Antibiotics and endocrine disruptors in sewage sludge samples in terms of its agriculture use

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ABSTRACT

The formation of sewage sludge in the wastewater treatment process is an inevitable component of this process. To manage this waste in an ecological manner, the qualitative and quantitative composition of the sludge should be considered. Studies of sewage sludge clearly indicate the presence of micropollutants in raw sewage, such as residues of pharmaceuticals. This broad group of compounds differs in terms of physicochemical properties and the ability to adsorb to sludge. Some of them are significantly reduced in the treatment process. There is a large group of hydrophilic compounds that are only slightly reduced and thus leave the treatment plant together with the treated sewage. Substances characterized by high adsorption to sewage sludge, such as endocrine compounds, may affect the possibility of its safe use. This paper focuses on the review of the methods of determination of antibiotics and endocrine disruptors in sewage sludge. Issues related to the selection of the sample type, the method of its preparation and the selection of the appropriate chromatographic analysis were discussed. The obtained data will make it possible to assess the safe use of sewage sludge in agriculture, which is important for the implementation of the circular economy in sewage sludge management.

Keywords: Sewage sludge; Micropollutants; Pharmaceuticals; Sewage sludge management

1. Introduction

In the process of sewage treatment, the stabilized sewage sludge produced in large quantities must be managed with minimal environmental pressure. The most frequently chosen method of sludge management in Poland in 2018 was soil reclamation and other agricultural procedures [1]. It is the cheapest method of sludge disposal from the wastewater treatment plant (WWTP). Although, the mineral richness of the sludge is the advantage of this method of utilization, there is a risk of spreading hazardous substances such as pesticides, heavy metals, micropollutants and pathogens [2,3]. Contaminants such as endocrine-disrupting compounds (EDCs) [4] are leached from soils treated with solid waste. This means that the quantitative and qualitative composition of the sludge introduced into the environment is an extremely important aspect, as the effect of the sludge on the soil on which it is spread is indisputable [5].

These hazardous substances include pharmacological compounds and their derivatives, personal care products, perfumes, UV filters, substances for everyday use, for example, bisphenol A or repellents. This broad, diverse group of compounds are generally referred to as 'new generation' micropollutants, or chemicals of emerging concern (CECs). Due to increasing consumerism and the desired higher

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standard of living, these substances are used by humans widely and in increasing amounts.

Some of these compounds are not reduced in wastewater treatment plants in any way [6]. This means that such substances leave the plant either with treated sewage or associated with sewage sludge [7]. CECs get into the environment by a direct route – through sewage discharge or indirectly – by the spreading of sewage sludge with adsorbed micropollutants onto agricultural land or it is irrigating with sewage [8–10]. In this way, not only the local environment is contaminated, but also micropollutants get through the natural water circulation to waters that are intended for consumption.

The European Commission also became interested in the problem of the presence of CECs in waters. In the Directive of the European Parliament and of the Council of 2013 on priority substances in the field of water policy, the so-called 'watch list' of substances that should be monitored in detail included such substances as diclofenac (a non-steroidal anti-inflammatory drug) and 17β-estradiol and 17α-ethinylestradiol (endocrine compounds) [11,12]. In 2018, estrone was added to 'the watch list' [13], and in 2020 also antibiotics: sulfamethoxazole and trimethoprim, the antidepressant venlafaxine and its metabolite O-desmethylvenlafaxine [14].

At the same time, in 2019, the Commission to the European Parliament issues a Communication, which states "the presence of residues of several pharmaceutical substances in surface and groundwater, soil and animal tissues" throughout the EU [15] and recommends taking measures to minimize the impact of pharmaceuticals and personal care products (PPCPs) on the natural environment by monitoring their presence and implementing technologies which reduce their content. This is of particular importance for the introduction of sludge in the circular economy. It is very probable that in the near future the maximum allowable concentration for individual micropollutants in treated sewage, drinking water or sludge used in agriculture will be determined to protect animal and human health.

The presence of micropollutants in the sewage sludge is observed at trace amounts (μg/kg or ng/kg), which makes the measurement of their content difficult. This forces one to seek very sensitive analytical methods [16]. An additional difficulty is the diversity of their chemical structures and properties, which affects the specificity and selectivity of the research method [6,7].

Undoubtedly, the constant exposure of a living organism to chemicals of emerging concern results in negative changes in its physiology and disruptions in natural biosystems [17].

The need to manage sludge in a circular manner requires detailed research on their composition. Widespread agricultural use (e.g., as fertilizers based on stabilized sewage sludge) contributes to the distribution of substances related to the sludge. Such compounds include some groups of micropollutants that strongly adsorb on the solid particles.

The article presents an overview of research methods for these compounds that are a potential threat to the natural environment in the case of sludge management for agricultural purposes. This work analyzes the results of research from 2011 to 2019. Special attention will be focused on hydrophobic substances like endocrine compounds and antibiotics [18]. The issues of reliable quantitative measurements of these substances and the possibility of their reduction from solid particles will also be addressed.

2. Micropollutants

CECs are detected practically in every type of water as well as in solid samples such as soils or sewage sludge. These compounds were detected all over the world in various concentrations, both in wastewater and waters [19–25]. Pharmaceuticals were detected in raw sewage at the level of several hundred ng/L, and in treated sewage this value dropped to even several dozen ng/L [19,20]. Due to the continuous circulation of water in nature, research was also undertaken on micropollutants in groundwater, surface waters and in waters intended for consumption [21–24]. The content of these compounds was lower than in the case of sewage and ranged from a few to several dozen ng/L [26]. The lowest concentrations were recorded for drinking water [19,27]. Pharmaceuticals were also detected in the solid samples, which will be discussed later in this work. They constitute a highly diversified group of chemical compounds. Among them, the following types of substances are distinguished along with examples of compounds found in the tested environmental samples [28]:

- painkillers: acetylsalicylic acid, paracetamol
- NSAIDs: diclofenac, ibuprofen, ketoprofen, naproxen, flunixin, mefenamic acid, niflumic acid, phenylbutazone
- psychotropic drugs: carbamazepine, fluoxetine
- hypolipemic drugs: clofibric acid, bezafibrate
- β-blockers: metoprolol, propanolol, atenolol
- antibacterial drugs: chloramphenicol, florfenicol, pyrimethamine, thiamphenicol, trimethoprim, macrolides, sulfonamides, quinolones
- hormones: 17α-ethinylestradiol, 17β-estradiol, estrone
- antibacterial and antifungal agents present in some consumer products, including toothpaste, soaps, detergents, for example, triclosan.

Moreover, CECs also include widely used chemicals as caffeine [29], illicit drugs together with their derivatives [25] and bisphenol A [30].

