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INFLUENCE OF 1,3,4-THIADIAZOLE DERIVATIVES ON THE BIOLOGICAL ACTIVITY OF THE SELECTED ENVIRONMENTAL BACTERIA

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Abstract: The paper presents the results of microbiological research carried out with the living microorganisms participation and in the domestic sewage and sludge from municipal sewage treatment plants environment. The influence of the selected heterocyclic compounds – the derivatives of 1,3,4-thiadiazole on susceptibility of aerobic Gram-positive and Gram-negative bacteria was investigated. For the research of biological activity the following environmental bacterial strains were selected: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Enterococcus faecium*, *Salmonella senftenberg,* and *Salmonella typhimurium.* For the group of the tested bacteria, 2-amino-1,3,4-thiadiazole turned out to be the most active chemical compound. It was found that evenly matched concentrations of this compound allow for the selective elimination of such microorganisms as: *Salmonella senftenberg*, *Bacillus cereus*, *Staphylococcus aureus* and *Enterococcus faecium*. In the case of *Escherichia coli*, the minimum inhibitory concentration (MIC) was detected at the level of 12.5 mg/cm^3 (determined in relation to 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide, bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide), and for 2-amino-1,3,4-thiadiazole: 0.5 mg/cm^3. Similar results were observed in case of *Enterococcus faecium* bacterium: MIC for 2-amino-1,3,4-thiadiazole was less than 0.25 mg/cm^3, and MIC for bis(2-acetylamino--1,3,4-thiadiazole)-5,5'-disulfonamide this level was equal to 25.0 mg/cm^3.

Keywords: 1,3,4-thiadiazole derivatives, microbiological research, sanitary status indicator

The interest in derivatives of 1,3,4-thiadiazole has increased since phenylhydrazine and hydrazine were found. Thus, the research widened our knowledge concerning the preparation and chemical properties of 1,3,4-thiadiazoles. Derivatives of this compound showing biological activity are known as medical preparations [1]. A lot of research is

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being carried out concerning the issue of the application of heterocyclic derivatives in viral and bacterial diseases treatment [2–4]. It is known that 1,3,4-thiadiazole derivatives possess such biological activity as: antibacterial [5, 6] antimycobacterial [7, 8], antidepressive [9] and cardiotonic [10]. Resent investigation has also detected the analgesic [11] and anti-inflammatory [12–14] activity for these heterocyclic compounds. The compositions containing this heterocyclic ring form reactive centres in many enzymes and coenzymes, and are included in the composition of structures such as nucleic acids, chlorophyll, haemin – red blood pigment, pesticides, medicines (such as: 4-dimethylamino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, penicillin G, diazepam, resirpin, acridine), hormones (serotonin, melatonin), amino acids (eg: tryptophan, histidine) and many others. Other 1,3,4-thiadiazole derivatives are practically obtained by the application of different methods and their preparation technologies are strictly related to water and the pollution of its derivative compounds as well as to sewage and solid waste formation. In the pharmaceutic industry, diuramid is mostly prepared from 2-acetylamine-5-mercapto-1,3,4-thiadiazole by the process of chlorination involving gaseous chlorine and the ammonolysis of the semi-product [15]. The compounds such as: 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (known under the trade name of diuramid, acetazolamide, diamox, diacarb, diluran), 2-amino-1,3,4-thiadiazole and bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide are found in the wastes and wastewaters from different diuramid production technologies [16]. These compounds also occur in nature and show high biological activity [17, 18].

The aim of the work is investigating the influence of the 1,3,4-thiadiazole derivatives on the activity of the selected environmental bacterial strains: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Enterococcus faecium*, *Salmonella senftenberg,* and *Salmonella typhimurium.*

Experimental part

The following heterocyclic compounds were applied in the biological research: *2-acetylamino-1,3,4-thiadiazole-5-sulfonamide* (ATS), *2-amino-1,3,4-thiadiazole* (AT) and *bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide* (BATD). They were prepared with the use of the procedures described below. Reagents of the analytically pure class produced by Sigma-Aldrich and POCh Gliwice were used for syntheses.

