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Review

Heavy Metal and Antimicrobial Residue Levels in Various Types of Digestate from Biogas Plants—A Review

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Abstract: Global population growth generates problems relating to increasing demand for sustainable energy and waste treatment. Proper solid waste management promotes material reuse, maximizes recovery and reduces anthropological pressure on natural resources. Anaerobic digestion (AD) is an alternative method of stabilizing organic substrates and generating biogas as a source of environmentally friendly energy. In addition, digestate is not only a waste product of that process but also a renewable resource with many potential applications. The circular economy concept encourages the use of digestate as a source of nutrients that promotes plant growth and improves soil properties. However, the stabilized substrates often contain various contaminants, including heavy metals (HMs) and antibiotics that are also detected in digestate. Therefore, the agricultural use of digestate obtained by AD could increase the pool of these pollutants in soil and water environments and contribute to their circulation in these ecosystems. Moreover, digestate may also increase the co-selection of genes determining resistance to HMs and antibiotics in environmental microorganisms. This article comprehensively reviews published data on the residues of various HMs and antimicrobial substances in different digestates around the world and maps the scope of the problem. Moreover, the potential risk of residual levels of these contaminants in digestate has also been evaluated. The review highlights the lack of legal standards regulating the concentrations of drugs introduced into the soil with digestate. The results of the ecological risk assessment indicate that the presence of medically important antimicrobials in digestate products, especially those used in agriculture, should be limited.

Keywords: anaerobic digestion; digestate; biogas plants; antibiotics; heavy metals; ecological risk; anthropogenic pressure

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1. Introduction

Due to the intensification of agriculture, progressing urbanization, and human population growth, the volumes of waste generated worldwide have become a significant burden on the natural environment [1]. The global production of various types of organic waste, including agricultural residues, animal manure, food waste, and sewage sludge, is estimated at 2, 120, 1.3, and 16.4 billion tons per year, respectively [2]. The global volume of organic waste, as well as the consumption and demand for energy, continues to increase. Organic biomass is biodegradable and can be used for energy generation under anaerobic conditions. During anaerobic digestion (AD), various organic wastes are converted into biogas—an environmentally friendly and renewable energy source that can be used to produce vehicle fuel, electricity, or heat. In recent years, AD of waste from agriculture, industry, and municipal facilities has become one of the most promising pathways for renewable energy generation [3]. Sustainable management of organic waste plays an important role in the circular economy by promoting material reuse and maximizing recovery. Energy recovery from organic waste provides environmental benefits by decreasing the following: (1) greenhouse gas emissions; (2) fossil fuel use; (3) water pollution; and (4) the volume of landfilled waste. Moreover, the AD process offers an additional advantage by promoting the simultaneous recovery of material in the form of digestate [4,5]. While biogas as a renewable energy source is discussed intensively, digestate still plays a minor role in political and scientific debates.

Digestate is a stable and valuable by-product of the AD process that is rich in nutrients. It includes a balanced mixture of micro- and macronutrients which are essential for plant growth [6]. Digestate generated by AD can be returned to farms that supply substrates to biogas plants, sold on the market or recirculated [7]. The use of digestate as fertilizer or soil improver offers one of the simplest solutions for managing this by-product, improving the economic sustainability of biogas production and minimizing its negative environmental impacts. Digestate obtained by AD is more stable, hygienic, and abundant in bioavailable nitrogen (in the form of N-NH_4) than undigested organic biomass. It is suitable for agricultural use due to specific properties that can improve the chemical and physical properties of soil, including soil structure, moisture retention, microbial activity, and organic carbon content. Moreover, the recovery of nutrients (such as nitrogen and phosphorus) limits soil erosion. Ultimately, digestate enhances soil quality and fertility. The agricultural use of digestate offers an excellent alternative to reducing the use of chemical fertilizers [5,8].

However, despite these advantages, the introduction of digestate into the soil environment entails some risks. In Europe, agricultural residues and animal manure are the dominant feedstocks for AD. Due to various waste management options and the legal restrictions related to the climate and energy policy, all types of organic waste and by-products are being increasingly used as substrates in biogas production. For this reason, sewage sludge, biowaste, and industrial waste are also commonly processed by AD [6]. It should be emphasized that the specific properties of the obtained digestate are influenced mainly by the type of substrates used in the AD process. Digestate composition and quality are determined by the composition and quality of the feedstock, as well as the efficiency of the AD process [9]. The substrate should be free of contaminants to prevent undesirable substances from reaching the digestate. There are concerns regarding the presence of hazardous compounds, especially heavy metals (HMs) and antibiotics, in digestates obtained from animal, household, or industrial wastes [10–12]. These types of pollutants are most widely encountered in animal manure and sewage sludge [13].

Heavy metals are widely distributed in wastewater and animal feed, and they are present in sewage sludge and manure, respectively [14–17]. In addition, these micropollutants are also detected in the waste stream [18]. Heavy metals are toxic and non-biodegradable, and they pose a serious environmental risk. Antibiotics also undermine the quality of digestate. According to estimates, up to 80% of ingested drugs can be excreted with feces and urine in unchanged form. The antibiotics used in human medicine reach wastewater treatment plants and accumulate in sewage sludge, while veterinary drugs have been detected in manure and slurry [1]. Antimicrobial substances reach anaerobic bioreactors and are released into the environment, posing a threat to the soil, water ecosystems, and plants. Even low concentrations of HMs and antibiotics in various ecosystems can have adverse consequences for the environment [12,19]. The agricultural use of digestate containing HMs and antibiotics leads to the accumulation of these contaminants in the soil, posing a risk for both the environment and public health [1,19,20]. Moreover, the use of contaminated digestate in agricultural land and crop production can exert selective pressure on bacteria, lead to the spread of HMs and promote antibiotic resistance in microorganisms [1,21]. It should be emphasized that, in Europe, the quality and potential applications of digestate are regulated by legal acts pertaining to fertilizers, waste, and soil protection, or a combination of these laws. Although HM concentrations are strictly regulated in digestate that is intended for use as fertilizer or soil improver, statutory limits on drug concentrations have not been introduced to date [13]. The lack of appropriate risk assessment for the safe management of digestate has also been highlighted by other authors [4].

The main objective of this study was to review the current knowledge on the presence of HMs and antibiotics in digestate derived from various feedstocks. The article describes the key reservoirs of these micropollutants and the role of the AD process in their release to the environment. The growing levels of microbial resistance to HMs and antibiotics pose a serious public health concern and an environmental issue worldwide, which is why the potential effects of micropollutant residues in digestate have also been discussed. An ecological risk assessment of HMs and drugs has been performed to determine the severity of the risk resulting from the introduction of digestate into the soil environment. However, the current study has several limitations. Firstly, despite a large number of scientific papers on the presence of HMs and antibiotics in potential AD substrates, including raw animal manure, agricultural residues, sewage sludge, and the organic fraction of municipal solid waste or mixed wastes [22–32], the concentrations of these micropollutants in the resulting digestates have been analyzed by very few authors. Secondly, most studies investigating the fate of HMs and antibiotics during AD included feedstock supplementation before the process, making it impossible to assess the real, environmental concentrations of these pollutants in digestates [33–37]. Moreover, many scientific papers examining the impact of soil fertilization with digestate focused only on the concentrations of HMs and antibiotics in soil samples, while disregarding AD by-products [38–40]. In addition, the solid−liquid separation of AD products has also received little attention in the existing literature. To the best of our knowledge, there is a general scarcity of comprehensive review studies addressing the levels of HM and antibiotic residues in various anaerobically stabilized organic wastes. The number of reviewed papers from various regions of the world is presented in Figure 1. The authors were unable to find any studies presenting data on the content of HMs and/or antibiotics in digestates from Africa and South America. The only published studies addressing digestate contamination on the Asian continent came from China (11 articles). This issue was most often studied by research teams from European countries (22 articles), but many of these articles present selective data. Residual contamination is a broad and complex area of research involving different types of feedstocks, AD conditions, digestate fractions, and their environmental impacts, which is why further research is needed to fill these knowledge gaps.

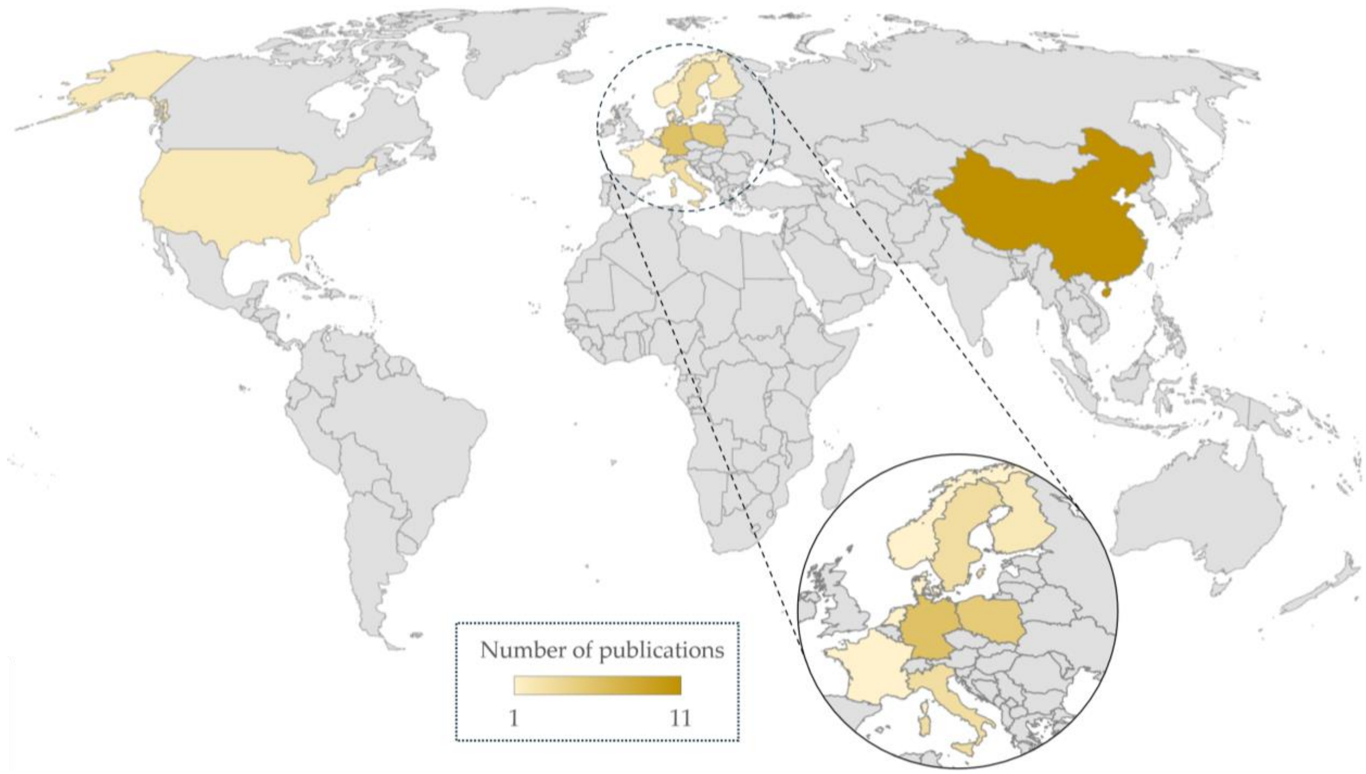

Figure 1. Map showing the regions of the world where the reviewed studies were conducted (including the number of articles from each country).

2. Materials and Methods

2.1. Data Sources

In compliance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines [41], the articles were selected according to the following four criteria: (i) identification; (ii) screening studies; (iii) eligibility; and (iv) inclusion. PubMed and Google Scholar scientific literature databases were surveyed to find papers published between 1 January 2004 and 30 November 2024.