Lipophilicity and acidity are essential features that determine the behavior of substances during traditional wastewater treatment using the activated sludge method. Lipophilicity is defined by the lipophilicity coefficient, the octanol/water partition coefficient ($\log K_{OW}$). It is estimated by determining the ratio of the concentration of the substances in the two immiscible phases: octanol/water. The higher this coefficient, the greater is the ability to adsorb to solid particles. The acidic nature of the compound, defined by the relationship between the pKa and the pH of the soil (sludge, suspension), determines whether the tested analyte will be in a dissociated form or not. When the pH of the environment is higher than the pKa of the analyte, the compound will exist predominately in its deprotonated form and will accumulate less on the sewage sludge and dissolve more easily in the liquid phase (e.g., ibuprofen, diclofenac, ketoprofen) [6]. Substances

Table 1
Determinations of micropollutants in sewage sludge

	Type of solid sample and its pretreatment	Sample pretreatment	Instrumental analysis	LOQ	Recovery efficiency	References
Antibiotics: chloramphenicol, thiamphenicol; Hormones: 17α-ethinylestradiol, 17β-estradiol, estrone	Lyophilized sewage sludge	• MAE (methanol:water, 3:2, v/v) • SPE (Oasis HLB)	GC-MS with derivatization	0.8–5.1 ng/kg	92%–99%	[36]
Quinolone antibiotic derivatives: pipemidic acid (PIP), enoxacin (ENO), norfloxacin (NOR), ciprofloxacin (CIP), ofloxacin (OFX), enrofloxacin (ENR), lomefloxacin (LOM), moxifloxacin (MOX), cinoxacin (CIN), nalidixic acid (NAL), oxolinic acid (OXO), flumequine, (FLU), piromidic acid (PIR), marbofloxacin (MAR), 2-phenyl-4-quinolinecarboxylic acid (cincophen, CIC)	Dried sewage sludge	• UAE (methanol:McIlvaine buffer, 1:1, v/v, pH = 3) • MAE (methanol:McIlvaine buffer, 1:1, v/v, pH = 3) • PLE (methanol:McIlvaine buffer, 1:1, v/v, pH = 3)	LC-MS/MS	• 6–18 ng/g • 6–15 ng/g • 4–18 ng/g	• 96.1%–103.8% • 96%–104.8% • 94.9%–104.8%	[37]
Estrogens: 17α-ethinylestradiol (EE2), 17β-estradiol (E2), estrone (E1), estriol (E3)	Lyophilized sediments from lakes and rivers	• UAE (water:methanol:acetone, 1:2:1, v/v/v) • SPE (carbograph 4)	UHPLC-MS/MS	1.28–7 ng/g	90%–108%	[38]
Hormones: estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethinylestradiol (EE2)	Lyophilized return sludge	• UAE (ethyl acetate) acidification with H_2SO_4 • clean-up: SPE (Oasis HLB-ethyl acetate); (Al_2O_3/silica gel)	GC-MS with derivatization (BSTFA)	1.2–5.5 ng/L	Not reported	[39]
Hormones: estrone (E1), 17β-estradiol (E2), estriol (E3), 17α-ethinylestradiol (EE2), bisphenol A (BPA)	Lyophilized sewage sludge	• clean-up: LLE, Florisil, aqueous alkali extraction (AAE), Oasis HLB	LC-MS/MS	0.1–0.5 ng/mL	15.0%–100.2%	[40]
Bisphenol A (BPA)	Dried sewage sludge	• PLE (ethyl acetate)	LC-MS	18 ng/g	99.4%–99.5%	[41]
Bisphenol A (BPA)	Drinking water treatment sludge	• LSE (ACN with acetic acid) • d-SPE • QuEChERS	LC-MS	50 μg/kg	87%	[42]
Sulfonamide antibiotics	Freeze-dried digested sewage sludge	• PLE (ACN:water; 25:75; v/v) • SPE (Oasis HLB)	HPLC-MS/MS	0.14–7.42 ng/g	60%–130%	[43]
Antibiotics: sulfathiazole (STZ), sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfapyridine (SPY)	Stabilized sewage sludge	• UAE (methanol:water, 1:1, 1:2, 1:3, v/v) • 0.2 μm nylon syringe filter	LC-MS/MS	Not reported	76%–131%	[44]

Analytes	Sample matrix	Extraction method	Analytical technique	LOD/LOQ	Recovery	References
EDCs: estradiol (E2), estrone (E1), estriol (E3), 17α-ethinylestradiol (EE2), diethylstilbestrol (DES), estriol 3-sulfate (E3-3S), estradiol 17-glucuronide (E2-17G), estrone-3-glucuronide (E1-3G), estriol-16-glucuronide (E3-16G), triclosan (TCS), methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), benzylparaben (BeP), triclocarban (TCC), BPA, tolyltriazole (TT), tris(2-butoxyethyl) phosphate (TBEP), tris(2-chloroethyl) phosphate (TCEP)	Lyophilized sewage sludge	• PLE (water:methanol:acetone, 1:2:1, v/v) • SPE (Oasis HLB)	TFC-LC-MS/MS	0.10–125 ng/g	40%–115%	[45]
Hormonal steroids: betamethasone, cortisol (hydrocortisone), cortisone, dexamethasone, flumethasone, methylprednisolone, prednisolone, prednisone, triamcinolone acetonide	Lyophilized sewage sludge	• PLE (methanol:acetone, 80:2) • SPE	UHPLC-MS/MS	1.0–+5.0 μg/kg	8%–73%	[46]
Antibiotics: ampicillin (AMP), amoxicillin (AMX), sulfamethoxazole (SMX), chloramphenicol (CAP), ciprofloxacin (CIP)	Air-dried sludge	• SPE (Phenomenex C$_{18}$)	HPLC-grade acetonitrile/ methanol	50 ng/g	Not detected	[47]
Antibiotics: sulfamethoxazole, trimethoprim Estrogens: 17α-ethinylestradiol, 17β-estradiol, estrone, estriol	Lyophilized primary, secondary and anaerobically digested sewage sludge	• UAE • SPE	HPLC	No data	No data	[48,49]
BPA	Freeze-dried sewage sludge	SPE (Oasis HLB or C18)	UHPLC	No data	68.4% ± 1.26%	[50]
Antibiotics: tetracyclines (TCs), sulfonamides (SAs)	Lyophilized sewage sludge	• PLE (citric acid:methanol, 1:1, v/v) • SPE (Oasis HLB)	ESI-LC-MS/MS	3.2–13 ng/g (TCs) 0.6–4.2 ng/g (SAs)	96.2%–100.9% (TCs) 90.4%–99.9% (SAs)	[51]
Antibiotics: tetracyclines (TCs), sulfonamides (SAs)	Freeze-dried sludge	SPE (methanol:acetone, 1:1, v/v; acetonitrile/5% oxalic acid, 8:2, v/v)	LC-QqQ-MS	No data	No data	[52]
Bisphenol A (BPA), estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2)	Activated sludge	• UAE (methanol) • SPME	GC-MS	5 ng/g (BPA) 4 ng/g (E1) 20 ng/g (E2) 10 ng/g (EE2)	No data	[53]

