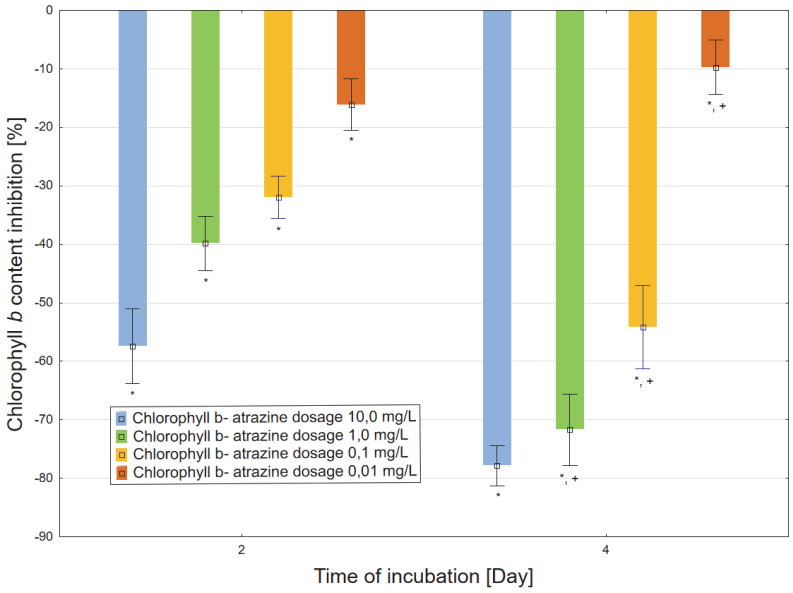

Fig. 3. Effect of studied doses of atrazine on chlorophyll *b* content in *Chlorella vulgaris*, presented as a percent (%) of the inhibition *- statistically significant difference at α= 0.05 between control sample, + - statistically significant difference at α= 0.05 between sample after 2 days. Error bars indicate the standard error of the mean of three independent replicates.

toxicity and protein degradation was observed after 24 hours of incubation of bacteria culture compared to 2 hours of *E. coli* RFM443 *grpE*:*luxCDABE* exposure to ATR. After 24 hours of bacteria culture incubation with ATR at the concentration of 10 mg/L, almost 50% inhibition of the activity of the *grpE* promoter compared to the control was obtained. This was probably due to the global toxic effect of ATR on bacteria, which inhibited the expression of the *luxCDABE* gene from the *grpE* promoter. For the remaining concentrations tested, induction of the *grpE* promoter was observed with the highest level of response of the *grpE:luxCDABE* gene construct in *E. coli* of over 60% at concentrations of 1 and 0.01 mg/L. Based on the obtained results, it was found that the time of exposure of the bacterial culture to ATR was the main factor affecting the sensitivity of the *grpE:luxCDABE* gene construct in *E. coli*.

The toxic effect of ATR was also estimated with the use of *E. coli* RFM443 *lac:luxCDABE* biosensor strain. The decrease in luminescence in *E. coli* RFM443 *lac:luxCDABE* culture is proportional to the toxic potency of tested ATR concentrations of 10, 1, 0.1 and 0.01 mg/L. In all tested concentrations of ATR, its strongest toxic effect was detected after 24 hours of

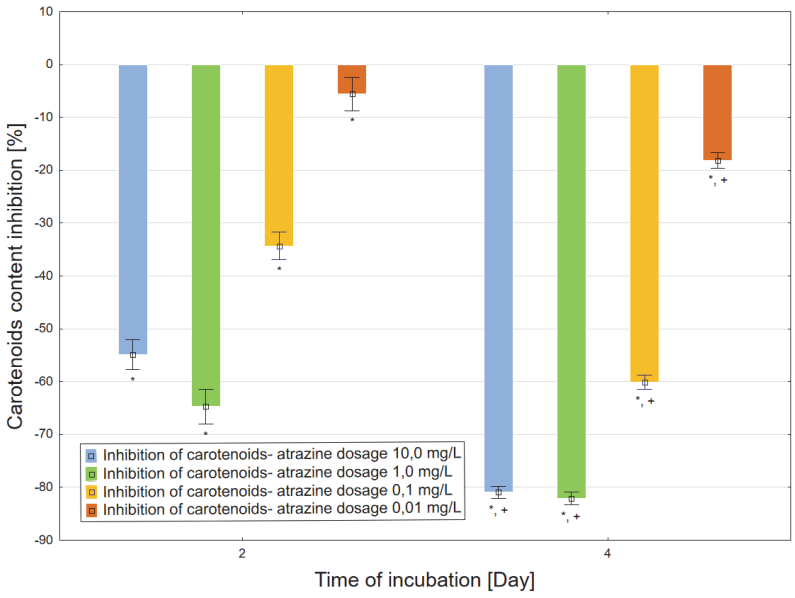

Fig. 4. Effect of studied doses of atrazine on carotenoids content in *Chlorella vulgaris*, presented as a percent (%) of the inhibition. *- statistically significant difference at α= 0.05 between control sample, + - statistically significant difference at α= 0.05 between sample after 2 days. Error bars indicate the standard error of the mean of three independent replicates.