2.2. Search Strategy

The search strategy is presented in Figure S1 in Supplementary Materials. The following keywords were used in the search strategy: ((anaerobic digestion) AND (digestate) AND ((antibiotic) OR (antimicrobial) OR (drug) OR (heavy metal)) AND ((residue) OR (degradation) OR (concentration) OR (content))) AND ((anaerobic digestion) AND (digestate) AND ((antibiotic) OR (antimicrobial) OR (drug) OR (heavy metal)) AND ((residue) OR (degradation) OR (concentration) OR (content))). These keywords were tailored to each database.

A preliminary search of published scientific articles relating to the subject of this review was conducted to identify the keywords for the advanced search process. The selected keywords are presented in Figure 2. In addition, a reference list of articles was checked manually to find adequate scientific publications for the review. After filtering the literature, 131 scientific publications were selected for this review article.

Figure 2. A co-occurrence map of scientific papers containing the following keywords: anaerobic digestion, digestate, antibiotic/antimicrobial/drug/heavy metal, and residue/degradation/concentration/content. The frame size represents the frequency of the keyword's co-occurrence, and the color of clusters denotes the publication date. The map was created in VOSviewer (v1.6.16; 2020; Center for Science and Technology Studies, Leiden University, the Netherlands).

2.3. Ecological Risk Assessment

The indicators related to the levels of pollution with HMs and pharmaceuticals were computed to evaluate the influence of digestates on the soil ecosystem and the associated hazards. The geo-accumulation index and ecological risk factors were calculated for HM contamination of digestates. The risk quotient (RQ) method was used to assess the effect of pharmaceutical contaminants in digestates on the soil ecosystem, including co-selection for antimicrobial resistance.

2.3.1. Geo-Accumulation Index

The geo-accumulation index (I_{geo}) measures the level of pollution with inorganic or organic trace substances and the presence of bioelements in sediment or soil. This index is commonly used to assess HM pollution, and it was calculated by comparing the concentrations of HMs in samples with the natural background levels of metals in soils with the use of the following equation [42]:

$$I_{geo} = \log_2\left(\frac{C_n}{1.5B_n}\right), \quad (1)$$

where

C_n is the concentration of the analyzed heavy metal in digestate ($mg\cdot kg^{-1}$);

B_n is the geochemical background value of the analyzed heavy metal in soil ($mg\cdot kg^{-1}$). Background values were derived from the elemental concentrations in the upper continental crust [43].

The values of the I_{geo} index were classified on a 5-point scale: <0, practically unpolluted; 0–1, unpolluted to moderately polluted; 1–2, moderately polluted; 2–3, moderately to strongly polluted; 3–4, strongly polluted; 4–5, strongly to extremely polluted; and >5, extremely polluted.

2.3.2. Potential Ecological Risk Index

The ecological risk index (E_r) was applied to evaluate the ecological risk posed by each HM individually. This index was calculated using the following formula [44]:

$$E_r = T_r \cdot C_f, \tag{2}$$

where

T_r is the toxicity factor of the analyzed HM; the following values were adopted: cadmium = 30, chromium = 2, mercury = 40, nickel = 5, lead = 5, zinc = 1, copper = 5, arsenic = 10, iron = 1, manganese = 1, cobalt = 5, molybdenum = 15;

C_f is the pollution factor.

The toxic response factors for manganese (Mn) and cobalt (Co) were not provided by [44]; therefore, the present study relied on the relevant values computed by other researchers [45–47]. The T_r values for silver (Ag) and aluminum (Al) were not available; therefore, the E_r values for these elements were not calculated.

The contamination factor (C_f) is the ratio of metal contamination levels to pre-industrial levels that are commonly found in the Earth's crust. This factor was calculated using the following formula:

$$C_f = \frac{C_n}{C_b}, \tag{3}$$

where

C_n is the concentration of the analyzed HM in digestate (mg·kg^{-1});

C_{ob} is the concentration of the analyzed HM in the soil (mg·kg^{-1}). Background values were derived from the elemental concentrations found in the upper continental crust [43].

The E_r values were classified on the following scale: very high risk ($E_r > 320$), high risk ($160 < E_r < 320$), considerable risk ($80 < E_r < 160$), moderate risk ($40 < E_r < 80$), and low risk ($E_r < 40$).

2.3.3. Predicted Environmental Concentrations (PECs)

To assess the influence of antibiotic residues in digestates on the soil ecosystem, the predicted environmental concentrations (PECs) of antibiotics in fertilizer-amended soil ($PEC_{(soil)}$) were calculated using the prescribed methodology [48] and the following equation:

$$PEC_{(soil)} = Co_{(soil)} + \frac{MEC_{(digestate)} \cdot APP_{(digestate)}}{DEPTH_{(soil)} \cdot RHO_{(soil)}} \tag{4}$$

where

$Co_{(soil)}$ is the background concentration of the analyzed antibiotic in soil before the application of digestate (assumed to be zero in this study);

$MEC_{(digestate)}$ is the concentration of the analyzed antibiotic in digestate (μg kg^{-1});

$APP_{(digestate)}$ is the typical rate of sludge application in soil, usually 0.5 kg m^{-2} for agricultural applications [49];

$DEPTH_{(soil)}$ is the mixing depth, usually set at 0.2 m for agricultural soil;

$RHO_{(soil)}$ *is* the bulk density of wet soil (1700 kg m^{-3}) [49].

The calculated $PEC_{(soil)}$ values were compared with the known predicted no-effect concentrations (*PNECs*) for resistance selection in the environment ($PNEC_{MIC}$) and ecotoxicity ($PNEC_{ENV}$) used in antibiotic environmental risk assessments for individual antibiotics detected in digestates. This analysis involved only antimicrobial substances with known values of $PNEC_{MIC}/PNEC_{ENV}$ [50,51].

The risk assessment based on $PNEC_{MIC}/PNEC_{ENV}$ values was conducted with the use of the RQ method according to the following formula:

$$RQ_{mic/env} = \frac{PEC_{(soil)}}{PNEC_{MIC/ENV}}, \quad (5)$$

RQ_{env} values were classified on the following scale: $RQ > 1$—high potential risk for soil-living organisms; $0.1 < RQ < 1$—moderate potential risk; and $RQ < 0.1$—low potential risk. $PEC_{(soil)}$ values exceeding the $PNEC_{MIC}$ for specific antibiotics ($RQ_{mic} > 1$) were regarded as contributors to selection for antibiotic resistance in soil.

3. Results

3.1. Heavy Metal Content of Various Digestates

The use of digestate as soil fertilizer or improver is an environmentally friendly and cost-effective method of managing AD by-products that enhances soil capability for agriculture, supplies plants with nutrients and promotes waste recycling. However, HM concentrations in various digestates raise serious concerns. Although AD has the potential to reduce microbial loads and antibiotics in the processed substrate, it is not effective in eliminating HMs that are highly stable [52]. Heavy metals can be introduced into the soil when agricultural land is amended with contaminated fertilizers. Although some HMs, including copper (Cu) and zinc (Zn), are essential for the growth of various organisms, high concentrations of these elements can be toxic and can cause damage to nucleic acids and the cell membrane [53,54].

Heavy metal concentrations can differ considerably in various digestates. The origin of green waste or biowaste feedstocks is a significant consideration because aerial deposition of HMs is higher in urban than in rural environments. Moreover, the season of substrate collection also plays an important role due to the peak deposition of HMs in winter [55,56]. However, these variations are related mainly to the type of feedstock processed by AD. In intensive livestock production systems, HMs (mainly Cu, Zn, and arsenic (As)) are used as feed additives to prevent disease, increase weight gains or boost egg production in poultry farming [57]. Even more than 80% of dietary Zn and Cu is excreted in active form by humans and animals, which leads to the accumulation of these HMs in various feedstocks that are processed by AD [24]. The above leads to an increase in the HM content of animal by-products, which is stabilized during the AD process. Animal manure is recognized as a valuable fertilizer, but it is also the main source of HMs [57,58]. Sewage sludge, yet another popular substrate for AD, is an equally important source of HMs [52]. The HMs that are most frequently identified in sewage sludge include Cu, Zn, Co, iron (Fe), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni), cadmium (Cd), and the highly toxic As and selenium (Se). Heavy metals can occur in sewage sludge in dissolved or precipitated form, and they can also be associated with solid particles [52]. Industrial development increases the concentrations of HMs in wastewater, directly leading to sewage sludge contamination [59]. Surface runoffs and industrial wastewater are the main sources of these micropollutants in sewage sludge [60]. It should be noted that HM concentrations in sewage sludge rarely exceed the regulatory limits, but prolonged application of sewage sludge in agriculture contributes to the accumulation of HMs in soil [61–63]. Soil fertilization with both digested manure and sewage sludge increases HM levels in soil, which poses serious environmental risks. As previously mentioned, HM concentrations in raw substrates for AD and in fertilized soil have been extensively researched. However, very few studies have examined the HM content of digestates. The concentrations of HMs in the by-products of AD of various feedstocks are presented in Table 1.

Table 1. Heavy metal concentrations in digestates derived from various feedstock sources.

Substrate for AD	HM	Concentration	Unit of Measure	Country	References
Mixed cattle manure and maize silage	Cd	0.9–1.6	$mg \cdot kg^{-1}$ DM	Poland	[11]
	Cr	26–40			
	Hg	0.31–0.49			
	Ni	12–19			
	Pb	7.9–20			
	Zn	595–790			
Mixed cattle manure, bovine slurry, and maize silage	Cd	0.2–0.8	$mg \cdot kg^{-1}$ DM	Poland	[64]
	Cr	1.5–6.5			
	Hg	0.01–0.023			
	Ni	5–15			
	Pb	2–11.5			
Mixed food waste, industrial waste, animal by-products/ manure, and slaughterhouse waste	Cd	0.34–0.37	$mg \cdot kg^{-1}$ DM	Sweden	[65]
	Cr	8.2–20			
	Cu	41–100			
	Hg	<0.1			
	Ni	7.2–15			
	Pb	2–7.4			
	Zn	180–540			
Mixed maize silage, slaughterhouse waste, potato pulp, and confectionery press cake	Cd	0.02–0.03	$mg \cdot kg^{-1}$ DM	Poland	[11]
	Cr	0.2–0.25			
	Hg	0.0037–0.0097			
	Ni	0.32–0.37			
	Zn	10.4–11.9			
Mixed plant residues and cattle manure	Cd	135.64	$mg \cdot kg^{-1}$	China	[66]
Mixed sewage sludge and food by-products	As	12.15–18.83	$mg \cdot kg^{-1}$	Poland	[67]
	Cd	7.57–10.79			
	Cr	143.65–217.47			
	Cu	1387–2059			
	Fe	4629–6685			
	Hg	19.62–37.16			
	Mn	124.78–180.53			
	Ni	56.9–81.38			
	Pb	18.6–63.83			
	Zn	3689–5530			
Mixed sewage sludge and green waste	Cu	501	$mg \cdot kg^{-1}$ DM	France	[68]
	Fe	10,400			
	Zn	842			