Table 1 Continued

	Type of solid sample and its pretreatment	Sample pretreatment	Instrumental analysis	LOQ	Recovery efficiency	References
Bisphenol A (BPA), estrone (E1)	Air-dried sewage sludge	• UAE (methanol:formic acid, 1:1, v/v) • SPE (Oasis HLB)	GC-MS with derivatization (MTBSTFA)	4.7 ng/g (BPA) 16 ng/g (E1)	80%–85% 78%–80%	[54]
Antibiotics: azithromycin, baquiloprim, cefoperazone, chlortetracycline, ciprofloxacin, clarithromycin, cloxacillin, daunorubicin, dicloxacillin, difloxacin, doxycycline, enoxacin, enrofloxacin, epirubicin, erythromycin, flumequine, ivermectine, josamycin, lincomycin, marbofloxacin, mevinolin, miconazole, minocycline, norfloxacin, ofloxacin, oxytetracycline, penicillin G, roxithromycin, sarafloxacin, sulfadiazine, sulfadimethoxine, sulfamethoxazole, sulfapyridine, tetracycline, tiamulin, tilmicosin, trimethoprim, tylosin, valnemulin, bithionol, chloramphenicol, novobiocin, spiramycin, triclocarban, triclosan Endocrine compounds: androstenedione, androsterone, androstenone, diosgenin, levonorgestrel, mestranol, norethindrone, progesterone, testosterone, dexamethasone, diethylstilbestrol, 17α-estradiol, 17β-estradiol, estriol, estrone, ethinylestradiol	Digested sludge	• QuEChERS extraction (PSA and MgSO₄) • d-SPE	LC-TOF-MS	10–50 ng/g (antibiotics) 667–3,000 ng/g (EDCs)	48%–127% (antibiotics) 70%–135% (EDCs)	[55]
Estrogens: 17α-ethinylestradiol (EE2), 17β-estradiol (E2), estrone (E1), estriol (E3)	Freeze-dried thickened and digested sludge	SPE (C18)	LC-MS/MS	0.01–2.5 ng/mL	70%–120%	[56]
Antibiotics: tetracyclines (TCs), sulfonamides (SAs), macrolides, fluoroquinolones	Freeze-dried sewage sludge	• UAE • SPE (Oasis HLB)	UHPLC-MS/MS	0.02–1.00 µg/kg	54%–130%	[57]
Antibiotics: trimethoprim, sulfamethoxazole (SMZ), azithromycin, tylosin, fluoxetine, norfluoxetine, clarithromycin EDCs: methylparaben (MeP), ethylparaben (EtP), bisphenol A, 17α-ethinylestradiol (EE2), 17β-estradiol (E2), estrone (E1), triclosan, tamoxifen	Freeze-dried digested sludge	• MAE (water:methanol, 50:50, v/v) • SPE (Oasis MCX, MAX)	UPLC-MS/MS	0.1–24.1 ng/g	40%–152%	[58]

Analytes	Matrix	Extraction	Instrumental method	LOD	Recovery	Reference
Estrogens: 17α-ethinylestradiol (EE2), 17β-estradiol (E2), estrone (E1), estriol (E3), BPA	Freeze-dried sewage sludge before and after anaerobic digestion	• UAE (methanol, acetone) • SPE (Oasis HLB)	LC-MS	16.6–33.3 pg absolute	95%–101%	[59]
Antibiotics: tetracyclines (TCs), sulfonamides (SAs), macrolides, quinolones, β-lactams	Lyophilized sewage sludge	• UAE (methanol, EDTA, citrate buffer) • SPE (Oasis HLB)	UPLC-MS/MS	0.3–3.2 ng/g	60.1%–92.7%	[60]
Antibiotics: lincomycin, clindamycin, trimethoprim	Freeze-dried sewage sludge	• UAE (methanol:water, 5:3, v/v) • SPE (C18)	HPLC-MS/MS	5.0 ng/g dry weight	No data	[61]
EDCs: nonylphenol (NP), nonylphenol monoethoxylate (NP1EO), nonylphenol diethoxylate (NP2EO), triclosan, bisphenol A	Freeze-dried thickened digested and dewatered sludge	• UAE • SPE	GC-MS	49–108 ng/g dry weight	64%–115%	[62]
Antibiotics: erythromycin (as hydrate, ERY), roxithromycin (ROX), azithromycin (as dehydrate, AZM), sulfamethoxazole (SMZ), sulfadiazine (SDZ), sulfamethazine (SM1), trimethoprim (TMP), ofloxacin (OFX), norfloxacin (NOR)	Freeze-dried primary and secondary sludge	• UAE • SPE (Oasis HLB)	HPLC-MS/MS	0.17–5.83 µg/kg	54%–139%	[63]
Antibiotics: ciprofloxacin, clarithromycin, erythromycin, trimethoprim, sulfamethoxazole Hormones: estrone	Lyophilized dewatered stabilised sludge	• PHWE • SPE (Oasis HLB)	UPLC-MS/MS	No data	17%–26%	[64]
Antibiotics: 4-epianhydrochlortetracycline, 4-epianhydrotetracycline, 4-epichlortetracycline, 4-epioxytetracycline, 4-epitetracycline, anhydrochlortetracycline, anhydrotetracycline, azithromycin, carbadox, cefotaxime, chlortetracycline, ciprofloxacin, clarithromycin, clinafloxacin, cloxacillin, demeclocycline, doxycycline, enrofloxacin, erythromycin-H₂O, flumequine, isochlortetracycline, lincomycin, lomefloxacin, minocycline, norfloxacin, ofloxacin, ormetoprim, oxacillin, oxolinic acid, oxytetracycline, penicillin G, penicillin V, roxithromycin, sarafloxacin, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulphanilamide, sulfathiazole, tetracycline, trimethoprim, tylosin, virginiamycin	Biosolids samples	• UAE (phosphate buffer/acetonitrile) • SPE	LC-MS/MS	No data	20%–150%	[65]

Table 1 Continued

	Type of solid sample and its pretreatment	Sample pretreatment	Instrumental analysis	LOQ	Recovery efficiency	References
Antibiotics: trimethoprim, azithromycin, clarithromycin, roxithromycin	Freeze-dried: • thickened • digested • dewatered sludge	• PLE (methanol) • SPE (Oasis HLB)	HPLC-MS/MS	0.2–16 ng/g (thickened) 0.2–14 ng/g (digested) 0.3–18 ng/g (dewatered)	31%–136% (thickened) 35%–126% (digested) 35%–133% (dewatered)	[66]
BPA	Lyophilized anaerobically digested sewage sludge	• UAE (methanol:acetic acid, 95:5, v/v) • d-SPE (C18)	LC-MS/MS	10.2 ng/g dry mass	89%	[67]
Antibiotics: sulfamethoxazole (SMX), sulfadimethoxine (SDM), tetracycline (TET), oxytetracycline (OXY) Estrogens: estrone (E1), 17β-estradiol (E2)	Lyophilized secondary sludge	• UAE (methanol:water) SPE (Oasis HLB, Strata SAX) – antibiotics • SPE (CarboPrep/NAX) – estrogens	HPLC-MS/MS	2.1–7.6 ng/g (antibiotics) 1.1–17 ng/g (estrogens)	17%–31% (antibiotics) 30%–59% (estrogens)	[68]
Antibiotics: amoxicillin, azithromycin, betamethasone, chloramphenicol, chlortetracycline, ciprofloxacin, clarithromycin, dicloxacillin, difloxacin, doxycycline, enrofloxacin, fluoxetine, sarafloxacin, sulfachloropyridazine, sulfaclozine, sulfadiazine, sulfadimethoxine, sulfadimidine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamonomethoxine, sulfamoxole, sulfapyridine, sulfathiazole, sulfisoxazole, tetracycline, thiamphenicol, tiamulin, trimethoprim, tylosin	Freeze-dried sludge	• UAE (methanol:Milli-Q water, 50:50, v/v) • no clean up	LC-MS/MS	3.1–66.3 ng/g	43%–107%	[69]

