Environmental development is defined as conscious and planned changes in the natural environment, the aim of which is the economic use of ecosystems or restoration of their ecological function. It can therefore be stated that environmental development is a deliberate and conscious impact on the natural environment, the purpose of which is to enrich it with features that are beneficial to humans and to increase the capacity of the environment. The primary objective of such measures is to create suitable conditions for environmental management that will meet the requirements of sustainable development, will ensure a higher quality of life for the residents and will increase the tourist attractiveness of a given area, while protecting the natural environment for future generations [1-5].

One of the most important elements of the natural environment is water and this is the only necessary medium for life. Any water pollution leads to various types of diseases and the death of living organisms. Contaminated water also causes diseases of aquatic plants. Harmful compounds accumulated in plants penetrate into human and animal organisms. Large amounts of industrial and municipal wastewater,
increasing every day, threaten the viability of lakes, rivers, seas and oceans [6-10].

At present, water purity is a worldwide problem. In developed countries, it is not possible to drink water directly from rivers or from the tap, which raises concerns as to its purity and harmfulness. Despite various prohibitions and penalties, we still have to deal with the contamination of rivers, lakes and groundwater, from which people draw their drinking water. Water used for consumption is obviously purified, but even the most advanced technologies are insufficient to eliminate all contaminants. In addition, wastewater generated by industry, agriculture and households leads to the increasing deterioration of waters [11-15]. It is therefore necessary to protect the natural environment, and water in particular.

Stilbene derivatives with antimicrobial properties may be used for environmental protection. The interest in antiseptic (decontaminant), preserving and disinfecting substances usually results from their negative effects on human health. At the same time, these substances are essential in a number of different products, e.g. they are commonly used as additives preventing the development of mould, bacteria and fungi [15]. Stilbene derivatives under study are characterised by demonstrated fungistatic and fungitoxic properties [16]. Considering the above properties of stilbene derivatives, it has been decided to use them for environmental protection.

The objective of this study was to demonstrate the possibility of using stilbene derivatives for surface water protection by determining their short- and long-term stability in the analysed water samples. Assays were carried out with the use of high-performance liquid chromatography.

Material and Methods

Study Area

Twenty samples of surface water were collected from each of the three rivers, i.e. the Bug River in the town of Wyszków, the Liwic River in the town of Węgrów and the Muchawka River in the city of Siedlce. The Muchawka River, with a length of 32.1 km, is a left-bank tributary of the Liwic River. The status of water in the Muchawka River was defined as moderate, mainly due to the exceeded annual average and maximum concentrations of benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene [17].

The Bug River, on the other hand, is a border river over a long distance and a receiver of large quantities of wastewater from Ukraine. The direct source of pollution in this river in the Mazowia province is the town of Wyszków, which discharges about 3,000 m³ per day of

<table>
<thead>
<tr>
<th>Compound</th>
<th>M.p. °C</th>
<th>IR (KBr), cm⁻¹, δ CH=CH</th>
<th>H-NMR δ, ppm, DMSO-d₆, δ CH₂-N</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A1)</td>
<td>227–230</td>
<td>970</td>
<td>5.81</td>
</tr>
<tr>
<td>(A3)</td>
<td>209–212</td>
<td>965</td>
<td>5.82</td>
</tr>
<tr>
<td>(A15)</td>
<td>218–221</td>
<td>980</td>
<td>5.90</td>
</tr>
<tr>
<td>(A17)</td>
<td>205–208</td>
<td>960</td>
<td>5.79</td>
</tr>
</tbody>
</table>
wastewater into the river, treated in a sewage treatment plant with increased nutrient removal. The Toczna and Cetynia rivers discharge significant loads of pollutants into the Bug River with wastewater from the town of Sokół Podlaski. The characteristic contamination of the Bug River are the total suspended solids.

The reaction of water samples collected from the Muchawka River was slightly alkaline (pH = 7.23), from the Liwiec River – also slightly alkaline (pH = 7.58), and from the Bug River – alkaline (pH = 8.31).