Table 1. *Cont.*

Substrate for AD	HM	Concentration	Unit of Measure	Country	References
Mixed textile dyeing sludge and soybean okara by-products	Ni	11.23	$mg \cdot kg^{-1}$ VS	China	[69]
	Cr	316.97			
	Cu	37.47			
	Zn	355.7			
Municipal organic waste	Cd	1.4	$mg \cdot kg^{-1}$ DM	Germany	[70]
	Cr	26.6			
	Cu	49.1			
	Ni	12.5			
	Pb	123.6			
	Zn	327.3			
Fine fraction of municipal waste	Cu	136	$mg \cdot kg^{-1}$ DM	France	[68]
	Fe	12,100			
	Zn	634			
Organic output of mechanical-biological waste treatment facilities	Cd	0.77–3.5 (SF) <0.58 (LF)	$mg \cdot kg^{-1}$ DM	Italy	[71]
	Cr	41.17–77.54 (SF) 2.31–21.78 (LF)			
	Cu	255.35–315.77 (SF) 1.91–57.58 (LF)			
	Ni	22.61–35.40 (SF) 7.81–32.49 (LF)			
	Pb	34.45–344.13 (SF) 0.26–45.10 (LF)			
	Zn	811.5–920.28 (SF) 14.28–224.63 (LF)			
Pharmaceutical-derived organic wastes	As	0.19	$mg \cdot kg^{-1}$ DM	Italy	[72]
	Cd	<0.2			
	Cr	<0.5			
	Cu	23.4			
	Hg	0.41			
	Ni	<0.5			
	Pb	<1			
	Zn	117.2			
Poultry manure	Ag	0.15	$mg \cdot kg^{-1}$	China	[73]
	As	106.65			
	Cd	0.39			
	Cr	61.2			
	Cu	71.72			
	Pb	22.97			
	Zn	370.24			
Rice straw	Cd	0.4–0.7	$mg\ L^{-1}$	China	[74]
	Cu	0.05–0.15			
	Pb	0.03–0.1			
	Zn	0.15–0.37			

Table 1. *Cont.*

Substrate for AD	HM	Concentration	Unit of Measure	Country	References
Sewage sludge	Al	3262.3	mg·kg^{-1}	China	[75]
	Fe	2017.8			
	Hg	6.37			[76]
	Cu	487	mg·kg^{-1} DM	France	[68]
	Fe	9840			
	Zn	988			
	Al	6270–8025		Poland	[77]
	Cd	2.3–5.4			
	Co	3.8–5.9			
	Cr	14–17.9			
	Cu	87.8–91.3			
	Fe	4001–4548			
	Mn	241–267			
	Mo	3–3.9			
	Ni	6.6–8			
	Pb	18.8–25.5			
	Zn	732–789			
	As	14.11	mg·kg^{-1}		[67]
	Cd	8.09			
	Cr	156.58			
	Cu	1,496.7			
	Fe	4836			
	Hg	23.07			
	Mn	132.51			
	Ni	61.67			
	Pb	42.38			
	Zn	4303			
	Cd	0.9–1.6	mg·kg^{-1} DM		[11]
	Cr	28–44			
	Hg	0.41–0.51			
	Ni	11–17			
	Pb	7.13–18			
	Zn	645–830			
Pig manure	Cu	788	mg·kg^{-1} DM	France	[68]
	Fe	3,180			
	Zn	842			
	As	7.49	mg·kg^{-1} VS	China	[78]
	Cd	0.31			
	Cu	781.33			
	Ni	23.13			
	Pb	7.02			
	Zn	2146.32			

Table 1. *Cont.*

Substrate for AD	HM	Concentration	Unit of Measure	Country	References
Textile dyeing sludge	Ni	21.74	mg·kg^{-1} VS	China	[69]
	Cr	525.92			
	Cu	57.46			
	Zn	473.08			

DM—dry matter; FW—fresh weight; VSs—volatile solids; LF—liquid fraction; SF—solid fraction; Al—aluminum; As—arsenic; Cd—cadmium; Co—cobalt; Cr—chromium; Cu—copper; Fe—iron; Hg—mercury; Ni—nickel; Pb—lead; Se—selenium; Zn—zinc.

According to the literature, digestates are most abundant in Zn, followed by Cu, Mn, and As [79]. Although the content of As, Cd, Cr, and Pb in various digestates is much lower relative to the commonly present Cu and Zn, these HMs are regarded as hazardous and highly persistent microelements [80]. The concentrations of HMs in digestates often exceed the values noted in the feedstock. Several studies reported on increasing concentrations of HMs after anaerobic treatment [64,81,82]. The above can be attributed to the production of methane during the AD of organic matter. This process leads to a decrease in the weight and volume of the substrate [83,84], which ultimately increases HM concentrations. Heavy metals are often closely bound to insoluble solids, which is why their concentrations are considerably higher in solid fractions after anaerobic treatment [82]. These micropollutants are present in the liquid fraction, and they are deposited and adsorbed by solid particles or colloids to form precipitates at the end of the treatment [78]. In addition, HM solubilization may be limited under alkaline conditions [71]. Due to the complexity of AD, HMs can be involved in many physical and/or chemical processes, including adsorption on the solid fraction and precipitation as carbonates, sulfides, and hydroxides [85]. The observed increase in HM concentrations in digestate indicates that the impact of this by-product on the soil environment should be examined, especially if digestate is used as a crop fertilizer. It should be emphasized that HM residues in soil, even at low concentrations, can be absorbed by roots and can accumulate in edible plant parts [52,86].

Digestate is a significant source of potential environmental contamination, which is why permissible HM levels for biomass incorporated into the soil are set by national and international laws. In Europe, the relevant limits have been prescribed by the EU Fertilizing Products Regulation (2019/1009). This regulation classifies the materials introduced into the soil into various categories. Digestate has been classified in four categories as organic fertilizer, soil improver, growing medium, and plant biostimulant. The permissible limits of HMs in each of these categories are presented in Table 2. The EU Fertilizing Products Regulation (EU 2019/1009, Table 2) lists eight HMs, and some of the AD by-products listed in Table 1 do not meet the European agricultural standards for fertilizers. In some studies, Ni, Zn, and Cu concentrations in the examined digestates, in particular digested animal manure, significantly exceeded the regulatory limits. The reported HM content of various digestates further highlights the environmental risks associated with the accumulation of these micropollutants in soils. However, some authors found that the application of digestate derived from animal manure and/or plant material can reduce the mobility and bioavailability of HM in soil through complexation, adsorption, and precipitation [28].

Table 2. Permissible levels of HMs in digestates classified as fertilizers (EU 2019/1009).

HM	Unit of Measure	Types of Digestate Used as Fertilizers			
		Organic Fertilizer	Organic Soil Improver	Growing Medium	Plant Biostimulant
Cd	$mg \cdot kg^{-1}$ DM	1.5	2	1.5	1.5
Cr		2	2	2	2
Hg		1	1	1	1
Ni		50	50	50	50
Pb		120	120	120	120
As		40	40	40	40
Cu		300	300	200	600
Zn		800	800	500	1500

DM—dry matter; As—arsenic; Cd—cadmium; Cr—chromium; Cu—copper; Hg—mercury; Ni—nickel; Pb—lead; Zn—zinc.

3.2. Antimicrobial Residue Levels in Digestates

Intensive use of antimicrobial substances in agriculture, industry, human, and veterinary medicine has led to an increase in the concentrations of antibiotic residues in various types of organic wastes that may be applied as feedstocks for AD [24,87]. The continuous release of antibiotics into the environment and their accumulation in organic biomass pose a great concern. Most drugs are not completely metabolized by humans and animals, and a significant percentage of administered antibiotics enter anaerobic bioreactors with the processed feedstock, including agricultural residues, animal manure, and sewage sludge [1]. The concentrations of antimicrobial residues in raw substrates and digestates vary significantly and range from nanograms to micrograms per kg or mL. Although antibiotics can be degraded during AD, drug removal rates vary considerably due to differences in treatment parameters, such as temperature, organic load rate, or hydraulic retention time [87,88]. Moreover, antibiotic degradation rates during AD are influenced by the chemical structure of the drug, as well as the type and characteristic parameters of the feedstock. The reported rates of drug degradation differ between studies [24,81,89]. The efficiency of drug removal is determined by process conditions and the type of processed raw material, and it has been estimated at 7–98% for tetracyclines, 36–95% for MLS antibiotics, and 20–83% for fluoroquinolones [22,37,90]. Antibiotics persist in AD by-products, which can potentially lead to the emergence, persistence, or accumulation of these micropollutants in the environment when digestate is applied to soil [87,91]. The persistence and transformation of antibiotic residues in soil are influenced by numerous processes. Various antibiotic degradation rates in soil have been reported in the literature [87,92]. Digestate can be applied as a soil conditioner to promote sustainable biogas production and the circular economy, which is why the fate of antibiotics in substrates processed by AD should be analyzed in greater detail. The risks associated with drug residues in AD by-products have not been sufficiently investigated to date, which highlights the need for further research [93]. Therefore, the types and concentrations of antimicrobials identified in digestates derived from various feedstocks are presented in Table 3.

Table 3. Types and concentrations of antimicrobial substances detected in digestates derived from the AD of various substrates.

Antimicrobial Class and Substance		Concentration of Antimicrobial Substance: Average Value or Minimum–Maximum Range		Substrate	Country	References
β-lactams	Amoxicillin	460–960	μg kg^{-1} DM	Food waste	Norway	[94]
	penicillin G	510	μg kg^{-1} DM	Cattle manure	Norway	[94]
Fluoroquinolones	Ciprofloxacin	2100	μg kg^{-1}	Cattle manure	Italy	[12]
		776,000	μg kg^{-1} DM	Mixed animal substrates	Sweden, Finland, and Germany	[95]
		1620	μg kg^{-1} DM	Mixed animal substrates	Germany	[96]
		63,100	μg kg^{-1} DM	Mixed pig slurry and sewage sludge	Sweden, Finland, and Germany	[95]
		121,000	μg kg^{-1} DM	Poultry manure	Sweden, Finland, and Germany	[95]
		430	μg kg^{-1} DM	Sewage sludge	Norway	[94]
		16.05–45.26	μg kg^{-1}	Sewage sludge	China	[97]
		1068	μg L^{-1}	Pig manure	China	[98]
		57.3–122.4	μg kg^{-1}	Pig manure	China	[99]
	Danofloxacin	963	μg L^{-1}	Pig manure	China	[98]
		970	μg kg^{-1} DM	Mixed animal substrates	Germany	[96]
	Enrofloxacin	147,000	μg kg^{-1} DM	Cattle manure	Sweden, Finland, and Germany	[95]
		82,100	μg kg^{-1} DM	Mixed animal substrates	Sweden, Finland, and Germany	[95]
		Max 1090	μg kg^{-1} DM	Mixed animal substrates	Germany	[96]
		50	μg kg^{-1}	Mixed plants and animal manure	Poland	[100]
		47,700	μg kg^{-1} DM	Mix pig slurry and sewage sludge	Sweden, Finland, and Germany	[95]
		850,000	μg kg^{-1} DM	Poultry manure	Sweden, Finland, and Germany	[95]
		6.28–20.28	μg kg^{-1}	Sewage sludge	China	[97]
		147.1–387.9	μg kg^{-1}	Sewage sludge	Poland	[11]
		200–44,200	μg kg^{-1} DM	Pig manure	Sweden, Finland, and Germany	[95,101]
		98	μg L^{-1}	Pig manure	China	[98]
		58.7–61	μg kg^{-1}	Pig manure	China	[99]
	Fleroxacin	127	μg L^{-1}	Pig manure	China	[98]
	Lomefloxacin	409	μg L^{-1}	Pig manure	China	[98]
	Norfloxacin	132.54–444.27	μg kg^{-1}	Pig manure	China	[97]
		55.9–82.4	μg kg^{-1}	Pig manure	China	[99]