Preparing 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (ATS)

For the purpose of the biological study, concerning the subject of our research, we prepared acetazolamide with the use of hydrogen peroxide – hydrochloric acid. The synthesis was carried out as follows: First, 50.0 cm^3 (1.62 mole) of concentrated 36.0 % hydrochloric acid (d = 1.18 g/cm^3) was introduced into the reactor equipped with the reflux condenser, the mixer and the thermometer. Then, holding temperature within the range of: 5 to 10 ^oC, 80 cm^3 (2.64 mole) of 30.0 % hydrogen peroxide (d = 1.11 g/cm^3) was added in small doses. After introducing the whole amount of the oxidizer, 5.0 g (0.029 mole) of 2-acetylamino-5-mercapto-1,3,4-thiadiazole was added in doses. The

last stage included the introduction of 99.5 % (d = 1.05 g/cm^3) acetic acid in the total volume of 50.0 cm^3 (0.88 mole). All the components were stirred for 120 min in temperature ranging from 5 to 10 ^{o}C. The obtained precipitate of 2-acetylamino-5-sulfochloro-1,3,4-thiadiazole (7.7 g in the form of the moist substance) was filtered and washed with ice-cold distilled water and submitted to ammonolysis by applying 9-time surplus of 25 % aqueous ammonia (d = 0.90 g/cm^3). Later, 2-acetylamino-5-sulfochloro-1,3,4-thiadiazole was introduced into 68.6 cm^3 of ammonia with the temperature not exceeding 20 ^{o}C. Then, the reaction mixture was heated for 120 minutes at 50 ^{o}C in order to remove the excess of ammonia. After that, charcoal was added and heated for 10 minutes. The hot mixture was filtered and cooled down to 2–3 ^{o}C. Later it underwent acidification with 36.0 % hydrochloric acid up to pH = 1. The obtained acetazolamide was separated and recrystallised from hot, distilled water. 2.05 g (yield: 32.0 %) of 2-acetylamino-5-sulfonamido-1,3,4-thiadiazole at melting point (m.p.) 257 ^{o}C (lit. m.p. 258–259 ^{o}C [15]) was prepared.

Preparing 2-amino-1,3,4-thiadiazole (AT)

The next compound: 2-amino-1,3,4-thiadiazole (AT) was prepared from thiosemicarbazide and ethyl orthoformate using the method described in [19]. A mixture containing 4.5 g (0.05 mole) of thiosemicarbazide and 8.0 cm^3 (0.05 mole) of ethyl orthoformate was introduced into the reactor equipped with a reflux condenser, a mixer and a thermometer. Then the mixture was heated on a water bath for 6 hours. Then, 250 cm^3 (4.3 mole) of boiling ethyl alcohol was added. The hot mixture was filtered in order to remove *N,N'*-bis-(1,3,4-thiadiazole-2) formamide. The filtrate was concentrated in a vacuum evaporator until approx. 50 cm^3 and then cooled. As much as 2.31 g (yield: 32.6 %) of 2-amino-1,3,4-thiadiazole was obtained with the melting point ranging from 190 to 191 ^{o}C (lit. m.p. 191 ^{o}C [19]).

Preparing bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide (BATD)

Bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide (BATD) (dimer) was obtained according to the method of Sarbu and co-workers described in item [20]. First, 0.40 g (0.01 mole) of sodium hydroxide was diluted in 100 cm^3 of water. Then, 2.2 g (0.01 mole) of technical acetazolamide was introduced at temperature 5–10 ^{o}C. Within the time of 1 hour, wet 2-acetylamino-5-sulfochloro-1,3,4-thiadiazole was added. After introducing sulfochloride, the mixture was kept for 90 minutes at the temperature ranging from 5 to 10 ^{o}C. Residual, unreacted acetazolamide and sulfochloride were filtered out. Afterwards, the filtrate was treated with the 40 % aqueous solution of sodium hydroxide until it reached pH = 12. After 24 hours, a white precipitate was found in the filtrate, then it was separated and dissolved in the minimum amount of water, heated and acidified with 36.0 % hydrochloric acid (d = 1.18 g/cm^3) up to 1 to 3 pH. After cooling, white crystals were separated from the solution, which were filtered and dried in the ambient temperature to the constant weight. 0.645 g of the product was obtained (yield: 15.3 %); melting point: 307–311 ^{o}C (lit.: m.p. 318 ^{o}C [20]).