Analytes	Matrix	Extraction method	Analytical technique	LOD/LOQ	Recovery	Ref.
Antibiotics: ciprofloxacin, ofloxacin, azithromycin, sulfamethoxazole, clarithromycin, trimethoprim	Membrane biological reactor sludge (MBR)	PLE	UPLC-MS	No data	No data	[70]
Antibiotics: trimethoprim, sulfamethoxazole, clindamycin, lincomycin, miconazole, tiabendazole	Freeze-dried sludge	• UAE (methanol:water, 5:3, v/v) • SPE (Oasis HLB)	HPLC-MS/MS	0.5–50 ng/g	No data	[71]
Antibiotics: sulfamerazine, sulfameter, sulfamethoxazole, sulfadimethoxine, ofloxacin, sarafloxacin, oxytetracycline, tetracycline	Freeze-dried dewatered sludge	• MSPD (C18 sorbent, methanol, acetonitrile/oxalic acid; 8:2, v/v)	LC-MS/MS	0.595–4.25 µg/kg	50.3%–102.3%	[72]
Antibiotics: sulfonamides (sulfadiazine SDZ, sulfamethazine SMZ, sulfamethoxazole SMZ), tetracyclines (oxytetracycline OTC, doxycycline DOC, chlortetracycline CTC, tetracycline TC)	Freeze-dried: • primary sludge • waste sludge • dewatered sludge	• PLE (acetonitrile/water; 7:3) • SPE (Oasis HLB)	HPLC-MS	No data	49%–95%	[73,74]
Antibiotics: Quinolones	Compost from sewage sludge	• MAE (acetonitrile:m-phosphoric acid) • SALLE	UHPLC-MS/MS	0.6–1.5 ng/g	95.3%–106.2%	[75]
EDCs: estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2), estriol (E3), mestranol (MEST), progesterone (PROG), testosterone (TEST), androsterone (ANDR), diethylstilbestrol (DES), dienestrol (DNS)	Thermally dried sludge-amended soils	MSPD (C18 sorbent, acetonitrile:methanol, 90:10, v/v)	GC-MS/MS with derivatization (BSTFA and pyridine)	0.13–0.99 ng/g	80%–110%	[76]

Abbreviations: MAE – microwave-assisted extraction; SPE – solid-phase extraction; GC-MS – gas chromatography-mass spectrometry; UAE – ultrasound-assisted extraction; PLE – pressurized liquid extraction; LC-MS – liquid chromatography–mass spectrometry; UHPLC-MS/MS – ultra high-performance liquid chromatography–tandem mass spectrometry; BSTFA – N,O-bis(trimethylsilyl)trifluoroacetamide; LLE – liquid–liquid extraction; LC-MS/MS – liquid chromatography–tandem mass spectrometry; LSE – liquid-solid extraction; ACN – acetonitrile; HPLC-MS – high-performance liquid chromatography–tandem mass spectrometry; TFC-LC-MS/MS – turbulent-flow chromatography–liquid chromatography–tandem mass spectrometry; ESI-LC-MS/MS – electrospray ionization–liquid chromatography–tandem mass spectrometry; LC-QqQ-MS – liquid chromatography–triple quadrupole-mass spectrometry; SPME – solid-phase microextraction; MTBSTFA – N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide; QuEChERS – quick, easy, cheap, effective, rugged and safe; LC-TOF-MS – liquid chromatography–time of flight–mass spectrometry; PHWE – pressurized hot water extraction; ASE – accelerated solvent extraction; MSPD – matrix solid-phase dispersion

that are more easily adsorbed on sewage sludge, for example, antibiotics, will be characterized by higher or close to the soil pH, that is, they will have basic properties [6]. The most frequently detected antibiotics in the sludge in one of the wastewater treatment plants in Sweden were norfloxacin, ofloxacin, ciprofloxacin and doxycycline [31]. Other research results have shown that only two classes of antibiotics tend to adsorb to sewage sludge, that is, tetracyclines and fluoroquinolones. Other groups of antibiotics were degraded in WWTPs [32].

Micropollutants identified both in wastewater and in sewage sludge are also characterized by different volatility and polarity. Due to all these features, CECs are degraded to a different extent in the sewage treatment system, and their content may fluctuate within the concentration limits of μg/kg or even mg/kg in the sludge [33].

3. Analytical procedure

To consider the presence and content of CECs in raw sewage sludge or products based on it, particular attention should be paid to the preparation of appropriate analytical procedures. The variety of emerging contaminants and trace amounts present in environmental samples mobilize to develop highly sensitive and selective methods of qualitative and quantitative analysis with a low limit of detection (LOD) and limit of quantification (LOQ) [16]. Obtaining a sufficiently low limit of quantification is important in the context of the legal regulation of the introduction of maximum permissible concentrations of micropollutants in waters. Reliable results of PPCPs tests in sludge can only be obtained if an appropriate analytical procedure is developed. The precise analysis of sewage sludge is important for determining standards for the quality of sludge (fertilizers) used in agriculture. The analyses of the content of various micropollutants in sewage sludge carried out so far showed differences in the type of a tested sample. It is important to clarify at which stage of wastewater treatment and sludge treatment the sample was taken [16,34,35]. Many factors affect the measurement, hence it is advisable to focus on the nature of the analyzed analyte and to develop a method specific for it. Table 1 presents a list of tests for the antibiotics and endocrine disruptors. The type of sample, the method of its preparation, the type of extraction and the instrumental methods used, as well as percentage of substance recovery and the limit of quantification, were taken into account. In addition, the individual steps of the analytical procedures used in the analysis of selected analytes are briefly discussed.

3.1. Preparation of samples

The analytical procedure is a multi-step process (Fig. 1), starting with the sampling process. In the analyses performed so far, the research object was the following: I. sludge after dehydration, II. sludge immediately after anaerobic or aerobic stabilization, III. crude sludge (after primary settling tank), IV. excessive sludge (after secondary settling tank).

The amount of collected sewage sludge should be in the range of 500 to 5,000 mL. The collected samples should be stored in containers made of materials neutral for the samples, enabling the preservation of all their features, protecting against the process of photodegradation of the sample, moisture and other external factors [16]. The most commonly used containers were glass bottles and jars cleaned with an organic solvent, as well as aluminum bags and foils. Until they are prepared for analysis, the samples should be stored at –20°C.

After proper sampling, sample pre-treatment is performed to facilitate the extraction of analytes. The most important step is to dehydrate the sample, which will allow the solvent to reach the matrix effectively during the extraction process. The methods of water removal include centrifugation and decantation, air drying [54], drying in laboratory dryers and freeze-drying. Lyophilization is the most frequently used method of removing water from the sample (Table 1). During lyophilization, the components of the sample are not degraded and the process itself is the least time-consuming compared to other methods. After dehydration, the samples are homogenized with a mortar. The acquired particles are subjected to sieving in order to obtain the uniform particle size, the dimensions of which vary between 425 and 0.2 μm.

The samples prepared in this way are taken to extraction. Various extraction techniques were used to extract the micropollutants, which will be briefly discussed further in this paper.

3.2. Extraction of analytes

Extraction of micropollutants from solid samples, such as activated sludge at various stages of sewage treatment or soil treated with waste sewage sludge, requires solid–liquid extraction. The extraction techniques used in line with the trend of 'green chemistry' have recently undergone dynamic changes, including the emergence of innovative solutions aimed at minimizing the use of organic solvents and saving time, work and energy [78].

The classic, time-consuming and labor-intensive methods that require the use of large volumes of solvents are replaced by modern, fast and often automated methods (Fig. 2).

3.2.1. Mechanical shaking

Mechanical shaking is a rarely used technique of a previously crushed and dried sample with a solvent. The action of shaking the sample with a new portion of the solvent is repeated many times, and the obtained portions of the extract are combined and then the extractant is evaporated. Mechanical shaking is practically not used to isolate micropollutants from sewage sludge.