Table 3. *Cont.*

Antimicrobial Class and Substance		Concentration of Antimicrobial Substance: Average Value or Minimum−Maximum Range		Substrate	Country	References
	Ofloxacin	628.89–1751.08	µg kg^{-1}			[97]
		49.9–60.2	µg kg^{-1}			[99]
		184	µg L^{-1}			[98]
	Orbifloxacin	33	µg L^{-1}			
	Sarafloxacin	445	µg L^{-1}			
	Sparfloxacin	140	µg L^{-1}			
MLS	Clarithromycin	353.28–369.84	µg L^{-1}	Pig manure	Denmark	[102]
		0.056–0.208	µg L^{-1} (LF)	Sewage sludge	Poland	[11]
		62.8 70.7	µg kg^{-1} (SF)			
	Clindamycin	0.08–0.268	µg L^{-1} (LF)			
	Erythromycin	7.8–624	µg L^{-1}	Pig manure	Denmark	[102]
Pleuromutilins	Tiamulin	148	µg kg^{-1}	Mixed plants and animal manure	Poland	[100]
Polyethermonocarboxylic acids	Monensin	220–720	µg kg^{-1}	Cattle manure	USA	[103]
Sulfonamides	Sulfadiazine	233,000	µg kg^{-1} DM		Sweden, Finland, and Germany	[95]
		n.d.–0.106	µg L^{-1} (LF)	Mixed cattle manure and maize silage	Poland	[11]
		105,000	µg kg^{-1} DM	Mixed animal substrates	Sweden, Finland, and Germany	[95]
		26,300	µg kg^{-1} DM	Mixed pig slurry and sewage sludge		
		239,000	µg kg^{-1} DM	Poultry manure	Sweden, Finland, and Germany	[95,96]
		0.098–0.136	µg L^{-1} (LF)	Sewage sludge	Poland	[11]
		12,400	µg kg^{-1} DM		Sweden, Finland, and Germany	[95]
		142,000	µg kg^{-1} DM	Pig manure		
		618	µg L−1		Denmark	[102]

Table 3. *Cont.*

Antimicrobial Class and Substance		Concentration of Antimicrobial Substance: Average Value or Minimum–Maximum Range		Substrate	Country	References
		n.d.–4.9	µg kg^{-1}		China	[99]
	Sulfadoxine	n.d.	µg kg^{-1} DM		Germany	[101]
		140	µg kg^{-1} DM	Food waste	Norway	[94]
	Sulfamethoxazole	70	µg kg^{-1}	Cattle manure	Italy	[12]
		n.d.–7.4	µg kg^{-1}	Pig manure	China	[99]
	Sulfamethazine	1400–200,900	µg kg^{-1} DM	Mixed animal substrates	Germany	[104]
		1.6	µg kg^{-1} DM	Mixed cattle and pig manure	Norway	[94]
		0.38	µg kg^{-1} DM	Mixed food waste and sewage sludge		
		66,100	µg kg^{-1} DM	Poultry manure	Sweden, Finland, Germany	[95]
		0.08	µg kg^{-1} DM	Sewage sludge	Norway	[94]
		176–3359.9	µg kg^{-1}	Pig manure	China	[99]
Tetracyclines	Chlortetracycline	240–340	µg kg^{-1}	Cattle manure	USA	[105]
		3500–36,500	µg kg^{-1} DM	Mixed animal substrates	Germany	[104]
		1300–10,100	µg kg^{-1} DM	Pig manure		[101]
		n.d.	µg kg^{-1} TS		China	[25]
		11,600	µg L^{-1} DM		USA	[106]
		1000–6000	µg L^{-1}			[103]
		36 ± 2	µg L^{-1}		China	[98]
		21,010.6–39,751.4	µg kg^{-1}			[99]
	Doxycycline	0.854–1.555	µg L^{-1} (LF)	Mixed cattle manure and maize silage	Poland	[11]
		396.7–1282.5	µg kg^{-1} (SF)			
		n.d.–3900	µg kg^{-1} DM	Pig manure	Germany	[101,107]
		360	µg L^{-1}		The Netherlands	[108]
		0.62 218.1–800.2	µg L^{-1} (LF) µg kg^{-1} (SF)	Sewage sludge	Poland	[11]
	Epichlortetracycline	99–170	µg kg^{-1} TF	Cattle manure	USA	[105]
	Epitetracycline	83–102	µg kg^{-1} TF			
	Oxytetracycline	196,000	µg kg^{-1} DM		Sweden, Finland, and Germany	[95]
		21–26	µg kg^{-1} TF		USA	[105]
		196,000	µg kg^{-1} DM	Mixed animal substrates	Sweden, Finland, and Germany	[95]
		346,000	µg kg^{-1} DM	Poultry manure		

Table 3. *Cont.*

Antimicrobial Class and Substance	Concentration of Antimicrobial Substance: Average Value or Minimum–Maximum Range		Substrate	Country	References
	1,256,000	µg kg^{-1} DM	Pig manure		
	n.d.	µg kg^{-1} TS	Pig manure	China	[25]
	34,911.8–84,687.9	µg kg^{-1}	Pig manure	China	[99]
	38.5	µg L^{-1}	Pig manure	The Netherlands	[108]
Tetracycline	290–450	µg kg^{-1} TF	Cattle manure	USA	[105]
Tetracycline	n.d.–17,030	µg kg^{-1} DM	Mixed animal substrates	Germany	[96,104]
Tetracycline	193,000	µg kg^{-1} DM	Mixed animal substrates	Sweden, Finland, and Germany	[95]
Tetracycline	438,000	µg kg^{-1} DM	Mixed pig slurry and sewage sludge	Sweden, Finland, and Germany	[95]
Tetracycline	16,399,000	µg kg^{-1} DM	Poultry manure	Sweden, Finland, and Germany	[95]
Tetracycline	0.27–0.36 464.8–1164.4	µg L^{-1} (LF) µg kg^{-1} (SF)	Sewage sludge	Poland	[11]
Tetracycline	885,000	µg kg^{-1} DM	Sewage sludge	Sweden, Finland, and Germany	[95]
Tetracycline	n.d.–6400	µg kg^{-1} DM	Pig manure	Germany	[101]
Tetracycline	1209.1–1769.7	µg kg^{-1}	Pig manure	China	[99]
Tetracycline	42.14	µg L^{-1}	Pig manure	The Netherlands	[108]

DM—dry matter; TF—total fraction; TSs—total solids; LF—liquid fraction; SF—solid fraction; n.d.—not detected.

The role of digestate as a source of antibiotics in soil ecosystems needs to be clarified. As indicated in Table 3, the concentrations of antimicrobial substances differ widely in various digestates. Antibiotic levels in digestates are determined mainly by the quality of the substrate. Sewage sludge and animal manure are more likely to be contaminated with drugs than agricultural residues. It has been reported that sulfonamide and fluoroquinolone antimicrobial agents are most prevalent in animal manure and sewage sludge [12,49,109]. Research has also shown that tetracycline and sulfonamide drugs can persist after the AD process [91]. As shown in Table 3, fluoroquinolone, sulfonamide, and tetracycline antibiotics have been most frequently detected in various AD by-products. However, the influence of AD on antibiotic concentrations in the substrates processed in anaerobic bioreactors remains unclear [12]. According to the literature, antimicrobial agents are degraded during anaerobic treatment, but this process has not been specifically designed to remove drugs [110]. As mentioned previously, the efficiency of antibiotic degradation depends on many factors. In some studies, antibiotics were not effectively removed during the AD process [104,111]. In turn, other authors have reported very high drug removal rates during AD and, consequently, very low drug concentrations in the obtained digestates [112].

The efficiency of drug removal and the concentrations of drug residues in digestates are also highly dependent on the chemical structure and specificity of antibiotics. Some antimicrobial substances are removed effectively, but their removal rates are very low (such as chlortetracycline and oxytetracycline that are characterized by high adsorption rates due to the presence of chlorine atoms and hydroxyl groups). Fluoroquinolones have a lower removal rate due to the presence of a fluorine atom [110]. In turn, β-lactams contain highly unstable β-lactam rings that are degraded by microbial β-lactamases during AD [113]. Moreover, antibiotic removal is considerably influenced by AD conditions. It has been reported that drug removal efficiency is strictly dependent on the total content of solids in the feedstock and the temperature of the anaerobic process [110]. Low drug removal rates are responsible for high concentrations of drug residues in AD by-products. The levels of ciprofloxacin, enrofloxacin, sulfadiazine, and oxytetracycline residues were particularly high in various digestates (Table 3). Due to their high stability and high adsorption potential, these antibiotics can persist in soil for long periods of time, thus posing a serious threat to the environment.

The lack of legal regulations concerning safe concentrations of drugs introduced into the soil with digestate gives serious cause for concern. Although the potential of digestate as an organic fertilizer has been studied to determine contamination with pathogens and HMs, as well as nutrient levels, the risks associated with antibiotic residues in digestate have been overlooked [21]. In Europe, the use of organic fertilizers (including digestate) is promoted by the EU Fertilizing Products Regulation (EU 2019/1009) which establishes the threshold values for total nitrogen, phosphorous, organic carbon, pathogens, and HMs that affect fertilizer quality. However, antibiotic residue levels have not been regulated to date [12]. Legal regulations concerning the permissible levels of antibiotics in digestates intended for soil fertilization should be urgently introduced. To the best of our knowledge, the presence of some antimicrobials should be banned, whereas the concentrations of other antibiotics should be restricted in fertilizer products (by defining limiting concentrations, as in the case of HMs). These measures are urgently needed to prevent the accumulation of pharmaceuticals in the environment and mitigate their negative effects on public health. At the beginning of 2024, the World Health Organization (WHO) published an updated list of medically important antimicrobials (MIAs) [114]. These drugs have been listed based on their importance in medicine, risk of antimicrobial resistance, and potential implications for public health resulting from inappropriate use, particularly in livestock farming. We believe that digestates containing third- and fourth-generation cephalosporins, quinolones, and polymyxins, i.e., drugs considered "the highest priority critically important antimicrobials", should be banned from agricultural use. In addition, maximum permissible levels should be introduced for antimicrobials classified as "critically important" and "highly important", especially aminoglycosides, macrolides, lincosamides, streptogramins, penicillins, pleuromutilins, sulfonamides, and tetracyclines, which were often detected in various digestates (Table 3).

3.3. Ecological Risks Associated with HM and Antibiotic Contamination

All HMs and some antimicrobial agents are considered persistent pollutants in agricultural ecosystems because long-term accumulation of these substances in the soil not only exerts highly toxic effects on various organisms and plants, but also contributes to the spread of microbial resistance [110]. When digestates contaminated with HMs or/and antibiotics are used as agricultural fertilizers, these substances pollute the environment and exert selective pressure on soil microorganisms [21,88]. Digestates and soil are colonized by diverse microorganisms, where bacteria harboring antibiotic resistance genes (ARGs) and heavy metal resistance genes (MRGs) pose a particular threat. Due to the widespread use of antibiotics in recent decades, ARGs have been classified as a new source of pollution that poses a threat to public health and safety [115]. High consumption of antibiotics promotes the emergence and persistence of antimicrobial resistance. Drugs exert selective pressure on ARGs [116]. In addition, HMs cannot be biodegraded; therefore, the selection pressure exerted on microorganisms in their presence is long-standing. It has been noted that high concentrations of HMs induce metal tolerance in communities of soil-dwelling bacteria [117]. Moreover, HMs also contribute to the spread of antimicrobial resistance. Research has shown that HM contamination can also promote the dissemination of ARGs among bacteria. At the molecular level, these phenomena can be interpreted as co-selection [118]. The presence of HMs in ecosystems can accelerate the development and propagation of ARGs [119], and the emergence of some ARGs may be directly associated with the presence of HMs in the environment [120]. There is scientific evidence to suggest that the release of HMs and antimicrobial substances into the environment is correlated with the presence of MRGs and ARGs. The co-selection of ARGs and MRGs has been reported in various environments [120–123]. In the context of digestate contamination, the impact of HMs and drugs on the soil environment is most meaningful because anaerobically stabilized organic matter is used primarily as a soil conditioner and fertilizer [124–127]. In one of the reviewed studies, the abundance of ARGs in the soil increased after exposure to HMs, and the observed increase was proportional to the rise in HM concentrations [128]. In another study, the spread of ARGs increased after HMs was released into antibiotic-contaminated soils [129]. However, HMs and antimicrobials may exert synergistic or antagonistic effects in the soil environment, which affects the correlation between ARG abundance and antibiotic levels [130]. Digestate-based fertilizers can release HMs and antimicrobial substances, as well as promote the spread of MRGs and ARGs in cultivated fields. These micropollutants may be transferred from the soil to groundwater and crops, ultimately reaching humans and animals [131].