The solutions of the synthesised compounds of concentration from 0.5 to 100.0 mg/cm^3, presented above, were prepared with the use of the dimethylsulfooxide (DMSO) solvent. A control sample with bactericidal properties was made for every strain of bacteria. The effect of tested heterocyclic compounds concerning their sensitivity to Gram-positive and Gram-negative aerobic bacteria, such as: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Enterococcus faecium*, *Salmonella senftenberg* and *Salmonella typhimurium* was determined. Bacterial strains were received from Hohenheim University in Stuttgart. The above microorganisms were multiplied within 24 hours and diluted in liquid substratum: Standard-I Broth and added to the solid medium: Standard-I Agar (Standard I-Nhragar, Merck No. 7881) in the amount equal to 0.5 cm^3 of suspended microbes per 250.0 cm^3 of agar. Then, Agar with vaccinated microbes was spilled on the Petri dishes and left to solidify. Afterwards, cylindrical wells with the radius of 4 mm were cut in the solidified bases, and filled with solutions of the tested compounds (this activity was performed twice each time and the amounts used were, respectively: 50.0 and 100.0 mg/cm^3; 25.0 and 50.0 mg/cm^3; 1.0 and 12.5 mg/cm^3; 0.25 and 0.5 mg/cm^3). The dissolvent itself was acting as the control. The samples were incubated in the temperature of 37 ^{o}C for 24 hours [21]. After the incubation, the areas of microbes growth inhibition (in mm) were measured and the *minimal inhibition concentrations* (MIC) were defined.

Results and discussion

The bactericidal properties of sulfonamides with free amine group in para-position in benzene ring [22] are discussed in the specialist literature. Seeking effective substances to eliminate pathogenic bacteria and, at the same time, the bacteria present in different types of pharmaceutical waste, a group of heterocyclic compounds was typed: *2-amino--1,3,4-thiadiazole* (AT) and *2-acetylamino-1,3,4-thiadiazole-5-sulfonamid* (ATS), and *bis(2-acetylamino-1,3,4-thiadiazole)-5,5'-disulfonamide* (BATD). In order to achieve this aim, studies on biological activity of all the derivatives of 1,3,4-thiadiazole for selected environmental bacteria: *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis, Enterococcus faecium*, *Salmonella senftenberg*, *Salmonella typhimurium*, *Escherichia coli* and *Staphylococcus aureus* were carried out. Pure compounds contained in solid wastes and waste water from diuramid production were used in the research. Tables 1–3 present the determined activities of the tested chemical compounds for different concentrations reacting with the individual bacteria and values necessary to determine their biological activity. The values presented in the form of a table describe: R – half of the zone of bacteria growth inhibition and r – radius of the well equal to 4.0 mm, respectively. Basing on R/r, the activity of a given compound was determined for individual tested bacteria strains within the range of 1,3,4-thiadiazole concentration. As the results clearly illustrate: if the relation $R/r < 1$ occurs, it means low activity of the studied chemical substance. The relation $R/r < 2$ indicates medium and $R/r \geq 2$ high activity [21]. As the data demonstrate, after 24 hours of incubation at temperature 37 ^{o}C, the zone of bacteria growth inhibition expressed in the form of minimum inhibitory concentration (MIC) depended on the type of the tested compound as well as on the

Table 1

Activity of 2-acetamide-1,3,4-thiadiazole-5-sulfonamide (ATS) for chosen bacterial species

Bacterial species	Concentration [mg/cm^3]											
	12.5			25.0			50.0			100.0		
	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity
Escherichia coli	4.0	1.0	++	4.0	1.0	++	4.0	1.0	++	4.0	1.0	++
Staphylococcus aureus	4.0	1.0	++	3.0	0.8	+	3.5	0.9	+	4.0	1.0	++
Bacillus subtilis	—	—	—	—	—	—	—	—	—	—	—	—
Bacillus cereus	3.0	0.8	+	3.5	0.9	+	4.0	1.0	++	n.s.	n.s.	n.s.
Proteus mirabilis	2.5	0.6	+	3.0	0.8	+	3.5	0.9	+	n.s.	n.s.	n.s.
Enterococcus faecium	3.5	0.9	+	2.5	0.6	+	2.5	0.6	+	n.s.	n.s.	n.s.
Salmonella senftenberg	3.0	0.8	+	3.5	0.9	+	3.5	0.9	+	n.s.	n.s.	n.s.
Salmonella typhimurium	2.5	0.6	+	3.0	0.8	+	3.0	0.8	+	n.s.	n.s.	n.s.

Where: n.s. – non-studied; +++ – high activity; ++ – middle activity; + – low activity; — – deficiency of activity.