The material of the study were also derivatives of (E)-azastilbene (Fig. 1), i.e. (E)-N-(m-chlorobenzyl)-4’-hydroxystilbazole-4 chloride, (E)-N-(m-chlorobenzyl)-2’-hydroxystilbazole-4 chloride, (E)-N-(o-bromobenzyl)-2’-hydroxystilbazoles-4 bromide, and (E)-N-(m-bromobenzyl)-2’-hydroxystilbazoles-4 bromide. Surface water samples were filtered through a membrane filter with a pore diameter of 3 µm, allowing bacteria to pass into the solution. The examined derivatives were obtained according to the published procedures [16]. Structures of derivatives were confirmed by nuclear magnetic resonance (Table 1) and the biological activity is presented in Table 2. The determination of the stability of derivatives was carried out between August 2018 and September 2019.

### Methods

Five surface water samples collected from each site were analysed in parallel. The analysed derivatives of (E)-azastilbene are well soluble in water. In order to analyse the surface water, four azastilbene derivatives were added to each sample to obtain a 1000 µg mL⁻¹ concentration each. All of the material was stirred and concentrated after 1 h, using the solid-phase extraction method. The extraction process was carried out on a naphthylpropyl extraction column (Fig. 2) [18]. The procedure of column conditioning consisted in washing the bed of 4 mL of cyclohexane (Merck, Darmstadt, Germany), 4 mL of methanol (Merck, Darmstadt, Germany) and then 4 mL of triple distilled water. Then the bed was dried in a stream of air for 15 sec. A volume of 100 mL of each solution of an analysed sample was passed through the thus prepared extraction column at a rate of 3-4 drops per second. The column was then dried in a stream of air for 10 min. The process of elution was performed using 8 cm³ of acetonitrile. Then 2 mL of methanol was added to 8 mL of eluent and the mixture was thoroughly stirred and concentrated in a stream of air to a volume of about 1 mL. Next, 1 mL of distilled water was added. Each sample was prepared the same way. The content of each derivative was analysed by high-performance liquid chromatography with UV/Vis detection. Chromatographic conditions of the analyses were as follows: the octadecyl column, the mobile phase – methanol (100%), flow rate – 1.0 mL · min⁻¹, wavelength – 410 nm, temperature – 25ºC.

When determining the stability of the analysed derivatives, consideration was given to their possible changes over time. Assays were carried out according to the above procedure, with four time intervals: 1 h, 7 days, 28 days and 12 months. Water samples, prepared for extraction, were stored during the year in plastic bottles at a temperature range of 18-30°C. Standard solutions with the following concentrations: 0, 50, 100, 300, 500, 750 and 1000 µg · mL⁻¹ were prepared to determine recovery values of individual derivatives and to perform quantitative analysis.

The standard solutions were introduced into a chromatographic device and calibration curves were plotted based on the obtained peak areas. Using the minimum values of correlation coefficients of 0.999, the range of concentrations was determined, at which the curves were linear. The limit of detection (LOD)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimal inhibitory concentration (µg · mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>100 500 500 1000 1000 &gt;500 &gt;500 &gt;500 &gt;500</td>
</tr>
<tr>
<td>A3</td>
<td>7.5 100 100 100 1000 1000 &gt;500 &gt;500 &gt;500</td>
</tr>
<tr>
<td>A15</td>
<td>5 500 500 100 1000 1000 &gt;500 &gt;500 &gt;500</td>
</tr>
<tr>
<td>A17</td>
<td>5 500 100 100 1000 1000 &gt;500 &gt;500 &gt;500</td>
</tr>
</tbody>
</table>

1–Staphylococcus aureus 209P FDA, 2–Streptococcus faecalis ATCC 8040, 3–Bacillus subtilis ATCC 1633, 4–Escherichia coli PZH 2686, 5–Klebsiella pneumoniae 231, 6–Pseudomonas aeruginosa 5 R1, 7–Candida albicans PCM 1409 PZH, 8–Microsporum gypseum K₁, 9–Aspergillus fumigatus C1.