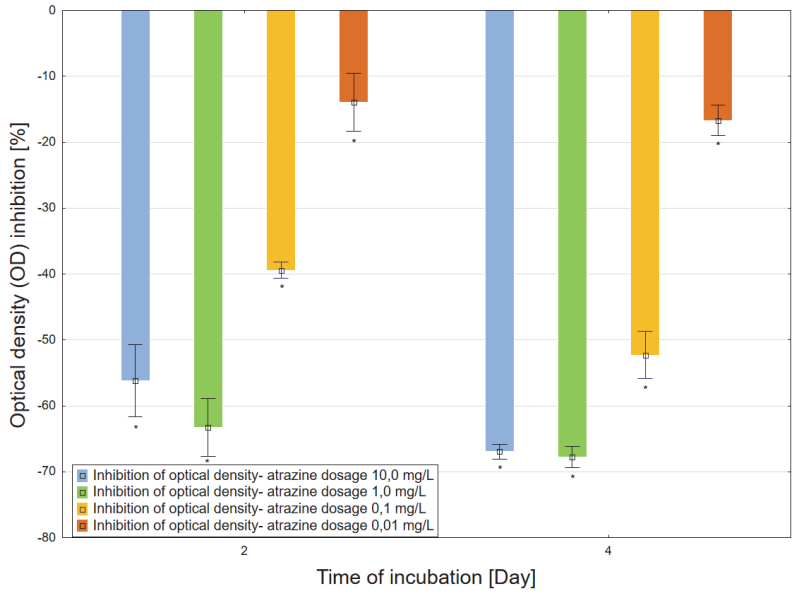

Fig. 5. Effect of studied doses of atrazine on the growth of *Chlorella vulgari*, presented as a percent (%) of the inhibition *- statistically significant difference at α= 0.05 between control sample, + - statistically significant difference at α= 0.05 between sample after 2 days. Error bars indicate the standard error of the mean of three independent replicates.

incubation of the *E. coli* RFM443 *lac:luxCDABE* culture in comparison to 2 hours of incubation (Fig. 7). Compared to the control sample, the strongest toxic effect of ATR was detected at the highest tested concentrations of 10 and 1 mg/L and was over 60% and about 20%, respectively.

ATR toxicity analysis with gfp gene as reporter

The use of the *E. coli* strain MM294-GFP allows for the assessment of ATR toxicity based on its effect on the GFP protein. Our results indicated that ATR at all tested concentrations had a toxic effect in *E. coli* MM294-GFP culture after 2 and 24 hours of incubation (Fig. 8). After 2 h of ATR treatment on a culture of *E. coli* MM294-GFP, the fluorescence inhibition of GFP protein ranged from over 2% to over 14% with the highest value of 14.525% at the highest tested ATR concentration of 10 mg/L. Extending the incubation time of the bacterial culture to 24 h, resulted in the toxic effect intensification of the herbicide in all tested ATR concentrations. This intensification was manifested by an increase in the GFP protein inhibition value in the *E. coli* MM294-GFP culture to over 15 and 30% at the highest tested ATR concentrations of 10 mg/L and 1 mg/L, respectively.

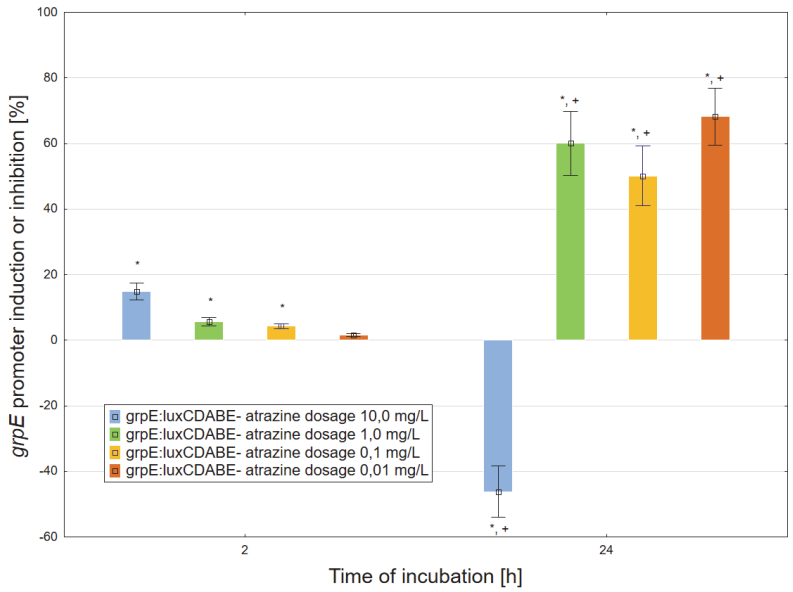

Fig. 6. Effect of studied doses of atrazine on *grpE* promoter response in *E. coli* RFM443 *grpE:luxCDABE* (toxicity and protein damage), presented as a percent (%) of the induction or inhibition. *- statistically significant difference at α= 0.05 between control sample, + - statistically significant difference at α= 0.05 between sample after 2 days. Error bars indicate the standard error of the mean of three independent replicates.