Table 2

Activity of 2-amide-1,3,4-thiadiazole (AT) for chosen bacterial species

Bacterial species	Concentration [mg/cm^3]																	
	0.5			1.0			12.5			25			50			100		
	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity
Escherichia coli	3.0	0.8	+	4.0	1.0	++	5.0	1.3	++	6.0	1.5	++	6.5	1.6	++	7.0	1.8	++
Staphylococcus aueus	3.5	0.9	+	7.5	1.9	++	14.5	3.6	+++	11.5	2.9	+++	11.0	2.8	+++	12.0	3.0	+++
Bacillus subtilis	4.0	1.0	++	7.5	1.9	++	11.0	2.8	+++	10.0	2.5	+++	11.0	2.8	+++	n.s.	n.s.	n.s.
Bacillus cereus	3.0	0.8	+	4.0	1.0	++	6.5	1.6	++	5.5	1.4	++	8.0	2.0	+++	n.s.	n.s.	n.s.
Proteus mirabilis	—	—	—	2.5	0.6	+	2.5	0.6	+	5.5	1.4	++	7.0	1.8	++	n.s.	n.s.	n.s.
Enterococcus faecium	3.0	0.8	+	3.5	0.9	+	4.0	1.0	++	7.5	1.9	++	13.0	3.2	+++	n.s.	n.s.	n.s.
Salmonella senftenberg	2.5	0.6	+	3.0	0.8	+	4.0	1.0	++	9.0	2.2	+++	11.0	2.8	+++	n.s.	n.s.	n.s.
Salmonella typhimurium	2.5	0.6	+	2.5	0.6	+	3.5	0.9	+	16.0	4.0	+++	17.0	4.2	+++	n.s.	n.s.	n.s.

Where: n.s. – non-studied; +++ – high activity; ++ – middle activity; + – low activity; — – deficiency of activity.

Table 3

Activity of bis(2-acetamide-1,3,4-thiadiazole)-5,5'-disulfonamide (BATD) for chosen bacterial species

Bacterial species	Concentration [mg/cm^3]											
	12.5			25.0			50.0			100.0		
	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity	R	R/r	Activity
Escherichia coli	2.5	0.6	+	—	—	—	2.5	0.6	+	3.0	0.8	+
Staphylococcus aueus	—	—	—	3.0	0.8	+	3.5	0.9	+	6.5	1.6	++
Bacillus subtilis	—	—	—	—	—	—	3.0	0.8	+	n.s.	n.s.	n.s.
Bacillus cereus	2.5	0.6	+	3.5	0.9	+	4.0	1.0	++	n.s.	n.s.	n.s.
Proteus mirabilis	3.0	0.8	+	3.0	0.8	+	3.5	0.9	+	n.s.	n.s.	n.s.
Enterococcus faecium	—	—	—	2.5	0.6	+	3.0	0.8	+	n.s.	n.s.	n.s.
Salmonella senftenberg	2.5	0.6	+	2.5	0.6	+	3.5	0.9	+	n.s.	n.s.	n.s.
Salmonella typhimurium	—	—	—	3.0	0.8	+	3.5	0.9	+	n.s.	n.s.	n.s.

Where: n.s. – non-studied; +++ – high activity; ++ – middle activity; + – low activity; — – deficiency of activity.

bacteria strain. Table 1 puts together values referring to determined activities of ATS for the tested species of bacteria. Table 2 contains the results obtained for AT and Table 3 – these for BATD. Tables 1 and 3 present the results for the following concentrations applied in the tests: 12.5, 25.0, 50.0, and 100.0 mg/cm^3. Additionally, Table 2 – presents concentrations 0.5 and 1.0 mg/cm^3 for the most biologically active AT compound. Biological activity for all the derivatives of 1,3,4-thiadiazole with the concentration of 100.0 mg/cm^3 were not tested for: *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis, Enterococcus faecium*, *Salmonella senftenberg* and *Salmonella typhimurium*.

On the basis of the research carried out it was found that ATS shows medium activity of identical values in relation to *Escherichia coli* in all tested concentrations (Table 1). Low activity of ATS with concentrations: 12.5, 25.0, and 50.0 mg/cm^3 was found for: *Proteus mirabilis*, *Enterococcus faecium*, *Salmonella senftenberg* and *Salmonella typhimurium*. It was found that for *Staphylococcus aureus* and *Bacillus cereus* low activity of ATS prevailed. No activity was detected for *Bacillus subtilis* in the whole range of concentrations. It could be the result of ATS chemical structure and the difference between its functional groups and reactive groups of *Bacillus subtilis*. Therefore, ATS is characterised by low biological activity in relation to the tested strains of bacteria.