Fig. 2. Scheme of chemical structure of bonded stationary phase.
and the limit of quantification (LOQ) under the chromatographic conditions and in the linearity range were expressed as the concentration of a compound for which signal/noise size ratios were 3:1 and 10:1, respectively [19-23].

All \(^1\)H NMR spectra were registered on Bruker-400 in CDCl\(_3\), using the internal standard HMDS, whereas the IR spectra were registered in potassium bromide using Nicollet Magna-IR 760 (Thermo Fisher Scientific, Waltham, GB). The extraction process was carried out using the naphthylpropyl extraction column at the Chair of Environmental Chemistry and Bioanalytics, Nicolaus Copernicus University in Toruń. High-performance liquid chromatography SPD-6A (Shimadzu, Kyoto, Japan) equipped with a UV detector (Shimadzu C-R6A) and an LC-6A pump with an Rheodyne dispenser (Berkeley, CA, USA), model 7125 with a volume of 20 \(\mu\)L, were used for qualitative and quantitative analysis.

## Results and Discussion

The main objective of this study was to demonstrate the possibility of using biologically active azastilbene derivatives to protect surface waters on the basis of their short- and long-term stability in the analysed water samples. The research was conducted with the use of surface waters collected from three rivers. The following substances were included in the study: (E)-N-(m-chlorobenzyl)-4'-hydroxystilbazole-4 chloride, (E)-N-(m-chlorobenzyl)-2'-hydroxystilbazole-4 chloride, (E)-N-(m-bromobenzyl)-4'-hydroxystilbazole-4 chloride, (E)-N-(o-bromobenzyl)-2'-hydroxystilbazole-4 bromide, and (E)-N-(m-bromobenzyl)-2'-hydroxystilbazole-4 bromide. The obtained results are presented in Tables 3-5.

<table>
<thead>
<tr>
<th>Water from the river</th>
<th>Mean recovery values (%)</th>
<th>SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liwiec</td>
<td>91.4 95.5 96.1 93.9</td>
<td>± 3.4  ± 4.2  ± 3.9  ± 4.4</td>
</tr>
<tr>
<td>Muchawka</td>
<td>93.7 92.9 95.4 93.6</td>
<td>± 4.1  ± 3.4  ± 4.7  ± 4.5</td>
</tr>
<tr>
<td>Bug</td>
<td>87.3 83.5 86.1 85.6</td>
<td>± 4.3  ± 4.9  ± 4.2  ± 4.7</td>
</tr>
</tbody>
</table>

Table 3. Mean recovery values (1 hour after sample preparation) for chloride of (E)-N-(m-chlorobenzyl)-4'-hydroxystilbazole-4 (A1), chloride of (E)-N-(m-chlorobenzyl)-2'-hydroxystilbazole-4 (A3), bromide of (E)-N-(o-bromobenzyl)-2'-hydroxystilbazole-4 (A15), bromide of (E)-N-(m-bromobenzyl)-2'-hydroxystilbazole-4 (A17) obtained from different matrices in the naphthylpropyl column used in the study (\(n = 5\)).
2'-hydroxystilbazole-4 bromide, and (E)-N-(m-bromobenzyl)-2'-hydroxystilbazole-4 bromide in the analysed surface water samples after 1 h, 7 days, 28 days and 12 months is presented in Table 5. The obtained results indicate that the content of the analysed derivatives in the studied surface waters slightly decreased over time. Since the derivatives of (E)-azastilbene used in the study are hydrolytically stable and resistant to light and oxygen, their loss is most likely associated with their effects on microorganisms. A slight decrease in the content of the analysed derivatives over time could also be partly caused by reactions with other ions or compounds present in the analysed samples, as well as adsorption on the walls of containers in which the samples were stored.