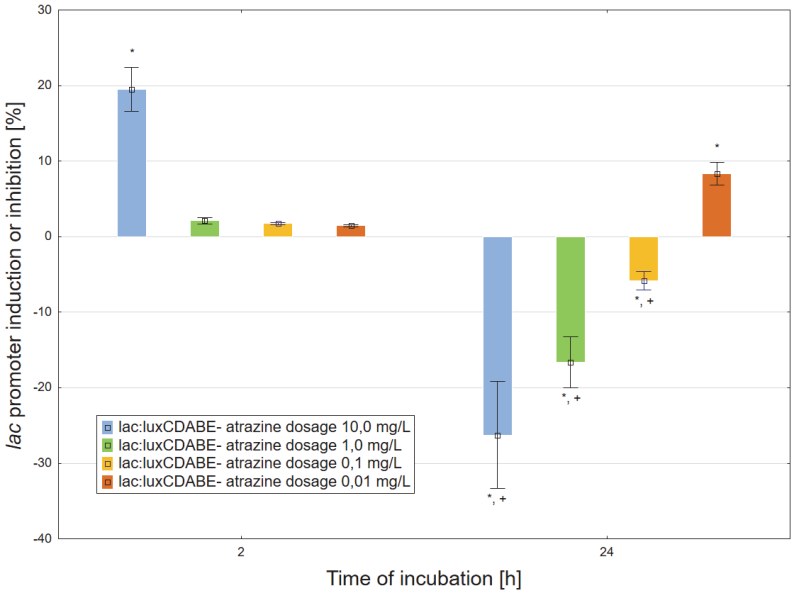

Fig. 7. Effect of studied doses of atrazine on *lac* promoter response in *E. coli* RFM443 *lac:luxCDABE* (toxicity), presented as a percent (%) of the induction or inhibition. *- statistically significant difference at α= 0.05 between control sample, + - statistically significant difference at α= 0.05 between sample after 2 days. Error bars indicate the standard error of the mean of three independent replicates.

ATR genotoxicity determination

The genotoxic potential of ATR was estimated with the use of *Escherichia coli* RFM443 *recA:luxCDABE* biosensor strain by analysis of the induction of *recA* promoter. A stronger genotoxic effect of ATR on bacterial cultures was detected after 24 hours of their exposure to the herbicide. ATR concentrations of 0.1 and 1 mg/L caused the strongest induction of the *recA* promoter, over 200% and almost 170%, respectively, compared to the control (Fig. 9). For the remaining concentrations of ATR, *recA* promoter induction levels ranged from greater than 75% to greater than 82%.

Statistical analysis

In the statistical analysis of the obtained results we applied Tukey's NIR test, which indicated statistical significance between chlorophyll *a*, chlorophyll *b*, carotenoids inhibition and optical density values in the control samples and between 2 and 4 days of exposure to the atrazine doses considered. Most parameters showed statistically significant differences, the exception to this observation being chlorophyll *b* values at atrazine concentrations of 0.01 mg/L. In the case of induction or inhibition of the *grpE*, *lac* and *recA* promoters in *E. coli* RFM443, especially after 24 hours of exposure to ATR and

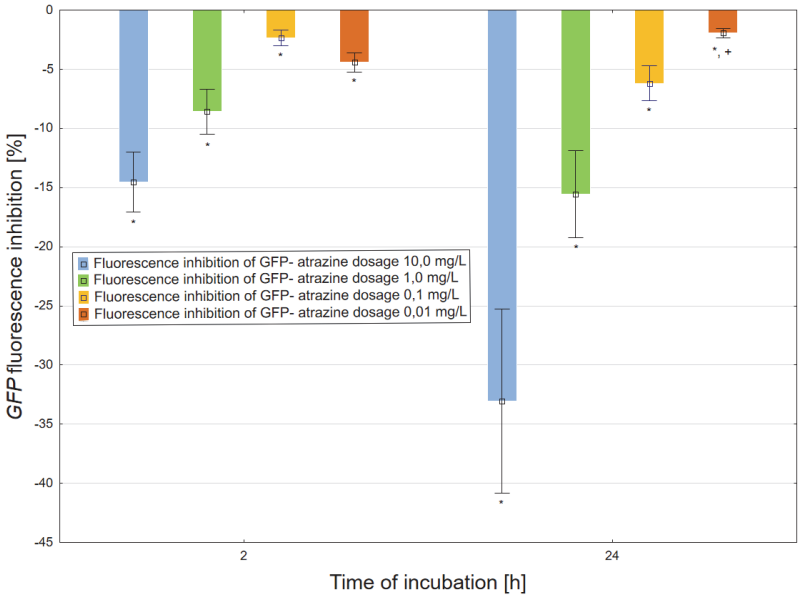

Fig. 8. Effect of studied doses of atrazine on *GFP* protein fluorescence inhibition in *E. coli* MM294 *trc:gfp* (toxicity), presented as a percent (%) of the inhibition. *- statistically significant difference at α= 0.05 between control sample, + - statistically significant difference at α= 0.05 between sample after 2 days. Error bars indicate the standard error of the mean of three independent replicates.