In the present study, the specific environmental risks associated with HMs and drugs were analyzed to determine the potential hazards resulting from the introduction of digestates into the soil. The levels of HM contamination were estimated by calculating the geo-accumulation index (I_{geo}) and the ecological risk factor (E_r) which indicate the extent to which the analyzed digestates induce changes in HM concentrations in soil and exert toxic effects on the ecosystem (Figure 3A,B, respectively). The values of I_{geo} and E_r calculated for Cd, Cu, Hg, Mo, and Ni were indicative of high pollution levels and a high ecological risk. Approximately 25% to 33% of the analyzed digestates were extremely contaminated with Cd and Hg ($I_{geo} > 5$), and approximately 45% to 50% of the digestates were characterized as posing a very high ecological risk ($E_r > 320$). Contamination with Mo was analyzed in only one publication, and the examined digestate was characterized by the highest level of pollution which exerted a potential risk for the ecosystem. None of the analyzed digestates were contaminated with Co, Fe, or Mn ($I_{geo} < 1$), and the concentrations of these HMs were indicative of low ecological risk ($E_r < 40$). In turn, the RQ method was used to assess the extent to which the presence of drug residues in various digestates can affect selection for antibiotic resistance in the environment (RQ_{mic}; Figure 3C) and ecotoxicity (RQ_{env}; Figure 3D) of fertilizer-amended soil. In most digestates (63–100%), the RQ_{mic} values for β-lactams (amoxicillin), fluoroquinolones (ciprofloxacin, enrofloxacin, and sparfloxacin), and tetracyclines (oxytetracycline) were considerably higher than 1, which points to a high risk of selection for antimicrobial resistance in soil. Drugs representing the same antibiotic classes (β-lactams—amoxicillin; fluoroquinolones—ciprofloxacin and enrofloxacin; tetracyclines—oxytetracycline and tetracycline) posed a high risk for soil-dwelling organisms ($RQ_{env} > 1$ in 33–100% of digestate samples). In turn, all RQ_{mic} values for clindamycin, erythromycin, and sulfamethoxazole (in the range of 0.0001–0.91), as well as RQ_{env} values for clindamycin, norfloxacin, and sulfamethoxazole (0.0006–0.37), were indicative of no risk or low risk for soils fertilized with various digestates.

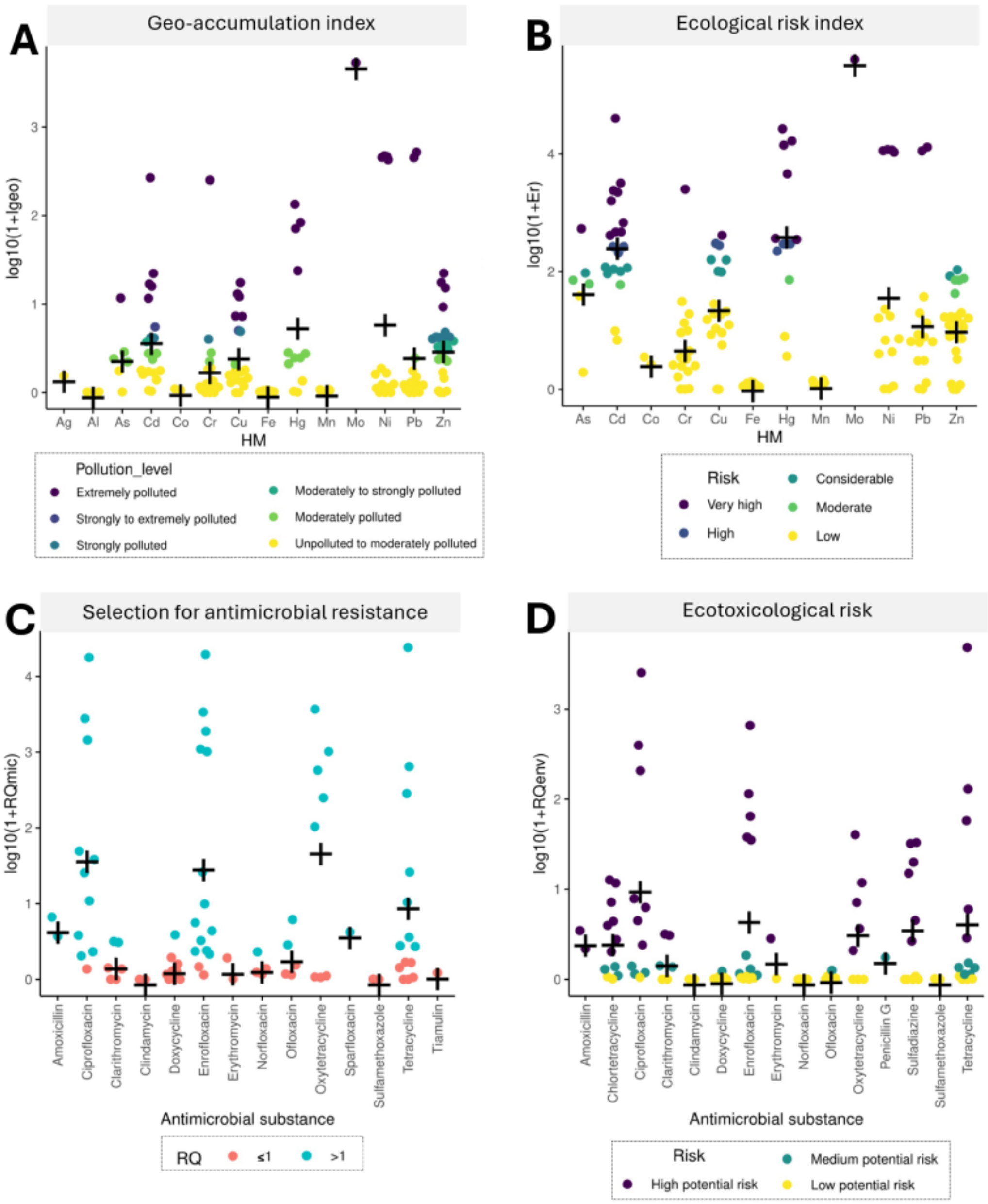

Figure 3. Ecological risk assessment of HMs and antibiotics detected in digestate based on the values of the geo-accumulation index (**A**), environmental risk factor (**B**), risk quotient (RQ) for selection for drug resistance in the environment (**C**), and ecotoxicity (**D**). The cross symbol indicates the average value.

The environmental risk assessment demonstrated that most digestates containing HMs and antibiotics could pose a high risk to the ecosystem and promote the spread of antimicrobial resistance if introduced into the soil. Moreover, the antibiotic classes considered critically important (quinolones) and highly important (β-lactams and tetracyclines) in the WHO MIA List tend to be present in high concentrations in various digestates and may exceed the alert limit as regards the risk of selection for drug resistance. The above reiterates the need for the establishment of legal limits concerning the maximum admissible concentrations of individual drugs in anaerobically stabilized organic matter that can be released into the soil environment. Considering the alarming levels of anthropogenic pollution, the fact that soil contamination with HMs and antimicrobials can exert long-term effects and promote widespread co-selection pressure for microbial resistance is of particular concern. Therefore, the close links between ARGs, MRGs and their co-transfer, especially in the presence of HMs and antibiotics, pose the greatest challenge for research in the field of environmental microbiology. The role of digestate in the environmental transfer of HMs and antibiotics is extremely important; therefore, the residual levels of these micropollutants in AD by-products should be systematically monitored and analyzed.

4. Conclusions and Future Perspectives

This review article analyzes the global scope of digestate contamination with HMs and antibiotics. Based on the results of the analysis, it can be concluded that the AD process and the resulting digestates directly contribute to the presence of HMs and antibiotics in the environment. The absence of effective methods for removing these micropollutants during anaerobic treatment promotes their further transfer into the ecosystem. Although digestates generally meet quality standards, their application in farming could pose a threat to the soil and water environment and, subsequently, to public health. This review demonstrated that HM and antibiotic residues in digestates pose a risk for soil-dwelling organisms and contribute to selection for microbial resistance in soil ecosystems. The presence of antimicrobial substances in digestates intended for agricultural use should be urgently addressed by legal regulations. We believe that a better understanding of the role of digestate as a source of anthropogenic micropollutants and the risks associated with its introduction into the soil environment will encourage a scientific debate and lead to the implementation of dedicated legislative initiatives.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su17020416/s1, Figure S1: PRISMA flowchart showing the results of the literature search and the screening process for this review.

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References

1. Czatzkowska, M.; Wolak, I.; Harnisz, M.; Korzeniewska, E. Impact of Anthropogenic Activities on the Dissemination of ARGs in the Environment—A Review. *Int. J. Environ. Res. Public Health* **2022**, *19*, 12853. [CrossRef]
2. Khan, N.; Bolan, N.; Joseph, S.; Anh, M.T.L.; Meier, S.; Kookana, R.; Borchard, N.; Sánchez-Monedero, M.A.; Jindo, K.; Solaiman, Z.M.; et al. Chapter One—Complementing compost with biochar for agriculture, soil remediation and climate mitigation. In *Advances in Agronomy*; Sparks, D.L., Ed.; Academic Press: Cambridge, MA, USA, 2023; Volume 179, pp. 1–90. [CrossRef]
3. Scarlat, N.; Dallemand, J.F.; Fahl, F. Biogas: Developments and perspectives in Europe. *Renew. Energy* **2018**, *129*, 457–472. [CrossRef]
4. Samoraj, M.; Mironiuk, M.; Izydorczyk, G.; Witek-Krowiak, A.; Szopa, D.; Moustakes, K.; Chojnacka, K. The challenges and perspectives for anaerobic digestion of animal waste and fertilizer application of the digestate. *Chemosphere* **2022**, *295*, 133799. [CrossRef]
5. Lamolinara, B.; Pérez-Martínez, A.; Guardado-Yordi, E.; Fiallos, C.G.; Diéguez-Santana, K.; Ruiz-Mercado, G.J. Anaerobic digestate management, environmental impacts, and techno-economic challenges. *Waste Manag.* **2022**, *140*, 14–30. [CrossRef]
6. Czekała, W.; Lewicki, A.; Pochwatka, P.; Czekała, A.; Wojcieszak, D.; Jóźwiakowski, K.; Waliszewska, H. Digestate management in polish farms as an element of the nutrient cycle. *J. Clean. Prod.* **2020**, *242*, 118454. [CrossRef]
7. Stürmer, B.; Pfundtner, E.; Kirchmeyr, F.; Uschnig, S. Legal requirements for digestate as fertilizer in Austria and the European Union compared to actual technical parameters. *J. Environ. Manag.* **2020**, *253*, 109756. [CrossRef] [PubMed]
8. Pastorelli, R.; Valboa, G.; Lagomarsino, A.; Fabiani, A.; Simoncini, S.; Zaghi, M.; Vignozzi, N. Recycling Biogas Digestate from Energy Crops: Effects on Soil Properties and Crop Productivity. *Appl. Sci.* **2021**, *11*, 750. [CrossRef]