3.2.2. Soxhlet extraction and Soxtec

Traditional Soxhlet solvent extraction is time-consuming and labor-intensive. It is a closed-loop process but requires the supply of fresh aliquots of solvent. Soxhlet extraction is still used in analytics, but attempts are being made to automate the process. The result of these works is the creation of an apparatus called Soxtec, in which the

SAMPLING PROCESSES			
(Type of samples)			
Primary settler sludge	Exceed sludge	Stabilized sludge (aerobical or anaerobical)	Dehydrated sludge (centrifugation, thickening)

SAMPLE PRETREATMENT - DEHYDRATION			
Centrifugation and decantation	Air Drying	Heating	Lyophilisation

HOMOGENISATION (grinding, sieving)

EXTRACTION

Soxhlet extraction	UAE	SPE	PLE	PHWE	MAE	MSPD	QuEChERS	SPME	MEPS	SFE	FUSLE

CLEAN UP

SPE

SEPARATIONAL ANALYSIS

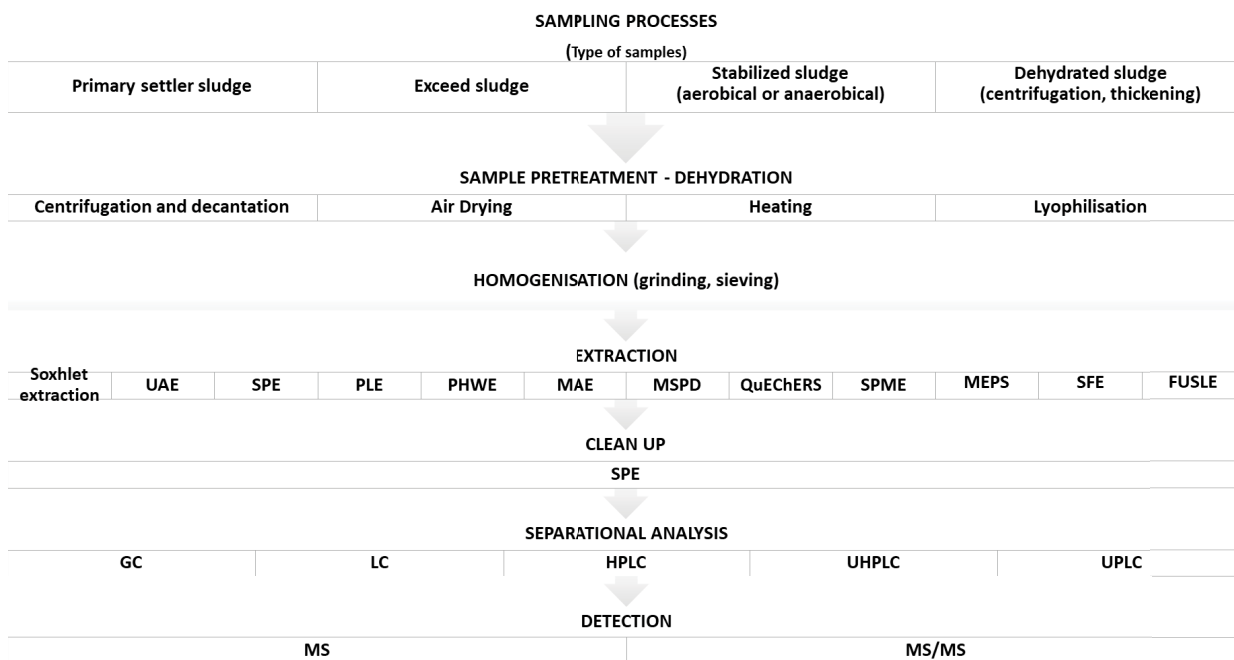| GC	LC	HPLC	UHPLC	UPLC

DETECTION

MS	MS/MS

Fig. 1. Stages of the analysis of the content of micropollutants in sewage sludge [16,34,35,77]. Abbreviations: UAE – ultrasound-assisted extraction; SPE – solid-phase extraction; PLE – pressurized liquid extraction; PHWE – pressurized hot water extraction; MAE – microwave-assisted extraction; MSPD – matrix solid-phase dispersion; QuEChERS – quick, easy, cheap, effective, rugged and safe; SPME – solid-phase microextraction; MEPS – microextraction by a packed sorbent; SFE – supercritical fluid extraction; FUSLE – ultrasonically assisted concentrated liquid extraction; GC – gas chromatography; LC – liquid chromatography; HPLC – high-performance liquid chromatography; UHPLC – ultra-high-performance liquid chromatography; UPLC – ultra-performance liquid chromatography; MS – mass spectrometry; MS/MS – tandem mass spectrometry.
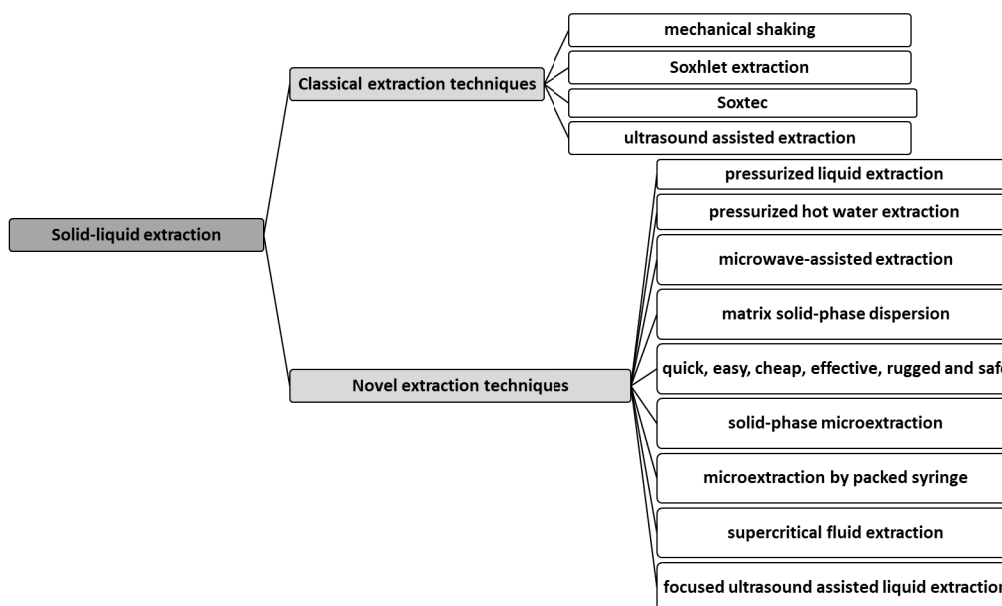
Fig. 2. Methods of extracting solid samples [16,34,35,77].

sample is dissolved in a hot solvent such as n-hexane/acetone (1:1, v/v), and the extract is sent to the purification stage [79]. Soxhlet extraction has not been used in the last 10 y to extract CECs from sewage sludge.

3.2.3. Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) consists of mixing the sample with a solvent and subjecting the mixture to ultrasound. As a result, the phenomenon of cavitation

occurs, that is, the formation of gas bubbles that burst under the influence of excess energy transmitted by the acoustic wave. This causes an increase in the solubility and transport of the analyte, which significantly shortens the extraction time and its efficiency. The most important advantages of this method are shortening the extraction time to even 30 min per sample, energy savings and the 10-fold reduction in the amount of solvent needed [35]. The disadvantage of this method has to do with the necessity to carry out a cleaning step on the extract. The most commonly used solvents include a mixture of water/methylene [36,44,68,72]. Other solvents are acetonitriles, acetonitriles with methanol, n-hexane/acetone.

3.2.4. Pressurized liquid extraction

Accelerated extraction with a solvent, also known as accelerated solvent extraction (ASE), requires the use of high temperature in the range of 100°C–200°C, which results in an increase in pressure in the closed vessel in which the process takes place. Raising the temperature increases the solubility of the substance and, consequently, faster desorption of the analyte from the solid phase to the liquid phase. The main advantages of pressurized liquid extraction (PLE) are automation, shorter reaction time and minimization of the amount of required organic solvents [77]. Extracting PLE may require an additional extraction step as it is not a highly selective method and the extracted analytes may be diluted. In this case, an additional sample cleaning phase (clean up) is required. Among the solvents used in the extraction of CECs is a wide variety of types and mixtures. So far, mixtures of methanol:McIlvaine buffer [37], ethyl acetate [41], acetonitrile:water [43,73,74], water: methanol:acetone [45], methanol:acetone [46], citric acid:methanol have been used [51], methanol [66]. Tests were also carried out on the influence of the solvents used and their mutual proportions on the extraction efficiency and on variables such as pressure, temperature and extraction time [75].