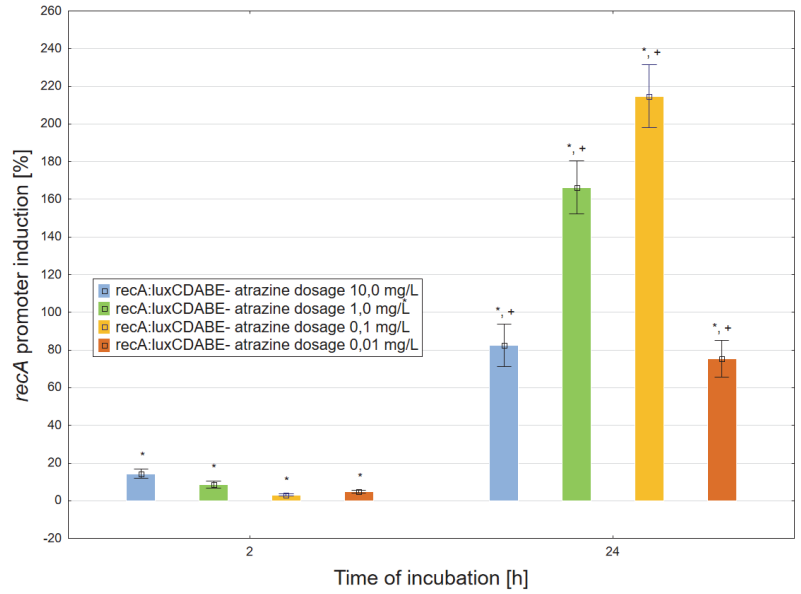

Fig. 9. Effect of studied doses of atrazine on *recA* promoter induction in *E. coli* RFM443 *recA:luxCDABE* (genotoxicity), presented as a percent (%) of the induction. *- statistically significant difference at α= 0.05 between control sample, + - statistically significant difference at α= 0.05 between sample after 2 days. Error bars indicate the standard error of the mean of three independent replicates.

in most of the tested concentrations, statistically significant differences in the values of the tested parameters were detected compared to the control and 2-hour incubation. No statistically significant differences were observed for the effect of an atrazine dose of 0.01 mg/L after 2 hours of exposure to the *grpE* promoter in *E. coli* strain RFM443 *grpE:luxCDABE*, which may have been due to the similar value to that observed in the control sample. A similar relationship was shown for the effect of atrazine doses of 0.01; 0.1 and 1.0 mg/L after an exposure time of 2 hours during *lac* promoter induction studies in *E. coli* strain RFM443 *lac:luxCDABE*. In addition, no statistically significant differences were observed for atrazine doses of 0.1, 1.0 and 10 mg/L between exposure times of 2 and 24 hours for induction of GFP protein fluorescence in *E. coli* strain MM294-GFP.

Discussion

Chlorophylls *a* and *b,* along with carotenoids, belong to the main pigments of *Chlorella vulgaris*, the content of which is often determined in toxicity tests. Chlorophylls are the main photosynthetic pigments. They are very important because they start the photosynthesis process by capturing photons and directing them *via* resonant transfer to the transmembrane structures in the thylakoid, i.e. photosystem II (PSII) (Barsanti and Gualtieri, 2014, Malcata, 2019). Carotenoids act as additional pigments for capturing and transferring energy, protecting against excessive irradiation. They also function as antioxidants and detoxifiers, protecting cells from damage caused by free radicals, including reactive oxygen species (Barsanti and Gualtieri, 2014, Ozturk et al. 2022, Sivathanu and Palaniswamy, 2012).

In our tests of ATR toxicity, we obtained the content inhibition of chlorophyll *a*, *b* and carotenoids in *Chlorella* after 2 and 4 days of exposure to ATR at concentrations of 10; 1; 0.1; 0.01 mg/L for these algae. The highest values of inhibition of the content of pigments up to over 80% were obtained after 4 days of algae incubation and at the highest tested concentrations of ATR 10 and 1 mg/L.

Majewska et al. (2021) described the phytocidal mechanism of atrazine. ATR can bind to the plastoquinone b (Q_b) binding site on the D1 protein of photosystem II (PSII), thus blocking the transfer of excitation energy from PSII to PSI. A decrease in the content of chlorophylls leads to a significant decrease in the efficiency of photosynthesis. Our experiments also showed a toxic effect of ATR on the growth of *Chlorella* cultures after both the 2 and 4 days of algae exposure to ATR. Our results of *Chlorella vulgaris* growth inhibition are in agreement with the study of Sun et al. (2020), who reported that atrazine concentrations of 0,004 to 1 mg/L caused a 50% decrease in *Chlorella vulgaris* growth (Sun et al. 2020). In turn, Camuel et al. (2017) found over 50% inhibition of the growth of *Chlorella vulgaris* cultures at ATR concentrations ranging from 0.02 to 4.6 µM. In the toxicity tests with the use of *Chlamydomonas reinhardtii*, the IC_{50} values were about 78 µg/L (Majewska et al. 2021).

Disturbances in the efficiency of the antioxidant enzyme system and the oxidation product generation system in living cells lead to oxidative stress. Oxidative stress causes damage to DNA, proteins, lipids, and cellular structures. Under conditions of oxidative stress, there are changes in the activity of the main antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione-S-transferase (GST), and glutathione reductase (GR), which can reflect the oxidative damage in the cell. Changes in the activity of antioxidant enzymes SOD, POD, CAT, GST and GR are considered to be markers of oxidative stress caused by environmental pollution, including herbicides (Yang et al. 2021a: Mofeed et al. 2013).

Previous work has documented that one of the main mechanisms of atrazine toxicity in algae is oxidative stress. Mofeed et al. (2013) in their work examined the effects of different ATR concentrations of 25, 50 and 100 µg/L on growth and oxidative stress on microalgae *Scenedesmus obliquus*. To determine the toxic effect of ATR on the microalgae the antioxidative enzyme activities were used as biomarkers. The authors in their work discovered that ATR significantly induced antioxidative enzyme activities of glutathione-S-transferase (GST), catalase activities (CAT) and glutathione reductase (GR) at different concentrations (Mofeed et al. 2013).