9. Czatzkowska, M.; Wolak, I.; Harnisz, M.; Korzeniewska, E. Microbial diversity and biosafety judgment of digestates derived from different biogas plants for agricultural applications. *J. Environ. Manag.* **2024**, *371*, 123329. [CrossRef] [PubMed]
10. Witorożec-piechnik, A.; Kopiński, J.; Markowska-strzemska, E.; Woźniak, M. Environmental safety aspects of using the digestate from an agricultural biogas plant. *Pol. J. Agron.* **2023**, *52*, 54–61. [CrossRef]
11. Wolak, I.; Bajkacz, S.; Harnisz, M.; Stando, K.; Męcik, M.; Korzeniewska, E. Digestate from Agricultural Biogas Plants as a Reservoir of Antimicrobials and Antibiotic Resistance Genes—Implications for the Environment. *Int. J. Environ. Res. Public Health* **2023**, *20*, 2672. [CrossRef]
12. Visca, A.; Rauseo, J.; Spataro, F.; Patrolecco, L.; Grenni, P.; Massini, G.; Mazzurco Miritana, V.; Barra Caracciolo, A. Antibiotics and antibiotic resistance genes in anaerobic digesters and predicted concentrations in agroecosystems. *J. Environ. Manag.* **2022**, *301*, 113891. [CrossRef]
13. Al Seadi, T.; Drosg, B.; Fuchs, W.; Rutz, D.; Janssen, R. 12—Biogas digestate quality and utilization. In *The Biogas Handbook*; Wellinger, A., Murphy, J., Baxter, D., Eds.; Woodhead Publishing Series in Energy; Woodhead Publishing: Cambridge, UK, 2013; pp. 267–301. [CrossRef]
14. Shamuyarira, K.K.; Gumbo, J.R. Assessment of Heavy Metals in Municipal Sewage Sludge: A Case Study of Limpopo Province, South Africa. *Int. J. Environ. Res. Public Health* **2014**, *11*, 2569–2579. [CrossRef]
15. Qasem, N.A.A.; Mohammed, R.H.; Lawal, D.U. Removal of heavy metal ions from wastewater: A comprehensive and critical review. *NPJ Clean Water* **2021**, *4*, 36. [CrossRef]
16. Buta, M.; Korzeniewska, E.; Harnisz, M.; Hubeny, J.; Zieliński, W.; Rolbiecki, R.; Bajkacz, S.; Felis, E.; Kokoszka, K. Microbial and chemical pollutants on the manure-crops pathway in the perspective of 'One Health' holistic approach. *Sci. Total Environ.* **2021**, *785*, 147411. [CrossRef]
17. Ji, X.; Shen, Q.; Liu, F.; Ma, J.; Xu, G.; Wang, Y.; Wu, M. Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; China. *J. Hazard. Mater.* **2012**, *235–236*, 178–185. [CrossRef] [PubMed]
18. Essien, J.P.; Ikpe, D.I.; Inam, E.D.; Okon, A.O.; Ebong, G.A.; Benson, N.U. Occurrence and spatial distribution of heavy metals in landfill leachates and impacted freshwater ecosystem: An environmental and human health threat. *PLoS ONE* **2022**, *17*, e0263279. [CrossRef]
19. Caracciolo, A.B.; Visca, A.; Rauseo, J.; Spataro, F.; Garbini, G.L.; Grenni, P.; Mariani, L.; Mazzurco Miritana, V.; Massini, G.; Patrolecco, L. Bioaccumulation of antibiotics and resistance genes in lettuce following cattle manure and digestate fertilization and their effects on soil and phyllosphere microbial communities. *Environ. Pollut.* **2022**, *315*, 120413. [CrossRef]
20. Catenacci, A.; Boniardi, G.; Mainardis, M.; Gievers, F.; Farru, G.; Asunis, F.; Malpei, F.; Goi, D.; Cappai, G.; Canziani, R. Processes, applications and legislative framework for carbonized anaerobic digestate: Opportunities and bottlenecks. A critical review. *Energy Convers. Manag.* **2022**, *263*, 115691. [CrossRef]
21. Yusuf, H.H.; Pan, X.; Cai, G.; Cai, J.; Huang, X.; Ye, Z.L. Semi-solid anaerobic co-digestion of source-separated fecal slag and food waste: Focusing on methane production, ecological risk assessment, and quality evaluation as fertilizer. *Environ. Sci. Pollut. Res.* **2022**, *29*, 66578–66590. [CrossRef] [PubMed]
22. Zubair, M.; Li, Z.; Zhu, R.; Wang, J.; Liu, X.; Liu, X. The Antibiotics Degradation and Its Mechanisms during the Livestock Manure Anaerobic Digestion. *Molecules* **2023**, *28*, 4090. [CrossRef] [PubMed]
23. Tytła, M. Assessment of Heavy Metal Pollution and Potential Ecological Risk in Sewage Sludge from Municipal Wastewater Treatment Plant Located in the Most Industrialized Region in Poland-Case Study. *Int. J. Environ. Res. Public Health* **2019**, *16*, 2430. [CrossRef] [PubMed]
24. Marutescu, L.G.; Jaga, M.; Postolache, C.; Barbuceanu, F.; Milita, N.M.; Romascu, L.M.; Schmitt, H.; de Roda Husman, A.M.; Sefeedpari, P.; Glaeser, S.; et al. Insights into the impact of manure on the environmental antibiotic residues and resistance pool. *Front. Microbiol.* **2022**, *13*, 965132. [CrossRef]
25. Yin, F.; Dong, H.; Ji, C.; Tao, X.; Chen, Y. Effects of anaerobic digestion on chlortetracycline and oxytetracycline degradation efficiency for swine manure. *Waste Manag.* **2016**, *56*, 540–546. [CrossRef] [PubMed]
26. Hao, H.; Cheng, G.; Iqbal, Z.; Ai, X.; Hussain, H.I.; Huang, L.; Dai, M.; Wang, Y.; Liu, Z.; Yuan, Z. Benefits and risks of antimicrobial use in food-producing animals. *Front. Microbiol.* **2014**, *5*, 288. [CrossRef] [PubMed]
27. Hao, H.; Tian, Y.; Zhang, H.; Chai, Y. Copper stressed anaerobic fermentation: Biogas properties, process stability, biodegradation and enzyme responses. *Biodegradation* **2017**, *28*, 369–381. [CrossRef] [PubMed]
28. Irshad, M.; Malik, A.H.; Shaukat, S.; Mushtaq, S.; Ashraf, M. Characterization of Heavy Metals in Livestock Manures. *Pol. J. Environ. Stud.* **2013**, *22*, 1257–1262.
29. Adegbeye, M.J.; Adetuyi, B.O.; Igirigi, A.I.; Adisa, A.; Palangi, V.; Aiyedun, S.; Alvarado-Ramírez, E.R.; Elghandour, M.M.M.Y.; Márquez Molina, O.; Oladipo, A.A.; et al. Comprehensive insights into antibiotic residues in livestock products: Distribution, factors, challenges, opportunities, and implications for food safety and public health. *Food Control* **2024**, *163*, 110545. [CrossRef]

30. Liu, W.-R.; Zeng, D.; She, L.; Su, W.-X.; He, D.-C.; Wu, G.-Y.; Ma, X.-R.; Jiang, S.; Jiang, C.-H.; Ying, G.-G. Comparisons of pollution characteristics, emission situations, and mass loads for heavy metals in the manures of different livestock and poultry in China. *Sci. Total Environ.* **2020**, *734*, 139023. [CrossRef]
31. Zha, Y.; Li, Q.; Liu, H.; Ge, Y.; Wei, Y.; Wang, H.; Zhang, L.; Fan, J.; Chen, Y.; Zhang, C.; et al. Occurrence and ecological risk assessment of antibiotics in manure and the surrounding soil from typical chicken farms in Hangzhou, China. *Front. Environ. Sci.* **2023**, *11*, 1241405. [CrossRef]
32. do Carmo Precci Lopes, A.; Ebner, C.; Gerke, F.; Wehner, M.; Robra, S.; Hupfauf, S.; Bockreis, A. Residual municipal solid waste as co-substrate at wastewater treatment plants: An assessment of methane yield, dewatering potential and microbial diversity. *Sci. Total Environ.* **2022**, *804*, 149936. [CrossRef]
33. Shi, J.C.; Liao, X.D.; Wu, Y.B.; Liang, J.B. Effect of antibiotics on methane arising from anaerobic digestion of pig manure. *Anim. Feed Sci. Technol.* **2011**, *166–167*, 457–463. [CrossRef]
34. Turker, G.; Akyol, Ç.; Ince, O.; Aydin, S.; Ince, B. Operating conditions influence microbial community structures, elimination of the antibiotic resistance genes and metabolites during anaerobic digestion of cow manure in the presence of oxytetracycline. *Ecotoxicol. Environ. Saf.* **2018**, *147*, 349–356. [CrossRef] [PubMed]
35. Álvarez, J.A.; Otero, L.; Lema, J.M.; Omil, F. The effect and fate of antibiotics during the anaerobic digestion of pig manure. *Bioresour. Technol.* **2010**, *101*, 8581–8586. [CrossRef]
36. Coban, H.; Ertekin, E.; Ince, O.; Turker, G.; Akyol, Ç.; Ince, B. Degradation of oxytetracycline and its impacts on biogas-producing microbial community structure. *Bioprocess Biosyst. Eng.* **2016**, *39*, 1051–1060. [CrossRef]
37. Czatzkowska, M.; Harnisz, M.; Korzeniewska, E.; Rusanowska, P.; Bajkacz, S.; Felis, E.; Jastrzębski, J.P.; Paukszto, Ł.; Koniuszewska, I. The impact of antimicrobials on the efficiency of methane fermentation of sewage sludge, changes in microbial biodiversity and the spread of antibiotic resistance. *J. Hazard. Mater.* **2021**, *416*, 125773. [CrossRef]
38. Huang, Q.; Yu, Y.; Wan, Y.; Wang, Q.; Luo, Z.; Qiao, Y.; Su, D.; Li, H. Effects of continuous fertilization on bioavailability and fractionation of cadmium in soil and its uptake by rice (*Oryza sativa* L.). *J. Environ. Manag.* **2018**, *215*, 13–21. [CrossRef] [PubMed]
39. Pan, M.; Chu, L.M. Adsorption and degradation of five selected antibiotics in agricultural soil. *Sci. Total Environ.* **2016**, *545–546*, 48–56. [CrossRef]
40. Alengebawy, A.; Abdelkhalek, S.T.; Qureshi, S.R.; Wang, M.-Q. Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. *Toxics* **2021**, *9*, 42. [CrossRef]
41. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Med.* **2009**, *6*, e1000097. [CrossRef] [PubMed]
42. Muller, G. Index of geoaccumulation in sediments of the rhine river. *GeoJournal* **1969**, *2*, 108–118.
43. Reimann, C.; Caritat, P. *Chemical Elements in the Environment*; Springer: Berlin/Heidelberg, Germany, 1998. [CrossRef]
44. Håkanson, L. An ecological risk index for aquatic pollution control. A sedimentological approach. *Water Res.* **1980**, *14*, 975–1001. [CrossRef]
45. Zhao, S.; Feng, C.; Yang, Y.; Niu, J.; Shen, Z. Risk assessment of sedimentary metals in the Yangtze Estuary: New evidence of the relationships between two typical index methods. *J. Hazard. Mater.* **2012**, *241–242*, 164–172. [CrossRef] [PubMed]
46. Chen, H.; Chen, Z.; Chen, Z.; Ou, X.; Chen, J. Calculation of Toxicity Coefficient of Potential Ecological Risk Assessment of Rare Earth Elements. *Bull. Environ. Contam. Toxicol.* **2020**, *104*, 582–587. [CrossRef] [PubMed]
47. Bengtsson-Palme, J.; Larsson, D.G.J. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. *Environ. Int.* **2016**, *86*, 140–149. [CrossRef] [PubMed]
48. EMA. Committee for Medicinal Products for Human Use (CHMP) Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use. 2024. Available online: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-environmental-risk-assessment-medicinal-products-human-use-revision-1_en.pdf (accessed on 9 October 2024).
49. Ajibola, A.S.; Zwiener, C. Occurrence and Risk Assessment of Antibiotic Residues in Sewage Sludge of Two Nigerian Hospital Wastewater Treatment Plants. *Water Air Soil Pollut.* **2022**, *233*, 405. [CrossRef]
50. Vestel, J.; Caldwell, D.J.; Tell, J.; Constantine, L.; Häner, A.; Hellstern, J.; Journel, R.; Ryan, J.J.; Swenson, T.; Xei, W. Default predicted no-effect target concentrations for antibiotics in the absence of data for the protection against antibiotic resistance and environmental toxicity. *Integr. Environ. Assess. Manag.* **2022**, *18*, 863–867. [CrossRef] [PubMed]
51. AMR Industry Alliance. AMR Alliance Science-Based PNEC Targets for Risk Assessments. 2024. Available online: https://www.amrindustryalliance.org/wp-content/uploads/2023/02/AMR-Table-1-Update-20230222_corrected.pdf (accessed on 7 October 2024).
52. Buta, M.; Hubeny, J.; Zieliński, W.; Harnisz, M.; Korzeniewska, E. Sewage sludge in agriculture—The effects of selected chemical pollutants and emerging genetic resistance determinants on the quality of soil and crops—A review. *Ecotoxicol. Environ. Saf.* **2021**, *214*, 112070. [CrossRef]
53. Seiler, C.; Berendonk, T.U. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Front. Microbiol.* **2012**, *3*, 399. [CrossRef]