3.2.5. Pressurized hot water extraction

A special type of high-temperature extraction is extraction with water. The condition is to maintain a pressure of 5 MPa and a temperature of 250°C, which changes the properties of water and prevents it from changing its state of aggregation into gas. Pressurized hot water extraction (PHWE) extraction separated estrone and sulfamethoxazole adsorbing on the sludge [64]. However, the recovery efficiency of this method was low (17%–26%).

3.2.6. Microwave-assisted extraction

Extraction with a solvent assisted by microwave extraction, which consists in direct absorption of the microwave dose by the extractant molecules, which significantly accelerates heating and energy saving. The solvents used are polar solvents characterized by a dipole moment other than zero and absorbing microwaves. CECs were extracted, among others in methanol and water [58,80], McIlvaine buffer (citrate-phosphate buffer) and water [37], a mixture of phosphoric acid and acetonitrile [75]. It can also be ethanol, water, acetone, ethyl acetate, acetonitrile. The greatest advantages of microwave-assisted extraction (MAE) are the need for a small volume of samples, low solvent consumption, and shortening the extraction time up to 10 min [79]. On the other hand, MAE is expensive and also requires a further stage step [35].

3.2.7. Matrix solid-phase dispersion

Matrix solid-phase dispersion (MSPD) is the extraction of an analyte from a solid sample using a sorbent with which the analyzed sample is spread. The sorbent is selected based on the type of matrix, analyte and interfering substances. Examples of sorbents are silica with C8 or C18 hydrocarbon chains, silica gel and aluminum oxide. In the second stage of extraction, the analyte is washed out with a mixture of solvents such as methanol, acetonitrile, m-phosphoric acid, and oxalic acid [72,76]. It takes place at atmospheric pressure and room temperature, which results in low costs of the process. Other advantages are that the required solvent and sample are minimized and the extraction time is shortened.

3.2.8. Quick, easy, cheap, effective, rugged and safe

Quick, easy, cheap, effective, rugged and safe (QuEChERS) is one of the newest techniques. It was used for the first time after 2012 [35]. QuEChERS extraction saves time as it does not require a clean-up stage, for which the sample would have to be prepared separately. Instead, there are two stages, with the second stage being dispersive solid-phase extraction (d-SPE), which replaces the clean-up stage. QuEChERS was used to analyze bisphenol A [42] and other EDCs and antibiotics [55]. The recovery of these substances was imprecise (48%–127% for antibiotics, 70%–135% for EDCs).

3.2.9. Solid-phase microextraction

Micro-amounts of organic compounds can be extracted into the solid phase by microextraction. They are deposited on the stationary phase composed of polysiloxanes (e.g., polydimethylsiloxane/divinylbenzene, divinylbenzene/carboxy/polydimethylsiloxane) or polyacrylate covering the glass fiber. Subsequently, the desorption of the analytes to the gas phase is carried out in the injector of the gas chromatograph or by the eluent to the high-performance liquid chromatography (HPLC) column of the liquid chromatography. Solid-phase microextraction (SPME) was used in the analysis of EDCs in the sewage sludge after UAE extraction [53].

3.2.10. Other extraction techniques

Microextraction by a wrapped syringe (MEPS) is a miniaturized solid-phase extraction (SPE). Microextraction into the sorbent filling a special syringe is often connected directly to a liquid or gas chromatography. It is a fast and fully automated method for the testing of organic trace amounts in samples. It is not used in the extraction of antibiotics and common endocrine compounds.

Supercritical fluid extraction (SFE) is an extraction that can be carried out statically providing an average contact

time with the extractant, or dynamically when the values are easily dissolved in the solvent. Even though the use of extractants is small, the extraction time is shortened, and the standard deviations are achieved more efficiently for SFE than in Soxhlet extraction, this technique was not used to extract antibiotics or endocrine compounds [79].

Ultrasonically assisted concentrated liquid extraction (FUSLE) is the intensified version of the UAE. The energy emitted by the ultrasonic probes is repeatable and remains constant for a long time, which has a positive effect on the efficiency of extraction. FUSLE was not used to extract antibiotics or EDC from sewage sludge.

3.3. Clean-up

The sample pre-treatment stage is used when a contaminated sample is obtained after extraction or it is necessary to lower the LOD. Solid–liquid extractions are non-selective, hence the need for an additional extract purification step. Most of the studies performed include the clean-up stage. Only one of the studies did not require an additional SPE stage [69], where a properly selected proportion of water and methanol turned out to be sufficient to perform an effective extraction. In the remaining studies, the clean-up phase was performed using SPE. For sewage sludge, the matrix of which is very complex, SPE is the most commonly used method of purifying analytes. Three classes of sorbents are used to perform SPE extraction: normal phase with a polar sorbent, reverse phase with a non-polar sorbent, and a mixed-mode, where the sorbents interact with different functional groups on a single analyte. Reversed-phase SPE is mainly used for the extraction of antibiotics and endocrine compounds, and the sorbents are divinylbenzene/N-vinylpyrrolidone copolymer (Oasis HLB) or silica gel C18. Polar sorbents were used only in two cases [39,40].

3.4. Instrumental analysis

After the solid analytes were extracted from samples, they underwent chromatographic analysis: liquid (LC) or gas (GC) coupled with mass spectrometry (MS) or tandem mass spectrometry (MS/MS). The choice of technique depends on the physicochemical properties of the analytes. Volatile compounds are determined by GC and less volatile and polar compounds by LC.

3.4.1. Gas chromatography

Some CECs require several derivatization reactions to increase their volatility and prepare them for gas chromatographic analysis [77]. Polar EDCs require an acylation or silylation reaction. Such measures increase the sensitivity and selectivity of the method. In the conducted studies, silylation reactions with BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) [39,76] or MSTFA (N-methyl-N-(trimethyl silyl)-trifluoroacetamide) were used [54].

3.4.2. Liquid chromatography

Liquid chromatography is a less demanding technique than GC. LC is used to determine any organic compound, even of high molecular weight. There is no need for a derivatization phase. The analysis is fast, sensitive and selective. Most of the emerging contaminants analyses performed were based on LC, using tandem mass spectrometry as a detector.

3.5. Most commonly used analytical methods

To be able to unequivocally determine the safety of using sewage sludge for the production of agricultural fertilizers, a meaningful analysis of the composition of the sludge and fertilizer should be performed. The trace presence of pharmaceuticals with an affinity for adsorption to sewage sludge prompts one to seek precise and sensitive research methods. Table 1 presents research papers about antibiotics and endocrine disruptors detected in sewage sludge for the last 9 y.

The collected data show that the most frequently tested sludge was activated sewage sludge and digested sludge. The sludge from the primary and secondary settling tanks as well as sludge from the thickening and dewatering stages were also analyzed. The dominant technique of sample preparation for analysis was definitely lyophilizing – in approx. 70% of the cited publications. In addition, air drying and heating were also used. Although studies comparing the content of antibiotics and endocrine compounds in different types of sludge [59,62,66,73,74] did not show significant differences in the obtained recovery levels. The presence of these compounds in samples of sewage sludge from the final stages of processing of sludge, that is, after anaerobic stabilization, which is the final raw material for fertilizer production confirms that their analysis is important, in the context of the production of fertilizers from sewage sludge.