The next tested compound: 2-amino-1,3,4-thiadiazole (AT) demonstrates: high activity for *Staphylococcus aureus* with concentrations within the range 12.5–100.0 mg/cm^3 (determined MIC = 12.5 mg/cm^3), medium activity with concentration 1.0 mg/cm^3, and low activity with concentration 0.5 mg/cm^3 (Table 2). Diversified activity was found for *Bacillus cereus*, where high activity for concentration 50.0 mg/cm^3, medium activity – for 1.0 mg/cm^3 and low activity – for 0.5 mg/cm^3; for *Enterococcus faecium* these are, respectively: high activity – MIC = 50.0 mg/cm^3, medium – MIC = 12.5 mg/cm^3, low MIC = 0.25 mg/cm^3 (the only activity value possible to be determined at the concentration of 0.25 mg/cm^3 is not included in the table); *Salmonella senftenberg*: MIC for high activity – 25.0 mg/cm^3, MIC for medium activity – 12.5 mg/cm^3, and MIC for low activity – 0.5 mg/cm^3. In case of *Escherichia coli*, medium activity of AT was found within the range of concentrations from 1.0 to 100.0 mg/cm^3 (MIC of medium activity: 1.0 mg/cm^3) and low activity for 0.5 mg/cm^3. Only *Bacillus subtilis* showed high and medium activities (MIC, respectively: 12.5 mg/cm^3 and 0.5 mg/cm^3) while *Salmonella typhymurium* showed high and low activities (MIC, respectively: 25.0 and 0.5 mg/cm^3). The relation between AT and *Proteus mirabilis* appeared to be an interesting case in which medium activity was determined for concentrations 25.0 and 50.0 mg/cm^3, low activity – for 1.0 and 12.5 mg/cm^3, and zero activity for 0.5 mg/cm^3. It was found that AT was characterised by the most diversified activity amongst the tested 1,3,4-thiadiazole derivatives in relation to *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecium* and *Salmonella senftenberg* bacteria, which could be determined by a suitable selection of MIC.

Dimer bis(2-acetamido-1,3,4-thiadiazole)-5,5'-disulfonamide (BATD) is characterised by its lack of activity or low activity in relation to the tested strains of bacteria. As in the case of ATS towards *Bacillus subtillis*, the dimer shows no activity and low

activity for the concentration of 50.0 mg/cm^3. The lack of BATD activity in relation to *Bacillus subtilis* may be interpreted by similarity of chemical structure of functional groups to ATS.

It was found that the low activity of dimer occurred for *Escherichia coli*, *Proteus mirabilis*, *Salmonella senftenberg*, *Enterococcus faecium,* and *Salmonella typhimurium.* Interestingly enough, the lack of activity was observed for the concentration of 12.5 mg/cm^3. The most diverse activities were observed for *Staphylococcus aureus*: from medium activity for the concentration of 100.0 mg/cm^3, through low activity for the concentrations of 25.0 and 50.0 mg/cm^3, and the lack of activity for the concentration of 12.5 mg/cm^3. Medium activity was found for *Bacillus cereus* for the concentration of 50.0 mg/cm^3, and the deficiency of activity for the concentrations of 12.5 and 25.0 mg/cm^3.

Figures 1–4 present the values' dependence on the growth zone inhibition [in mm] for the given individual bacterial strains as a function relating to the concentration of 1,3,4-thiadiazole derivatives selected for the tests.

Figure 1 shows the values' dependence on the size of growth zone inhibition for *Proteus mirabilis*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella senftenberg*, *Salmonella typhimurium* bacteria towards concentrations of biologically most active compound 2-amino-1,3.4-thiadiazole (AT). For all the tested strains of bacteria with the increase of AT concentration there is the increase in size of growth zone inhibition. The most steady increase of the growth zone inhibition was observed for *Proteus mirabilis, Bacillus cereus* and *Bacillus subtilis*. The difference between values of the growth zone inhibition was observed in case of: *Salmonella senftenberg* and *Salmonella typhimurium* for AT concentration of 25.0 mg/cm^3, which for the concentration of 12.5 mg/cm^3 was higher than the preceding value by 2.25 and 4.6 times, respectively. The values of growth zone inhibition of *Staphylococcus aureus* are worth considering. In this case a significant increase the trend was observed up to AT