According to the literature, fungistatic and fungitoxic properties of a preparation are considered suitable if no significant decrease in antiseptic, preservative or disinfectant content occurs after 28 days at a certain temperature in a matrix. The average content of the analysed derivatives, i.e. (E)-N-(m-chlorobenzyl)-4'-hydroxystilbazole-4 chloride, (E)-N-(m-chlorobenzyl)-2'-hydroxystilbazole-4 chloride, (E)-N-(o-bromobenzyl)-2'-hydroxystilbazole-4 bromide, and (E)-N-(m-bromobenzyl)-2'-hydroxystilbazole-4 bromide in the analysed surface water samples after 1 h, 7 days, 28 days and 12 months is presented in Table 5. The obtained results indicate that the content of the analysed derivatives in the studied surface waters slightly decreased over time. Since the derivatives of (E)-azastilbene used in the study are hydrolytically stable and resistant to light and oxygen, their loss is most likely associated with their effects on microorganisms. A slight decrease in the content of the analysed derivatives over time could also be partly caused by reactions with other ions or compounds present in the analysed samples, as well as adsorption on the walls of containers in which the samples were stored.

According to the literature, fungistatic and fungitoxic properties of a preparation are considered suitable if no significant decrease in antiseptic, preservative or disinfectant content occurs after 28 days at a certain temperature in a matrix.
contaminated with microorganisms [35]. Therefore, it can be inferred from the data in Table 5 that small differences in the decline of individual derivatives were observed after 1 h, regardless of sampling sites. The analysed (E)-azastilbene derivatives showed a slight decrease in their content of about 13%, and thus good stability, also after 7 days in all the collected surface water samples. On the other hand, the average decrease in the content of individual derivatives varied significantly in the case of the 28-day time interval. The difference in the content of derivative (A1) in water from the Liwiec River compared to the content after 7 days was 192.6 µg · L⁻¹, i.e. 33%, in water from the Muchawka River – 201.0 µg · L⁻¹, i.e. 35%, and in the water from the Bug River – 111.5 µg · L⁻¹, i.e. 20%. Similar results were also obtained for two derivatives – (A3 and A15). Derivative (A17), on the other hand, showed the highest average drop of nearly 38% in the case of water collected from the Liwiec River. After 7 and 28 days, the difference was 210.9 µg · L⁻¹. A slightly smaller difference (191.6 µg · L⁻¹) was recorded for samples of water collected from the Muchawka River, i.e. 33% compared to the concentration after 7 days. The smallest difference of 136.7 µg · L⁻¹ was found for water samples from the Bug River, i.e. 24%.

A further decrease in the content of the analysed derivatives was observed after 12 months. In the case of derivative (A1) and water samples from the Liwiec River, the difference in the content between the last two time intervals was 295.9 µg · L⁻¹, which accounts for a loss of 50%, for derivative (A3) – 47%, derivative (A15) – 50%, and derivative (A17) – 57%. Similar results were obtained for samples collected from the Muchawka River, i.e. for derivatives (A1 and A3) – a decrease by 49% each, for derivative (A15) – a decrease by 47%, derivative (A17) – a decrease by 59%. The content of derivative (A1) in water samples collected from the Bug River decreased by 48%, derivative (A3) by 49%, derivative (A15) by 52% and derivative (A17) also by 52%.

It can be concluded that the conducted research enables the use of the analysed derivatives of (E)-azastilbene for surface water treatment. Due to the reduction in the content of individual (E)-azastilbene derivatives, they can also be used with other substances in the form of mixtures that would be synergistically active when combined in solution. Literature data show that no substance has been invented so far that would fully meet all the requirements specified for antiseptics, preservatives or disinfectants. Each of the chemicals currently used has certain limitations and therefore mixtures of such substances with a synergistic effect are used in many products.

Conclusions

The research on short- and long-term stability of four biologically active derivatives of (E)-azastilbene in surface waters of three rivers was carried out. After 12 months, the highest reduction (59%) of derivative (A17) was recorded in water samples from the Muchawka River. The smallest reduction (47%) in the content of derivative (A3) was found in water samples collected from the Liwiec River. The obtained results indicate that the waters of the Muchawka River are the most susceptible to microbiological contamination. At the same time, it should be emphasised that the volume of water in this river and the flow rate are the smallest compared to the others, which undoubtedly has a negative impact on the process of dilution of pollutants and the self-purification of waters in this river. The research on short- and long-term stability indicates that the analysed derivatives of (E)-azastilbenes can be used for surface water treatment, preferably with other synergistic substances.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

References