54. Kang, W.; Zhang, Y.-J.; Shi, X.; He, J.-Z.; Hu, H.-W. Short-term copper exposure as a selection pressure for antibiotic resistance and metal resistance in an agricultural soil. *Environ. Sci. Pollut. Res.* **2018**, *25*, 29314–29324. [CrossRef] [PubMed]
55. Kovačić, Đ.; Lončarić, Z.; Jović, J.; Samac, D.; Popović, B.; Tišma, M. Digestate Management and Processing Practices: A Review. *Appl. Sci.* **2022**, *12*, 9216. [CrossRef]
56. Kupper, T.; Bürge, D.; Bachmann, H.J.; Güsewell, S.; Mayer, J. Heavy metals in source-separated compost and digestates. *Waste Manag.* **2014**, *34*, 867–874. [CrossRef] [PubMed]
57. Wan, Y.; Liu, J.; Zhuang, Z.; Wang, Q.; Li, H. Heavy Metals in Agricultural Soils: Sources, Influencing Factors, and Remediation Strategies. *Toxics* **2024**, *12*, 63. [CrossRef] [PubMed]
58. Zhen, H.; Jia, L.; Huang, C.; Qiao, Y.; Li, J.; Li, H.; Chen, Q.; Wan, Y. Long-term effects of intensive application of manure on heavy metal pollution risk in protected-field vegetable production. *Environ. Pollut.* **2020**, *263*, 114552. [CrossRef] [PubMed]
59. Cheng, M.; Wu, L.; Huang, Y.; Luo, Y.; Christie, P. Total concentrations of heavy metals and occurrence of antibiotics in sewage sludges from cities throughout China. *J. Soils Sediments* **2014**, *14*, 1123–1135. [CrossRef]
60. Fijalkowski, K.; Rorat, A.; Grobelak, A.; Kacprzak, M.J. The presence of contaminations in sewage sludge—The current situation. *J. Environ. Manag.* **2017**, *203*, 1126–1136. [CrossRef] [PubMed]
61. Iglesias, M.; Marguí, E.; Camps, F.; Hidalgo, M. Extractability and crop transfer of potentially toxic elements from mediterranean agricultural soils following long-term sewage sludge applications as a fertilizer replacement to barley and maize crops. *Waste Manag.* **2018**, *75*, 312–318. [CrossRef]
62. Kelepertzis, E. Accumulation of heavy metals in agricultural soils of Mediterranean: Insights from Argolida basin, Peloponnese, Greece. *Geoderma* **2014**, *221–222*, 82–90. [CrossRef]
63. Liu, C.; Tong, Q.; Li, Y.; Wang, N.; Liu, B.; Zhang, X. Biogas production and metal passivation analysis during anaerobic digestion of pig manure: Effects of a magnetic Fe_3O_4/FA composite supplement. *RSC Adv.* **2019**, *9*, 4488–4498. [CrossRef] [PubMed]
64. Derehajło, S.; Tymińska, M.; Skibko, Z.; Borusiewicz, A.; Romaniuk, W.; Kuboń, M.; Olech, E.; Koszel, M. Heavy Metal Content in Substrates in Agricultural Biogas Plants. *Agric. Eng.* **2023**, *27*, 315–329. [CrossRef]
65. Golovko, O.; Ahrens, L.; Schelin, J.; Sörengård, M.; Bergstrand, K.-J.; Asp, H.; Hultberg, M.; Wiberg, K. Organic micropollutants, heavy metals and pathogens in anaerobic digestate based on food waste. *J. Environ. Manag.* **2022**, *313*, 114997. [CrossRef]
66. Guo, Q.; Ji, J.; Ling, Z.; Zhang, K.; Xu, R.; Leng, X.; Mao, C.; Zhou, T.; Wang, H.; Liu, P.; et al. Bioaugmentation improves the anaerobic co-digestion of cadmium-containing plant residues and cow manure. *Environ. Pollut.* **2021**, *289*, 117885. [CrossRef] [PubMed]
67. Szaja, A.; Montusiewicz, A.; Lebiocka, M. Variability of Micro- and Macro-Elements in Anaerobic Co-Digestion of Municipal Sewage Sludge and Food Industrial By-Products. *Int. J. Environ. Res. Public Health* **2023**, *20*, 5405. [CrossRef] [PubMed]
68. Legros, S.; Levard, C.; Marcato-Romain, C.-E.; Guiresse, M.; Doelsch, E. Anaerobic Digestion Alters Copper and Zinc Speciation. *Environ. Sci. Technol.* **2017**, *51*, 10326–10334. [CrossRef]
69. Zhou, W.; Chen, X.; Wang, Y.; Tuersun, N.; Ismail, M.; Cheng, C.; Li, Z.; Song, Q.; Wang, Y.; Ma, C. Anaerobic co-digestion of textile dyeing sludge: Digestion efficiency and heavy metal stability. *Sci. Total Environ.* **2021**, *801*, 149722. [CrossRef] [PubMed]
70. Knoop, C.; Tietze, M.; Dornack, C.; Raab, T. Fate of nutrients and heavy metals during two-stage digestion and aerobic post-treatment of municipal organic waste. *Bioresour. Technol.* **2018**, *251*, 238–248. [CrossRef]
71. Carchesio, M.; Di Addario, M.; Tatàno, F.; de Rosa, S.; Gambioli, A. Evaluation of the biochemical methane potential of residual organic fraction and mechanically-biologically treated organic outputs intended for landfilling. *Waste Manag.* **2020**, *113*, 20–31. [CrossRef] [PubMed]
72. Cucina, M.; Tacconi, C.; Ricci, A.; Pezzolla, D.; Sordi, S.; Zadra, C.; Gigliotti, G. Evaluation of benefits and risks associated with the agricultural use of organic wastes of pharmaceutical origin. *Sci. Total Environ.* **2018**, *613–614*, 773–782. [CrossRef]
73. Riaz, L.; Wang, Q.; Yang, Q.; Li, X.; Yuan, W. Potential of industrial composting and anaerobic digestion for the removal of antibiotics, antibiotic resistance genes and heavy metals from chicken manure. *Sci. Total Environ.* **2020**, *718*, 137414. [CrossRef] [PubMed]
74. Xin, L.; Guo, Z.; Xiao, X.; Peng, C.; Zeng, P.; Feng, W.; Xu, W. Feasibility of anaerobic digestion on the release of biogas and heavy metals from rice straw pretreated with sodium hydroxide. *Environ. Sci. Pollut. Res.* **2019**, *26*, 19434–19444. [CrossRef] [PubMed]
75. Guo, Y.; Guo, Y.; Gong, H.; Fang, N.; Tan, Y.; Zhou, W.; Huang, J.; Dai, L.; Dai, X. Variations of heavy metals, nutrients, POPs and particle size distribution during 'sludge anaerobic digestion-solar drying-land utilization process': Case study in China. *Sci. Total Environ.* **2021**, *801*, 149609. [CrossRef]
76. Liu, J.; He, X.; Xu, Y.; Zuo, Z.; Lei, P.; Zhang, J.; Yin, Y.; Wei, Y. Fate of mercury and methylmercury in full-scale sludge anaerobic digestion combined with thermal hydrolysis. *J. Hazard. Mater.* **2021**, *406*, 124310. [CrossRef] [PubMed]
77. Montusiewicz, A.; Szaja, A.; Musielewicz, I.; Cydzik-Kwiatkowska, A.; Lebiocka, M. Effect of bioaugmentation on digestate metal concentrations in anaerobic digestion of sewage sludge. *PLoS ONE* **2020**, *15*, e0235508. [CrossRef] [PubMed]