E. coli microbial biosensor strains with *lux* and *gfp* reporter genes are used for toxicity, genotoxicity, and bioavailability bioassays, which respond to a variation of xenobiotics and herbicides (Ali et al. 2021). In our studies, we also detected toxic activity of ATR with the use of *E. coli* strains RFM 443 with the gene constructs *grpE:luxCDABE* and *lac:luxCDABE*. A 24-hour exposure of the bacterial culture to ATR resulted in an intensification of the toxic effect of the herbicide, compared to 2-hours. In the obtained results, in most of the tested ATR concentrations, the induction of the *grpE* promoter was observed, even up to more than 60%, which proves the toxicity of this herbicide and its potency of degradation of cellular proteins. Similar results were obtained using the *lac:luxCDABE* gene construct in *E. coli* RFM443, where 24-hour incubation with ATR caused over 26% inhibition of luminescence in bacterial cells. Also, using the *E. coli* strain MM294-GFP, the strongest inhibition of GFP protein fluorescence, up to over 30%, was obtained after 24-hour incubation with ATR at the highest concentration of 10 mg/L.

The widespread use of herbicides induces oxidative stress in microbial populations. We know the most about the effect of ATR on soil microorganisms. Yang et al. (2021) documented that ATR significantly reduced the diversity of bacterial populations in cultivated soil layers of chernozem. Our studies have shown that ATR at concentrations of 10; 1; 0.1 and 0.01 mg/L affects the level of expression of the *luxCDABE* gene and causes a toxic effect and protein degradation in *E. coli* RFM443 cells. Zhang et al. (2012) in their work documented the inhibitory effect of ATR on cultures of *E. coli* K-12 and *Bacillus subtilis* B19. They also showed that ATR induced superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and total antioxidant capacity (T-AOC). Exposure to atrazine led to oxidative stress responses in the shrimp *Palaemonetes argentinus*, in red swamp crayfish *Procambarus clarkii* and in copepods (Yang et al. 2021, Silverya et al. 2022). It is very possible that oxidative stress was the mechanism of ATR-induced toxicity and protein degradation in the *E. coli* RFM443 (*luxCDABE*) and *E. coli* MM294 (*gfp*) strains we studied.

In our studies, for the determination of ATR genotoxic potency we used *E. coli* RFM443 strain with plasmid transcriptional fusion of *recA* promoter with *luxCDABE* reporter gene. In our experiment with the application of *E. coli* RFM443 *recA:luxCDABE* strain the significant genotoxic activity of ATR was noticed. A very clear increase in ATR genotoxicity, even up to over 200% compared to the control, was detected after 24 hours of incubation of the bacterial culture with the herbicide. According to literature data ATR can accumulate in the tissue, destroy DNA, and cause genotoxicity which may lead to mutations, immunosuppression, as well as damage to reproduction, growth, and development (Roustan et al. 2014, Yang et al. 2021). So far, in studies with the use of fish as model organisms' teratogenicity, genotoxicity, and oxidative stress have been reported, together with several histopathological effects. In crustaceans exposed to atrazine, teratogenic effects were observed, which could be caused by damage to the genetic material of embryos (Silverya et al. 2022). Akhtar et al. (2021) in their work and with the use of *Comet assay* proved, the genotoxic activity of ATR at the concentration of 24 ppm to snow trout (*Schizothorax plagiostomus*). Genotoxic effects of ATR have already been observed in the fish *Puntius lineatus*. The main mechanism of genotoxicity of ATR is that this herbicide can bind directly to DNA through intercalation mechanisms and the formation of adducts between the herbicide and the adenine and guanine bases (Santos et al. 2014). This mechanism may also be the cause of the genotoxic effect of the ATR tested in this study in the *E. coli* strain RFM443 *recA:luxCDABE*.

Conclusions

In the presented studies, the toxicity of a known herbicide – atrazine was determined on *Chlorella vulgaris* and *E. coli* microbial biosensor strains. In the assessment of ATR toxicity, we defined the inhibition of the content of chlorophyll *a*, *b*, carotenoids in *Chlorella vulgaris* and the inhibition of the growth of this algae culture. *E. coli* RFM443 strains with gene constructs *grpE:luxCDABE*, *lac:luxCDABE*, *recA:luxCDABE* and *E. coli* strain MM294 *trc:luxCDABE* were used to determine toxicity, degradation of cellular proteins and genotoxicity. Based on the obtained results, the following conclusions were made.

1. Atrazine in the tested concentrations shows a toxic effect in relation to *Chlorella vulgaris*.
2. ATR is toxic in *E. coli* RFM443 strains with *grpE:luxCDABE*, *lac:luxCDABE* gene constructs and causes degradation of cellular proteins.
3. ATR is toxic to the GFP protein in *E. coli* strain MM294-GFP.
4. ATR is genotoxic in *E. coli* strain RFM443 *recA:luxCDABE*.

Atrazine in surface and drinking water is detected in small concentrations (µg/L), but long-term exposure of organisms to low concentrations of pesticides contained in water intended for consumption may adversely affect sensitive populations. Caution should be exercised, because scientific studies have shown a correlation between human exposure to ATR and cancer incidence.

Conflict of interest

No conflict of interest has been declared.