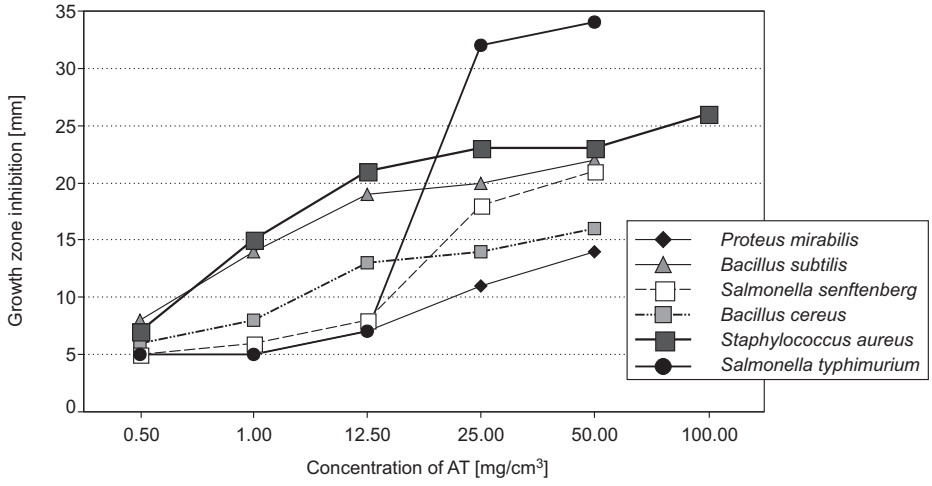

Fig. 1. Effect of AT concentration on the level of the growth zone inhibition for selected strains of bacteria

concentration of 12.5 mg/cm^3, and then the values of this parameter were kept on the same level.

Figures 2–4 present the characteristics for *Escherichia coli* and *Enterococcus faecium* bacteria as its dependence on the growth zone inhibition in the function of ATS (Fig. 2), AT (Fig. 3), and BATD (Fig. 4) concentrations. These bacteria are considered to be an environmental sanitary indicator.

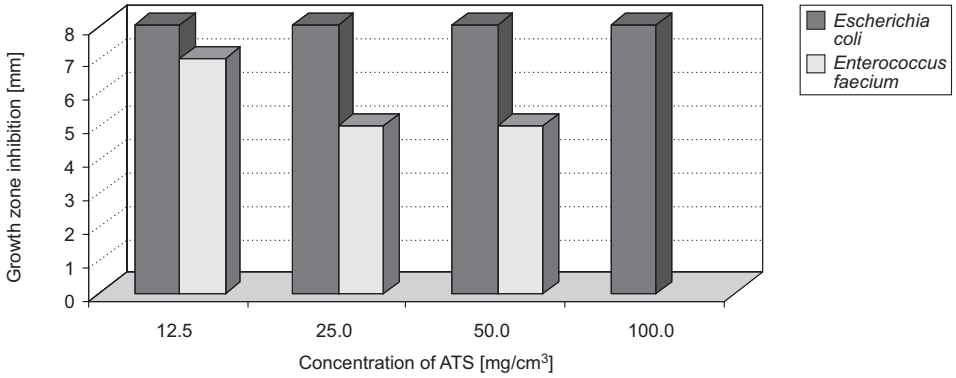

Fig. 2. Effect of ATS concentration on the level of the growth zone inhibition for *E. coli* and *Enterococcus faecium*

Together with the increase of ATS concentration, values of the growth zone inhibition for *Escherichia coli* remained unchanged at 8.0 mm. As far as the case of *Enterococcus faecium* is concerned, a different fact was observed: the value of the growth zone inhibition increased with the decrease of ATS. It might be determined that the optimal ATS concentration which inhibits the *Enterococcus faecium's* growth zone is 12.5 mg/cm^3.

Figure 3 indicates a very steady increase of *Escherichia coli* growth zone inhibition with the increase of AT concentration within the range of 0.5–100.0 mg/cm^3. A more