To extract the analytes, innovative extraction methods (PLE, PHWE, MSPD) were used. However, the most frequently chosen extraction technique was the UAE, followed by additional SPE extraction. Oasis HLB cartridges were most frequently used for clean-up, separation and concentration. LC was chosen more frequently than GC for instrumental analysis. GC is a chromatography that often requires a derivatization step, which significantly increases the cost of the research and the time it takes to perform it. More preferred methods include LC, HPLC, ultra-high-performance liquid (UHPLC) and ultra-performance liquid (UPLC) chromatography coupled with tandem mass spectrometry, providing high sensitivity of the analyses.

Methods with low limits of quantification are desirable because of the trace amounts of emerging contaminants in the sewage sludge. A comparison of the data is presented in Table 1, the results are imprecise. Determination of a research method to estimate the content of micropollutants in sewage sludge will enable its quality control and determination of standards. Exceeding the highest acceptable concentration will limit the given sewage sludge as waste intended for agricultural use and for the production of organic and mineral fertilizer from it. This will prevent any potential secondary soil contamination of emerging contaminants [81]. Obtaining the results concerning the content of pharmaceuticals in the sewage sludge will enable one to determine the possibility of their agricultural management.

An additional argument that determines the choice of the research method will be associated with the consideration of the ecological aspect, relying on the use of the so-called 'green chemistry'. Where possible, the use of solvents or samples should be minimized to reduce the generation of hazardous waste. The implemented modern and automated methods of extraction and chromatography undoubtedly fit the trend of the so-called 'sustainable chemistry'.

4. Fate of micropollutants in a wastewater treatment plant

The decomposition of micropollutants depends not only on their physicochemical properties but also on the environmental conditions in which they will find themselves, that is, types of treatment technology, sewage temperature, insulation, the length of the day, the manner of digestion, contribution of the microbial community, seasonal variation of wastewater, flows and size of the population.

CECs end up in the municipal wastewater treatment plant along with raw sewage, and here their fate differs. Emerging contaminants may undergo the following transformations or fates [34,82]:

- mineralization due to decomposition to CO_2 and H_2O in the process of:
  - biological wastewater treatment with the use of activated sludge,
  - anaerobic fermentation of sewage sludge;
- sludge adsorption;
- leaving the wastewater treatment plant system with the outflow of treated sewage.

Most of all, polar substances with a low capacity to adsorb on the activated sludge are mineralized to CO_2 and H_2O by biological treatment with activated sludge [6,7]. If no decomposition of such a substance occurs during wastewater treatment, these compounds will be released into the environment along with the treated wastewater discharge. Research shows that, for example, diclofenac is difficult to remove by the activated sludge method, although quite varied results of its degradation were obtained - the reduction of this drug was manifested in approx. 17% [83], as well as 71% [84]. On the other hand, another anti-inflammatory agent, ketoprofen, is removed in 98% by the activated sludge method [84].

Compounds characterized by lipophilicity, the pKa coefficient of which is higher than soil pH, assume an alkaline nature and adsorb to activated sludge flocs and are removed from the system with it [28]. Tests and analyses of the content of micropollutants in sewage sludge are constantly carried out.

Among the antibiotics, the following groups were determined: macrolides, quinolones, sulfonamides and tetracyclines. In the sewage sludge, antibiotics from the group of sulfonamides and tetracyclines were mainly detected. The highest concentrations were ofloxacin from the fluoroquinolone group. The concentration of this antibiotic was 8,546.21 µg/kg dry weight in the anoxic sludge [57]. Other studies have shown its content at the level of 2,300 µg/kg dw [52], 2,921.4 µg/kg dw in the recirculated sludge in membrane biological reactor-based WWTP [70],

690 µg/kg dw [65]. Similar data were obtained for ciprofloxacin, another fluoroquinolone antibiotic: 6,500 µg/kg dw [65], 3,726.8 µg/kg dw [70] and 303 µg/kg dw [64] and for norfloxacin: 2,796.68 µg/kg dw [57], 620 µg/kg dw [65]. Oxytetracycline, an antibiotic from the tetracycline group, was determined at 7,105.54 µg/kg dw [57] and 742.5 µg/kg dw [51], and the tetracycline content was 4,457 µg/kg dw [57]. From the group of sulfonamides, the most common and in the highest concentrations are sulfamethazine [43,44] and sulfamethoxazole [47,51,63,73].

The content of antibiotics in the sludge differed depending on the type of sludge tested [73]. The concentration of sulfonamides and tetracyclines in primary, excess and dehydrated sludge was analyzed. The highest contents were found in the primary sludge, the lowest in the excess sludge. Different results were obtained for sulfamethoxazole, the most of which was found in excess sludge, and the least in dehydrated sludge [73].

A similar study was undertaken by analyzing the primary sludge, secondary sludge and anaerobically stabilized sewage sludge in the context of the presence of endocrine compounds [48]. Estrone was not measured by the low concentration, the estriol content increased imperceptibly in the secondary sludge, and the highest level of 17β-estradiol (293.5 µg/kg) was determined in stabilized sludge. The highest amount of 17α-ethinylestradiol was observed in the secondary sludge (97.8 µg/kg) [48]. In other studies, the average concentration of this compound in the sewage sludge was a maximum 0.45 µg/kg [36].

The bisphenol A content in the sludge ranged from 92.9 µg/kg [40], through 155 µg/kg [50] to 3,590 µg/kg [45].

The existing literature clearly defines the content of the tested compounds in the sewage sludge at a low level. Both the concentration of some antibiotics and EDCs were outside the limits of the quantification of the method. The maximum recorded content of these compounds reached almost 10,000 µg/kg dry weight.

At the same time, the techniques and analytical methods used are improved, and the result of these activities is the development of more and more sensitive methods with low limits of quantification, which is important due to the low concentrations of the tested compounds.

The data presented in Table 1 show that the quantification limits of antibiotics and endocrine compounds in the methods used are as high as 0.01 ng/g. This proves the quality of the UAE and MAE methods used in combination with GC and advanced LC (UHPLC and UPLC). On the other hand, QuEChERS extraction turned out to be a technique that enables one to obtain relatively high limit values estimating the content of micropollutants.

The results which were obtained are satisfactory. Most of the studies allowed for the recovery of up to 100% of the analyzed compound. In several studies, the recovery efficiency was less than 50% at its lower end. Two studies showed maximum recovery efficiency of only 30%, and both focused on research on sulphonamides [64,68].

The necessity to manage the resulting by-product, which is stabilized sewage sludge, economically and ecologically, requires a thorough analysis of the sludge content, assuming the possibility of its application for agricultural purposes. The presence of micropollutants in stabilized

sludge or fertilizer produced from it will result in contamination of soils [4], surface waters, and then also groundwater with 'new generation' pollutants, the toxicity of which affects living organisms [85].

Environmental risk assessment of endocrine compounds and antibiotics is also under investigation. Importantly, the introduction of micropollutants into the environment will not be equal to their negative impact. First of all, attention should be paid to the ability to leach these substances from sewage sludge or potential fertilizers based on these sludge into solutions, which may result in the accumulation of these substances [49,86,87].

The desorption coefficient (K_d) is used to test the ability of a compound to transfer from a solid phase (e.g., an organic-mineral fertilizer) to a liquid phase (e.g., water penetrating the soil, fertilized with the test fertilizer). It is represented by the following equation:

$$K_d = \frac{C_{\text{solid}}}{C_{\text{water}}} \left[\text{L/kg} \right] \tag{1}$$

where C_{solid} is the concentration of the pharmaceutical compound in the solid phase (µg/kg dry matter (dm) and C_{water} is the concentration of the pharmaceutical compound in the aqueous phase (µg/L) [48].