Funding

Research carried out as part of statutory project WZ/WB-IIŚ/2/2023.

Acknowledgements

Authors are very grateful to Prof. Shimshon Belkin, Dr Tal Elad and Dr Sharon Yagur-Kroll, The Hebrew University of Jerusalem, Jerusalem, Israel for providing bioluminescent biosensor strains and as well as for scientific cooperation. Authors' gratitude also go to Prof. Imrich Barak, Slovak Academy of Science, Bratislava for providing *E. coli* MM294-GFP fluorescent biosensor strain.

References

Akhtar, N., Khan, M.F., Tabassum, S. & Zahran, E. (2021). Adverse effects of atrazine on blood parameters, biochemical profile, and genotoxicity of snow trout (*Schizothorax plagiostomus*). *Saudi Journal of Biological Sciences*, 28, pp. 1999–2003. DOI: 10.1016/j.sjbs.2021.01.001

Ali, S.A., Mittal, D. & Kaur G. (2021). In situ monitoring of xenobiotics using genetically engineered whole cell based microbial biosensors: recent advances and outlook. *World Journal of Microbiology and Biotechnology*, 7, pp. 37–81. DOI: 10.21203/rs.3.rs-264683/v1

Bae, J.W., Seo, H.B., Belkin, S. & Gu M.B. (2020). An optical detection module-based biosensor using fortified bacterial beads for soil toxicity assessment. *Analitical and Bioanalitical Chemistry*, 412, pp. 3373–3381, DOI: 10.1007/s00216-020-02469-z

Barsanti, L. & Gualtieri, P. (2014). Algae. Anatomy, Biochemistry, and Biotechnology (2 ed). CRC Press. Taylor & Francis Group, DOI: 10.1201/b16544

Camuel, A., Guieysse, B., Alcántara, C., & Béchet, Q. (2017). Fast algal ecotoxicity assessment: influence of light intensity and exposure time on *Chlorella vulgaris* inhibition by atrazine and DCMU. *Ecotoxicology and Environmental Safety*, 140, pp. 141–147, DOI: 10.1016/j.ecoenv.2017.02.013

Chen, J., Liu, J., Wu, S., Liu, W., Xia, Y., Zhao, J., Yang, Y., Peng, Y. & Zhao, S. (2021). Atrazine promoted epithelial ovarian cancer cells proliferation and metastasis by inducing low dose reactive oxygen species (ROS). *Iran Journal of Biotechnology*, 19, pp. 2623 – 2635, DOI: 10.30498/IJB.2021.2623

Dębowski, M. (2018). The use of microalgae biomass in engineering and environmental protection technologies. *Polish Journal of Natural Sciences*, 27, pp. 151–164, DOI: 10.3390/en14196025

Fareed, A., Hussain, A., Nawaz, M., Imran, M., Ali, Z. & Haq, S.U. (2021). The impact of prolonged use and oxidative degradation of Atrazine by Fenton and photo-Fenton processes. *Environmental Technology and Innovation*, 24, pp. 18–32. 101840. DOPI:10.1016/j.eti.2021.101840

Jiang, B., Li, G., Xing, Y., Zhang, D., Jia, J., Cui, Z., Luan, X. & Tang, H. (2017). A whole cell bioreporter assay for quantitative genotoxicity evaluation of environmental samples. *Chemosphere*, 184, pp. 384–392, DOI: 10.1016/j.chemosphere.2017.05.159

Kamaz, M., Jones, S.M., Qian, X., Watts, M.J., Zhang, W. & Wickramasinghe, S.R. (2020). Atrazine removal from municipal wastewater using a membrane bioreactor. *International*

Journal of Environmental Research and Public Health, 17, pp. 2567–2578, DOI: 10.3390/ijerph17072567

Kopcewicz, J., Lewak, S., Jaworski, K., Tretyn, A., Gniazdowska, A., Szmidt-Jaworska, A., Kęsy, J., Gabryś, H., Szymańska, M., Hawrylak-Nowak, B., Strzałka, K., Ciereszko, I., Rychter, A.M. & Tyburski, J. (2020). Plant physiology Polish Scientific Publishers PWN, Warsaw, Poland. (in Polish).

Lu, Q., Zhou, X., Liu, R., Shi, G., Zheng, N., Gao, G. & Wang, Y. (2023). Impacts of a bacterial algicide on metabolic pathways in *Chlorella vulgaris*. *Ecotoxicology and Environmental Safety*, 249, pp. 1–13, DOI: 10.1016/j.ecoenv.2022.114451

Majewska, M., Harshkova, D., Pokora, W., Baścik-Remisiewicz, A., Tułodziecki, S. & Aksmann, A. (2021). Does diclofenac act like a photosynthetic herbicide on green algae? Chlamydomonas reinhardtii synchronous culture-based study with atrazine as reference. *Ecotoxicology and Environmental Safety*, 208, 111630, DOI: 10.1016/j.ecoenv.2020.111630

Malcata, F.X. (2019). Marine macro- and microalgae: an overview. CRC Press Taylor & Francis Group, DOI: 10.1201/9781315119441

Matejczyk, M., Ofman, P., Dąbrowska, K., Świsłocka, R. & Lewandowski, W. (2020a). The study of biological activity of transformation products of dicoflenac and its interaction with chlorogenic acid. *Journal of Environmental Sciences*, 91, pp. 128–141, DOI: 10.1016/j.jes.2020.01.022