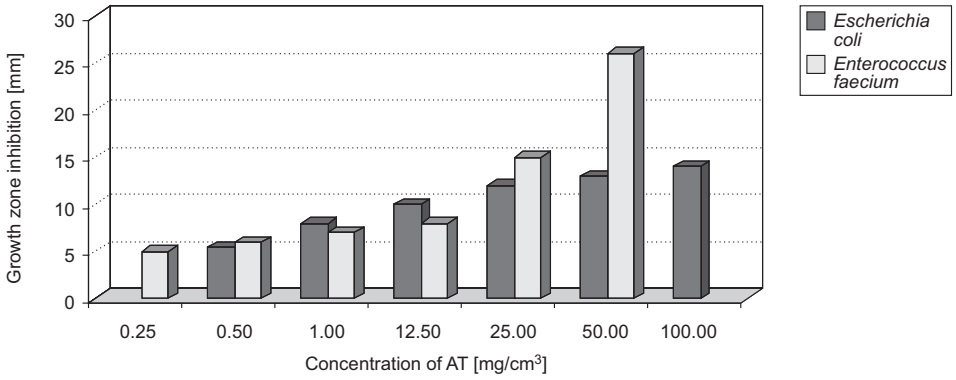

Fig. 3. Effect of AT concentration on the level of the growth zone inhibition for *E. coli* and *Enterococcus faecium*

rapid increase of the *Enterococcus faecium* growth zone inhibition (even up to 25.5 mm) is observed with the increase of AT concentration within the range from 0.25 to 50.0 mg/cm^3.

A small increase in the value of *Escherichia coli* and *Enterococcus faecium* growth zone inhibition (by one unit only) is characterised by the increase of BATD concentration within the range from 25.0 to 50.0 mg/cm^3 for *Enterococcus faecium*, and from 12.5 mg/cm^3 (50.0) to 100.0 mg/cm^3 for *Escherichia coli* (Fig. 4).

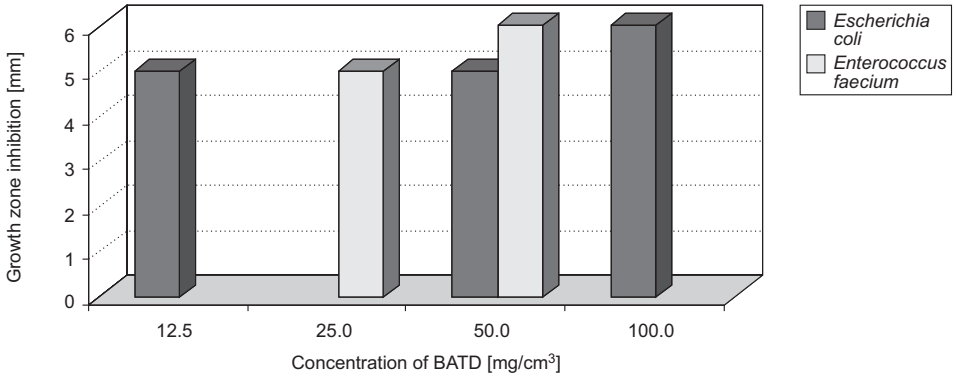

Fig. 4. Effect of BADT concentration on the level of the growth zone inhibition for *E. coli* and *Enterococcus faecium*

For each strain of the tested bacteria, a control test of organic solvent used for dissolving the chemical compounds was carried out. The tests were conducted in order to define a possible effect of the solvent used on the tested microorganisms. The studied microbes are the representatives of pathogenic microorganisms that are generally identified in the environment [23] and belong to the group of the most thermo-tolerable bacteria. Only in case of *Proteus mirabilis* and *Salmonella senftenberg* bacteria an occasional minimum bright area of diameter not exceeding the sensitivity of the method was found.

Conclusion

On the basis of the research it was found out that 2-amino-1,3,4-thiadiazole (AT) presented the highest bacteriological activity in relation to all the tested micro-organisms. It was found that the activity inhibiting the growth of the tested bacteria in this case amounted to 0.25 mg/cm^3. However, bis(2-acetylamino-1,3,4-thiadiazole--5,5'-disulfonamide) (BATD) was characterized by the lowest bacteriological activity, because MIC for this compound was 12.5 mg/cm^3.

2-amino-1,3,4-tiadiazol (AT) turned up to be the most effective as far as inhibiting the growth of the *Salmonella typhimurium* and *Salmonella senftenberg* bacteria is concerned. These microbes belong to the *Enterobacteriaceae* family which contains numerous species and strains living on different organisms and environments [24].