An example is the study of sorption of 17α-ethinylestradiol and estriol, the results of which indicate a high K_d, which means that these compounds tend to remain on the sludge [48].

An equally important test is the toxicity assessment of these micropollutants towards living organisms. It is carried out using the risk quotient (RQ) coefficient [48,49]. RQ value of each pharmaceutical residue in sludge was defined as the ratio between its measured environmental concentration (MEC) in sludge and the concentration below which no adverse effect is expected to occur (predicted no-effect concentration: PNEC) [49,88]. An RQ_f factor is also proposed which takes into account coefficient F, which represents the frequency of MECs exceeding PNEC [89]. Depending on the RQ_f values, they are divided into 5 groups: if RQ_f is higher than 1 ($RQ_f \geq 1$), the environmental risk is high; if RQ_f is between 0.1 and 1 ($1 > RQ_f \geq 0.1$), the environmental risk is moderate; if RQ_f is between 0.1 and 0.01 ($0.1 > RQ_f \geq 0.01$) the risk is endurable; if RQ_f is below 0.01 ($0.01 > RQ_f > 0$) the effect of this compound is negligible; if RQ_f is zero ($RQ_f = 0$) the substance is safe [89].

The studies show that the toxicity risk is diversified and specific for a given compound and each substance should be assessed separately [89].

5. Impact of micropollutants on the environment

The negative effect of many micropollutants detected in both treated sewage and sewage sludge on the environment has been proven. The constant circulation of water in nature facilitates the migration of proven and leaked pharmaceuticals from the discharged treated sewage and sludge to the soil, and then to surface and groundwater. These, in turn, are a source of drinking water necessary for human life. Micropollutants detected in drinking waters are a direct threat to the health of the population [90,91]. Another issue is the impact on animals when contaminants appear. Endocrine compounds that are ingredients of hormonal agents and their derivatives have an impact on the reduced reproduction of male aquatic organisms [17]. Studies have shown that embryo development may also be inhibited, which may reduce the population of a given species [17]. The phenomenon of feminization of male fish was also observed, resulting from the loss of their secondary sexual characteristics [92]. Bisphenol A is one of the excellent endocrine-disrupting effects on the reproductive system of living organisms [93,94].

An important phenomenon observed in the context of micropollutants is the reduced sensitivity of pathogens to drugs, the so-called drug resistance. If a microorganism comes into contact with an antibiotic, it can alter its metabolism, rendering it insensitive to the drug. The genes responsible for acquired drug resistance will be easily transported to other cells of the pathogen [24,95,96]. Therefore, there is a widespread concern that spreading sludge on farmland would contribute to the development or spread of antibiotic resistance. Antibiotics can accumulate in food webs, and even more alarmingly, antibiotic resistance genes can be transferred between environmental bacteria and human pathogens. Antibiotics and their impact on the environment have become an important topic in environmental science.

6. Possibilities of reducing the content of micropollutants

Research is constantly carried out to develop an effective method of reducing ECs. Studies on anaerobic sludge stabilization have been shown to remove endocrine compounds [97–101].

The influence of mesophilic and thermophilic fermentation on estrone (E1) and 17β-estradiol (E2) transformations was compared. The results of the research showed that anaerobic stabilization at higher temperatures intensifies the processes of decomposition of these estrogens. Additionally, it has been shown that the reduction of these compounds is greater in crude sludge subjected to anaerobic processes than in mixed sludge [99].

This is confirmed by other studies that indicate modifications of anaerobic decomposition in the form of anaerobic membrane bioreactors [98]. Their advantage is the intensification of CECs reduction through increased biodegradation and an increase in biogas production. When one compared the content of pharmaceuticals in sludge formed at individual stages of wastewater treatment, it turned out that the highest average concentration of CECs was found for secondary sludge (310 µg/kg dw), and then for primary sludge (179 µg/kg dw). On the other hand, the smallest amount of pharmaceuticals was found in the anaerobically fermented sludge (8 µg/kg dw) [102]. Thus, the anaerobic digestion significantly reduced the concentration of the majority of the compounds tested.

Estrogenic compounds are effectively removed by the thermal hydrolysis process [103]. Other tested methods of reducing pharmaceuticals (pasteurization, advanced oxidation processes using the Fenton reaction, treatment with ammonia, thermophilic dry fermentation) have shown a

positive effect, for example, carbamazepine or propanolol, but natural estrogens have been reduced to a small extent [102,103].

The use of ozone in the reduction of antibiotics yields the desired results not only for the water phase [104], but also for the activated sludge [105]. The effect of sludge ozonation on the content of tetracycline, oxytetracycline, doxycycline and azithromycin was investigated. A clearly reducing effect of ozonation on the content of antibiotics in the sewage sludge was demonstrated, as 86.4%–93.6% of the antibiotics present in the sludge were removed. This effect was enhanced by the alkaline environment and the increasing ozone dose [105].

7. Conclusions

The economical and ecological way of sewage sludge management is one of the main tasks of wastewater treatment plant operators. The introduction of the desired circular economy and the possibility of using sewage sludge in a closed cycle motivates scientists to create innovative solutions. The production of organic and mineral agricultural fertilizers based on sewage sludge from wastewater treatment plants is in line with the prevailing trend. The condition is its appropriate physicochemical composition, lack of toxic substances, pharmaceuticals, micropollutants and high nutrient content.

In this study, one devoted attention to micropollutants with an affinity for adsorption to solid particles (antibiotics and endocrine disruptors). Due to their sorption properties, these groups of pollutants remain in sewage sludge and, together with the produced fertilizer, may be transferred to the natural environment. The research demonstrated the presence of these compounds not only in sewage sludge but also in soil samples. Permanent contact of living organisms with this type of substance may contribute, for example, to the uncontrolled development of resistance to antibiotics or changes in the endocrine system.

Data on the research methodology of these compounds were collected. Research procedures were varied. Not only the sewage sludge after aerobic and anaerobic stabilization was investigated, but also the sludge after the primary settling tank, excess sludge and thickened sludge. The collected samples were dehydrated in the process of air drying, mechanical dehydration or lyophilization. Analytes were obtained from the samples by means of various types of extraction: from traditional ones such as UAE to modern, automated ones with a shortened reaction time and reduced amounts of necessary reagents (e.g., PLE, PHWE). The obtained extracts are pre-treated with SPE extraction with a polar, non-polar sorbent or in a mixed-mode. Liquid and gas chromatographies are used for the detection of micropollutants. The latter requires a sample derivatization process, hence they are less frequently selected by researchers. There are many variable factors in the selection of a precise and accurate methodology. This results in differences in the obtained data. Therefore, the question of specifying the research method remains open.

Scientific research confirms the presence of pollutants not only in treated sewage and sludge but also in natural waters. The impact of micropollutants poses a real threat to living organisms. Considering the safety of using sewage sludge in the form of fertilizers, the aspect of reducing CECs in the composition of sewage sludge should be considered. Technologies based on photodegradation or ozonation of wastewater show promising results.

The increasing amount of sludge generated in WWTPs and the increase in consumption of CECs prompts further research. In the near future, the research procedures should be standardized, which will be the basis for the creation of maximum permissible concentrations in agricultural sewage sludge. At the same time, special attention should be devoted to modern technologies of removing pharmaceuticals from wastewater. It will be necessary to develop an additional stage of the reduction of micropollutants in the wastewater treatment process, taking into account the technical and economic aspects.

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