Matejczyk, M., Ofman, P., Dąbrowska, K., Świsłocka, R. & Lewandowski, W. (2020b). Evaluation of the biological impact of the mixtures of diclofenac with its biodegradation metabolites 4'-hydroxydiclofenac and 5-hydroxydiclofenac on *Escherichia coli*. DCF synergistic effect with caffeic acid. *Archives of Environmental Protection*, 46, pp. 32–53, DOI: 10.24425/aep.2020.135760

Matejczyk, M., Ofman, P., Dąbrowska, K., Świsłocka, R. & Lewandowski, W. (2020c). Synergistic interaction of diclofenac and its metabolites with selected antibiotics and amygdalin in wastewaters. *Environmental Research*, 186, 109511, DOI: 10.1016/j.envres.2020.109511

Matejczyk, M., Ofman, P., Parcheta, M., Świsłocka, R. & Lewandowski, W. (2022). The study of biological activity of mandelic acid and its alkali metal salts in wastewaters. *Environmental Research*, 205, 112429, DOI: 10.1016/j.envres.2021.112429

Melamed, S., Lalush, C., Elad, T., Yagur-Krol, S., Belkin, S. & Pedahzur, R. (2012). A bacterial reporter panel for the detection and classification of antibiotic substances: Detection and classification of antibiotics. *Microbiology and Biotechnology*, 5, pp. 536–548, DOI: 10.1111/j.1751-7915.2012.00333.x

Mofeed, J. & Moshleh, Y. (2013). Toxic responses and antioxidative enzymes activity of *Scenedesmus obliquus* exposed to fenhexamid and atrazine, alone and in mixture. *Ecotoxicology and Environmental Safety*, 95, pp. 234–240, DOI: 10.1016/j.ecoenv.2013.05.023

Moraskie, M., Roshid, H., O'Connor, G., Dikici, E., Zings, J.M., Deo, S. & Daunert, S. (2021). Microbial whole-cell biosensors: current applications, challenges, and future perspectives. *Biosensors and Bioelectronics*, 191, 113359, DOI: 10.1016/j.bios.2021.113359

Ozturk, M., Coskuner, K.A., Serdar, B., Atar, F. & Bilgili, E. (2022). Impact of white mistletoe (Viscum album ssp. abietis) infection severity on morphology, anatomy and photosynthetic pigment content of the needles of cilician fir (*Abies cilicica*). *Flora*, 294, 152135, DOI: 10.1016/j.flora.2022.152135

Qian, H., Sheng, G., Liu, W., Lu, Y., Liu, Z. & Fu, Z. (2008). Inhibitory effects of atrazine on *Chlorella vulgaris* as assessed by real-time polymerase chain reaction. *Environmental Toxicology and Chemistry*, 27, pp. 182–187, DOI: 10.1897/07-163.1

Rojas-Villacorta, W., Rojas-Flores, S., De La Cruz-Noriega, M., Espino, H.C., Diaz, F. & Cardenas, M.G. (2022). Microbial biosensors for wastewater monitoring: mini review. *Processes*, 10, pp. 2–13, DOI: 10.3390/pr10102002

Roustan, A., Aye, M., De Meo, M. & Giorgio, C.D. (2014). Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation. *Chemosphere,* 108, pp. 93–100, DOI: 10.1016/j.chemosphere.2014.02.079

Santos, K.C. & Martinez, C.B.R. (2014). Genotoxic and biochemical effects of atrazine and Roundups, alone and in combination, on the Asian clam *Corbicula fluminea*. *Ecotoxicology and Environmental Safety*, 100, pp. 7–14, DOI: 10.1016/j.ecoenv.2013.11.014

Shan, W., Hu, W., Wen, Y., Ding, X., Ma, X., Yan, W. & Xia, Y. (2021). Evaluation of atrazine neurodevelopment toxicity *in vitro*-application of hESC-based neural differentiation model. *Reproductive Toxicology*, 103, pp. 149–158, DOI: 10.1016/j.reprotox.2021.06.009

Silveyra, G.R., Medesani, D.A. & Rodríguez, E.M. (2022). Effects of the herbicide atrazine on Crustacean Reproduction. Mini--Review. *Frontiers in Physiology*, 13, pp. 1–5, DOI: 10.3389/fphys.2022.926492

Sivathanu, B. & Palaniswamy, S. (2012). Purification and characterization of carotenoids from green algae *Chlorococcum humicola* by HPLC-NMR and LC-MS-APCI. *Biomedical Prevention and Nutrition,* 2, pp. 276–282, DOI: 10.1016/j.bionut.2012.04.006

Song,Y., Jiang, B., Tian, S., Tang, H., Liu, Z., Li, C., Jia, J., Huang, W. E., Zhang, X. & Li, G. (2014). A whole cell bioreporter approach for the genotoxicity assessment of bioavailability of toxic compounds in contaminated soil in China. *Environmental Pollution*, 195, pp. 178–184, DOI: 10.1016/j.envpol.2014.08.024

Su, Y., Cheng, Z., Chou, Y., Lin, S., Gao, L., Wang, Z., Bao, R. & Peng, L. (2022). Biodegradable and conventional microplastics posed similar toxicity to marine algae *Chlorella vulgaris*. *Aquatic Toxicology*, 244, 106097, DOI: 10.1016/j.aquatox.2022.106097