Salmonella typhimurium is a representative of the particularly dangerous microbes causing many pathogenic infections. The data indicate that in order to inhibit the growth of microbes belonging to *Salmonella* type, AT should be applied in the dosage of up to 25.0 mg/cm^3 due to its high activity in this range of concentration. Also, the activity of this reagent with the *Bacillus* type microorganisms appeared to be quite effective, especially in the case of the tested *Bacillus subtilis* species. As it results from the data, its high activity was observed even in the case of the 12.5 mg/cm^3 concentration, and medium activity was found for the concentration of 0.25 mg/cm^3. The tested species of *bacilli,* which belong to the *Bacillus* type are the examples of the microorganisms living in the environment rich in organic substance characteristic for soil. Furthermore, from the clinical point of view they are a source of allergic chronic diseases and alimentary toxicoses [24]. The obtained results indicate that in order to eliminate *Bacillus subtilis* bacteria, high bactericidal activity was observed for the optimal AT concentration of 12.5 mg/cm^3, but medium activity was observed even for 0.25 mg/cm^3. It should be noticed that in case of determining bactericidal activity for this group of bacteria, the deficiency of activity was found in case of *Bacillus subtilis* for ATS and low activity in case of BATD for 50.0 mg/cm^3.

Escherichia coli and *Enterococcus faecium* bacteria were used as indicators of the environmental microorganisms (Fig. 2–4). The activity of ATS and AT, as far as their interaction with *Escherichia coli* is concerned, was found at the medium level for each tested concentration ranging respectively: from 12.5 to 100.0 and from 0.5 to 100.0 mg/cm^3. The presence of these bacteria in the environment, especially in the surface waters, is a basic quality indicator of the biologically clean waters and allows for classifying them into their respective quality classes. However, *Enterococcus faecium* bacteria which belong to *Enterococcus* type are the microbes characteristic for the environment as far as the pollution with *fecal streptococci* is concerned [23]. Furthermore, their presence points at different types of the environmental pollution with the substances of organic origin [25]. As far as their influence on *Enterococcus Faecium* microorganisms activity is concerned the tested compounds were characterized by low effectiveness in relation to ATS and BATD. It was found that AT activity is concentration-based and demonstrates high, medium and low activity with the concentrations: 50 mg/cm^3, 12.5 to 25.0 mg/cm^3 and 0.25 to 0.5 mg/cm^3, respectively. These data proved that the tested thiadiazole derivative could be selectively used to eliminate pollutions of fecal origin.

Furthermore, it can be found that properly selected AT concentrations allow to eliminate selectively such microorganisms as *Salmonella senftenberg*, *Bacillus cereus* and *Staphylococcus aureus*.

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WPŁYW POCHODNYCH 1,3,4-TIADIAZOLI NA AKTYWNOŚĆ WYBRANYCH BAKTERII ŚRODOWISKOWYCH

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Abstrakt: Przedstawiono wyniki badań mikrobiologicznych, prowadzonych z udziałem mikroorganizmów, występujących m.in. w środowisku biologicznym ścieków gospodarczo-bytowych oraz w osadach ściekowych z komunalnych oczyszczalni. Badano wpływ wybranych związków heterocyklicznych pochodnych 1,3,4-tiadiazolu na wrażliwość bakterii aerobowych Gram-dodatnich i Gram-ujemnych. Do badań aktywności biologicznej wytypowano szczepy bakterii środowiskowych: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Proteus mirabilis*, *Enterococcus faecium*, *Salmonella senftenberg* i *Salmonella typhimurium.* Dla badanej grupy bakterii najbardziej aktywnym związkiem chemicznym okazał się 2-amino-1,3,4-tiadiazol. Stwierdzono, że odpowiednio dobrane stężenia tego związku pozwalają na selektywną eliminację takich drobnoustrojów, jak: *Salmonella senftenberg, Bacillus cereus, Staphylococcus aureus* i *Enterococcus faecium*. W przypadku *Escherichia coli* najniższe stężenie hamujące wzrost bakterii (MIC) stwierdzono na poziomie 12,5 mg/cm^3 (wyznaczone względem 2-acetyloamino-1,3,4-tiadiazolo-5-sulfonamidu i bis(2-acetyloamino-1,3,4-tiadiazolo)-5,5'-disulfonamidu), a dla 2-amino-1,3,4-tiadiazolu 0,5 mg/cm^3. Podobne wyniki uzyskano w przypadku bakterii *Enterococcus faecium* z tym, że dla 2-amino-1,3,4-tiadiazolu MIC < 0,25 mg/cm^3, a dla bis(2-acetylamino-1,3,4-tiadiazolo)-5,5'-disulfonamidu MIC = 25,0 mg/cm^3.

Słowa kluczowe: pochodne 1,3,4-tiadiazolu, badania mikrobiologiczne, wskaźnik sanitarny środowiska