Sun, C., Xu, Y., Hu, N., Ma, J., Sun, S., Cao, W., Klobučar, G., Hu, C. & Zhao, Y. (2020). To evaluate the toxicity of atrazine on the freshwater microalgae *Chlorella sp*. using sensitive indices indicated by photosynthetic parameters. *Chemosphere*, 244, 125514, DOI: 10.1016/j.chemosphere.2019.125514

Węgrzyn, A. & Mazur, R. (2020). Regulatory mechanisms of photosynthesis light reactions in higher plants. *Postępy Biochemii (Advances in biochemistry)*, 66, pp. 134–42. (in Polish). DOI: 10.18388/pb.2020_325

Woutersen, M., Belkin, S., Brouwer, B., Wezel, A.P. & Heringa, M.B. (2011). Are luminescent bacteria suitable for online detection and monitoring of toxic compounds in drinking water and its sources? *Analitical and Bioanalitical Chemistry*, 400, pp. 915–929. DOI: 10.1007/s00216-010-4372-6

Xiong, J.Q., Kurade, M.B., Abou-Shanab, R.A.J., Ji, M.K., Choi, J., Kim, J.O. & Jeon, B.H. (2016). Biodegradation of carbamazepine using freshwater microalgae *Chlamydomonas mexicana* and *Scenedesmus obliquus* and the determination of its metabolic fate. *Bioresources Technology*, 205, pp. 183–90, DOI: 10.1016/j.biortech.2016.01.038

Yang, F., Gao, M., Lu, H., Wei, Y., Chi, H., Yang, T., Yuan, M., Fu, H., Zeng, W. & Liu, C. (2021). Effects of atrazine on chernozem microbial communities evaluated by traditional detection and modern sequencing technology. *Microorganisms*, 9, 1832. DOI: 10.3390/microorganisms9091832

Yang, H., Jiang, Y., Lu, K., Xiong, H., Zhang, Y. & Wei, W. (2021a). Herbicide atrazine exposure induce oxidative stress, immune dysfunction and WSSV proliferation in red swamp crayfish *Procambarus clarkii*. *Chemosphere*, 283, 131227. DOI: 10.1016/j.chemosphere.2021.131227

Zappi, D., Coronado, E., Soljan, V., Basile, G., Varani, G., Turems, M. & Giardi, M. (2021). A microbial sensor platform based on bacterial bioluminescence (*luxAB*) and green fluorescent protein (*gfp*) reporters for in situ monitoring of toxicity of wastewater nitrification process dynamics. *Talanta*, 221, pp. 1–8. DOI: 10.1016/j.talanta.2020.121438

Zhang, Y., Meng, D., Wang, Z., Guo, H. & Wang, Y. (2012). Oxidative stress response in two representative bacteria exposed to atrazine. *FEMS Microbiology Letters*, 334, pp. 95–101. DOI: 10.1111/j.1574-6968.2012.02625.x

Zhao, Y., Yunyang, L., Bao, H., Nan, J. & Xu, G. (2023). Rapid biodegradation of atrazine by a novel *Paenarthrobacter ureafaciens* ZY and its effects on soil native microbial community dynamic. *Frontiers in Microbiology*, 4, pp. 1–13, DOI: 10.3389/fmicb.2022.1103168

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, 1018124, DOI: 10.3389/fchem.2022.1018124

Toksyczność atrazyny w glonach *Chlorella vulgaris* oraz w biosensorowych testach *E. coli* z genami *gfp* i *lux*

Streszczenie: Atrazyna (ATR) to szeroko stosowany na całym świecie chlorowany herbicyd z grupy s-triazyn. Ze względu na powszechne stosowanie, ATR przedostaje się do środowiska i jest wykrywana w wodzie pitnej, przekraczając dopuszczalne przez WHO stężenie, które wynosi 2 µg/L. W przedstawionych badaniach określono toksyczność ATR w stężeniach 10; 1; 0.1; 0.01 mg/L na glonach *Chlorella vulgaris* oraz z zastosowaniem *E. coli* mikrobiologicznych biosensorów z genami reporterowymi *gfp* i *lux*. Toksyczność oszacowano na podstawie zawartości chlorofilu a, b, karotenoidów w *Chlorella vulgaris* oraz zahamowania wzrostu tej kultury alg. Szczepy *E. coli* RFM443 z konstruktami genowymi *grpE:luxCDABE*, *lac:luxCDABE*, *recA:luxCDABE* i szczep *E. coli* MM294 *trc:luxCDABE* wykorzystano do określenia toksyczności, degradacji białek komórkowych i genotoksyczności. W przeprowadzonych badaniach wykryto, że ATR w analizowanych stężeniach wykazuje działanie toksyczne w stosunku do *Chlorella vulgaris*. W przypadku ATR stwierdzono właściwości toksyczne i genotoksyczne oraz potencjał degradacji białek w szczepach *E. coli* RFM443 z promotorami *grpE*, *lac*, *recA*. Ponadto wykryto toksyczność ATR w stosunku do białka GFP w szczepie *E. coli* MM294-GFP. Biorąc pod uwagę udokumentowaną w badaniach własnych oraz w doświadczeniach innych naukowców toksyczność i genotoksyczność ATR, obecność tego herbicydu w wodach powierzchniowych i wodzie pitnej stanowi poważne zagrożenie dla organizmów żywych.