78. Zheng, X.; Liu, Y.; Huang, J.; Du, Z.; Zhouyang, S.; Wang, Y.; Zheng, Y.; Li, Q.; Shen, X. The influence of variables on the bioavailability of heavy metals during the anaerobic digestion of swine manure. *Ecotoxicol. Environ. Saf.* **2020**, *195*, 110457. [CrossRef] [PubMed]
79. Zhu, N.M.; Guo, X.J. Sequential extraction of anaerobic digestate sludge for the determination of partitioning of heavy metals. *Ecotoxicol. Environ. Saf.* **2014**, *102*, 18–24. [CrossRef]
80. Raj, D.; Maiti, S.K. Sources, bioaccumulation, health risks and remediation of potentially toxic metal(loid)s (As, Cd, Cr, Pb and Hg): An epitomised review. *Environ. Monit. Assess.* **2020**, *192*, 108. [CrossRef]
81. Kadam, R.; Khanthong, K.; Jang, H.; Lee, J.; Park, J. Occurrence, Fate, and Implications of Heavy Metals during Anaerobic Digestion: A Review. *Energies* **2022**, *15*, 8618. [CrossRef]
82. Jin, H.; Chang, Z. Distribution of Heavy Metal Contents and Chemical Fractions in Anaerobically Digested Manure Slurry. *Appl. Biochem. Biotechnol.* **2011**, *164*, 268–282. [CrossRef]
83. Qi, G.; Pan, Z.; Sugawa, Y.; Andriamanohiarisoamanana, F.J.; Yamashiro, T.; Iwasaki, M.; Kawamoto, K.; Ihara, I.; Umetsu, K. Comparative fertilizer properties of digestates from mesophilic and thermophilic anaerobic digestion of dairy manure: Focusing on plant growth promoting bacteria (PGPB) and environmental risk. *J. Mater. Cycles Waste Manag.* **2018**, *20*, 1448–1457. [CrossRef]
84. Dong, B.; Liu, X.; Dai, L.; Dai, X. Changes of heavy metal speciation during high-solid anaerobic digestion of sewage sludge. *Bioresour. Technol.* **2013**, *131*, 152–158. [CrossRef]
85. Choong, Y.Y.; Norli, I.; Abdullah, A.Z.; Yhaya, M.F. Impacts of trace element supplementation on the performance of anaerobic digestion process: A critical review. *Bioresour. Technol.* **2016**, *209*, 369–379. [CrossRef]
86. Jolly, Y.N.; Islam, A.; Akbar, S. Transfer of metals from soil to vegetables and possible health risk assessment. *SpringerPlus* **2013**, *2*, 385. [CrossRef]
87. Cycoń, M.; Mrozik, A.; Piotrowska-Seget, Z. Antibiotics in the soil environment—Degradation and their impact on microbial activity and diversity. *Front. Microbiol.* **2019**, *10*, 338. [CrossRef] [PubMed]
88. Gurmessa, B.; Pedretti, E.F.; Cocco, S.; Cardelli, V.; Corti, G. Manure anaerobic digestion effects and the role of pre- and post-treatments on veterinary antibiotics and antibiotic resistance genes removal efficiency. *Sci. Total Environ.* **2020**, *721*, 137532. [CrossRef] [PubMed]
89. Aziz, A.; Sengar, A.; Basheer, F.; Farooqi, I.H.; Isa, M.H. Anaerobic digestion in the elimination of antibiotics and antibiotic-resistant genes from the environment—A comprehensive review. *J. Environ. Chem. Eng.* **2022**, *10*, 106423. [CrossRef]
90. Koniuszewska, I.; Harnisz, M.; Korzeniewska, E.; Czatzkowska, M.; Jastrzębski, J.P.; Paukszto, Ł.; Bajkacz, S.; Felis, E.; Rusanowska, P. The Effect of Antibiotics on Mesophilic Anaerobic Digestion Process of Cattle Manure. *Energies* **2021**, *14*, 1125. [CrossRef]
91. Kasumba, J.; Appala, K.; Agga, G.E.; Loughrin, J.H.; Conte, E.D. Anaerobic digestion of livestock and poultry manures spiked with tetracycline antibiotics. *J. Environ. Sci. Health Part B* **2020**, *55*, 135–147. [CrossRef]
92. Lu, J.; Xu, S. Post-treatment of food waste digestate towards land application: A review. *J. Clean. Prod.* **2021**, *303*, 127033. [CrossRef]
93. Polianciuc, S.I.; Gurzău, A.E.; Kiss, B.; Ştefan, M.G.; Loghin, F. Antibiotics in the environment: Causes and consequences. *Med. Pharm. Rep.* **2020**, *93*, 231–240. [CrossRef]
94. Nesse, A.S.; Aanrud, S.G.; Lyche, J.L.; Sogn, T.; Kallenborn, R. Confirming the presence of selected antibiotics and steroids in Norwegian biogas digestate. *Environ. Sci. Pollut. Res.* **2022**, *29*, 86595–86605. [CrossRef]
95. Lehmann, L.; Bloem, E. Antibiotic residues in substrates and output materials from biogas plants—Implications for agriculture. *Chemosphere* **2021**, *278*, 130425. [CrossRef]
96. Ratsak, C.; Guhl, B.; Zühlke, S.; Delschen, T. Veterinärantibiotikarückstände in Gülle und Gärresten aus Nordrhein-Westfalen [Veterinary antibiotic residues in manure and digestates in Northrhein-Westfalia]. *Environ. Sci. Eur.* **2013**, *25*, 7. [CrossRef]
97. Sun, C.; Li, W.; Chen, Z.; Qin, W.; Wen, X. Responses of antibiotics, antibiotic resistance genes, and mobile genetic elements in sewage sludge to thermal hydrolysis pre-treatment and various anaerobic digestion conditions. *Environ. Int.* **2019**, *133 Pt A*, 105156. [CrossRef]
98. Yin, F.; Lin, S.; Zhou, X.; Dong, H.; Zhan, Y. Fate of antibiotics during membrane separation followed by physical-chemical treatment processes. *Sci. Total Environ.* **2021**, *759*, 143520. [CrossRef] [PubMed]
99. Liu, H.; Pu, C.; Yu, X.; Sun, Y.; Chen, J. Removal of tetracyclines, sulfonamides, and quinolones by industrial-scale composting and anaerobic digestion processes. *Environ. Sci. Pollut. Res.* **2018**, *25*, 35835–35844. [CrossRef] [PubMed]
100. Patyra, E.; Kwiatek, K.; Nebot, C.; Gavilán, R.E. Quantification of veterinary antibiotics in pig and poultry feces and liquid manure as a non-invasive method to monitor antibiotic usage in livestock by liquid chromatography mass-spectrometry. *Molecules* **2020**, *25*, 3265. [CrossRef] [PubMed]
101. Wolters, B.; Widyasari-Mehta, A.; Kreuzig, R.; Smalla, K. Contaminations of organic fertilizers with antibiotic residues, resistance genes, and mobile genetic elements mirroring antibiotic use in livestock? *Appl. Microbiol. Biotechnol.* **2016**, *100*, 9343–9353. [CrossRef]

102. Feng, L.; Casas, M.E.; Ottosen, L.D.M.; Møller, H.B.; Bester, K. Removal of antibiotics during the anaerobic digestion of pig manure. *Sci. Total Environ.* **2017**, *603–604*, 219–225. [CrossRef]
103. Varel, V.H.; Wells, J.E.; Shelver, W.L.; Rice, C.P.; Armstrong, D.L.; Parker, D.B. Effect of anaerobic digestion temperature on odour, coliforms and chlortetracycline in swine manure or monensin in cattle manure. *J. Appl. Microbiol.* **2012**, *112*, 705–715. [CrossRef] [PubMed]
104. Spielmeyer, A.; Ahlborn, J.; Hamscher, G. Simultaneous determination of 14 sulfonamides and tetracyclines in biogas plants by liquid-liquid-extraction and liquid chromatography tandem mass spectrometry. *Anal. Bioanal. Chem.* **2014**, *406*, 2513–2524. [CrossRef] [PubMed]
105. Wallace, J.S.; Garner, E.; Pruden, A.; Aga, D.S. Occurrence and transformation of veterinary antibiotics and antibiotic resistance genes in dairy manure treated by advanced anaerobic digestion and conventional treatment methods. *Environ. Pollut.* **2018**, *236*, 764–772. [CrossRef] [PubMed]
106. Stone, J.J.; Clay, S.A.; Zhu, Z.; Wong, K.L.; Porath, L.R.; Spellman, G.M. Effect of antimicrobial compounds tylosin and chlortetracycline during batch anaerobic swine manure digestion. *Water Res.* **2009**, *43*, 4740–4750. [CrossRef]
107. Widyasari-Mehta, A.; Suwito, H.R.K.A.; Kreuzig, R. Laboratory testing on the removal of the veterinary antibiotic doxycycline during long-term liquid pig manure and digestate storage. *Chemosphere* **2016**, *149*, 154–160. [CrossRef] [PubMed]
108. Bousek, J.; Schöpp, T.; Schwaiger, B.; Lesueur, C.; Fuchs, W.; Weissenbacher, N. Behaviour of doxycycline, oxytetracycline, tetracycline and flumequine during manure up-cycling for fertilizer production. *J. Environ. Manag.* **2018**, *223*, 545–553. [CrossRef] [PubMed]
109. Karcı, A.; Balcıoğlu, I.A. Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. *Sci. Total Environ.* **2009**, *407*, 4652–4664. [CrossRef] [PubMed]
110. Yang, G.; Xie, S.; Yang, M.; Tang, S.; Zhou, L.; Jiang, W.; Zhou, B.; Li, Y.; Si, B. A critical review on retaining antibiotics in liquid digestate: Potential risk and removal technologies. *Sci. Total Environ.* **2022**, *853*, 158550. [CrossRef]
111. Mitchell, S.M.; Ullman, J.L.; Teel, A.L.; Watts, R.J.; Frear, C. The effects of the antibiotics ampicillin, florfenicol, sulfamethazine, and tylosin on biogas production and their degradation efficiency during anaerobic digestion. *Bioresour. Technol.* **2013**, *149*, 244–252. [CrossRef] [PubMed]
112. Miritana, V.M.; Massini, G.; Visca, A.; Grenni, P.; Patrolecco, L.; Spataro, F.; Rauseo, J.; Garbini, G.L.; Signorini, A.; Rosa, S.; et al. Effects of Sulfamethoxazole on the Microbial Community Dynamics During the Anaerobic Digestion Process. *Front. Microbiol.* **2020**, *11*, 537783. [CrossRef]
113. Oberoi, A.S.; Jia, Y.; Zhang, H.; Khanal, S.K.; Lu, H. Insights into the Fate and Removal of Antibiotics in Engineered Biological Treatment Systems: A Critical Review. *Environ. Sci. Technol.* **2019**, *53*, 7234–7264. [CrossRef]
114. WHO. *WHO's List of Medically Important Antimicrobials A Risk Management Tool for Mitigating Antimicrobial Resistance Due to Non-Human Use*; WHO: Geneva, Switzerland, 2024.
115. Barancheshme, F.; Munir, M. Development of Antibiotic Resistance in Wastewater Treatment Plants. In *Antimicrobial Resistance*; Kumar, Y., Ed.; IntechOpen: Rijeka, Croatia, 2019; Chapter 5. [CrossRef]
116. Xiong, W.; Sun, Y.; Ding, X.; Wang, M.; Zeng, Z. Selective pressure of antibiotics on ARGs and bacterial communities in manure-polluted freshwater-sediment microcosms. *Front. Microbiol.* **2015**, *6*, 194. [CrossRef]
117. Santás-Miguel, V.; Núñez-Delgado, A.; Álvarez-Rodríguez, E.; Díaz-Raviña, M.; Arias-Estévez, M.; Fernández-Calviño, D. Tolerance of soil bacterial community to tetracycline antibiotics induced by As, Cd, Zn, Cu, Ni, Cr, and Pb pollution. *Soil* **2022**, *8*, 437–449. [CrossRef]
118. Chen, J.; Li, J.; Zhang, H.; Shi, W.; Liu, Y. Bacterial heavy-metal and antibiotic resistance genes in a copper tailing dam area in northern China. *Front. Microbiol.* **2019**, *10*, 1916. [CrossRef] [PubMed]
119. Imran, M.; Das, K.R.; Naik, M.M. Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: An emerging health threat. *Chemosphere* **2019**, *215*, 846–857. [CrossRef] [PubMed]
120. Gupta, S.; Graham, D.W.; Sreekrishnan, T.R.; Ahammad, S.Z. Effects of heavy metals pollution on the co-selection of metal and antibiotic resistance in urban rivers in UK and India. *Environ. Pollut.* **2022**, *306*, 119326. [CrossRef] [PubMed]
121. Pedro, F.; Espírito Santo, C.; Branco, R.; Francisco, R.; Santos, S.; Hansen, L.; Sorensen, S.; Morais, P.V. Natural Hot Spots for Gain of Multiple Resistances: Arsenic and Antibiotic Resistances in Heterotrophic, Aerobic Bacteria from Marine Hydrothermal Vent Fields. *Appl. Environ. Microbiol.* **2015**, *81*, 2534–2543. [CrossRef]
122. Fang, L.; Li, X.; Li, L.; Li, S.; Liao, X.; Sun, J.; Liu, Y. Co-spread of metal and antibiotic resistance within ST3-IncHI2 plasmids from *E. coli* isolates of food-producing animals. *Sci. Rep.* **2016**, *6*, 25312. [CrossRef]
123. Edet, U.O.; Bassey, I.U.; Joseph, A.P. Heavy metal co-resistance with antibiotics amongst bacteria isolates from an open dumpsite soil. *Heliyon* **2023**, *9*, e13457. [CrossRef]
124. van Midden, C.; Harris, J.; Shaw, L.; Sizmur, T.; Pawlett, M. The impact of anaerobic digestate on soil life: A review. *Appl. Soil Ecol.* **2023**, *191*, 105066. [CrossRef]

125. Gurmessa, B.; Cocco, S.; Ashworth, A.J.; Udawatta, R.P.; Cardelli, V.; Ilari, A.; Serrani, D.; Fornasier, F.; Del Gatto, A.; Foppa Pedretti, E.; et al. Short term effects of digestate and composted digestate on soil health and crop yield: Implications for sustainable bioWaste Management in the bioenergy sector. *Sci. Total Environ.* **2024**, *906*, 167208. [CrossRef] [PubMed]
126. Nikolaidou, C.; Mola, M.; Papakostas, S.; Aschonitis, V.G.; Monokrousos, N.; Kougias, P.G. The effect of anaerobic digestate as an organic soil fertilizer on the diversity and structure of the indigenous soil microbial and nematode communities. *Environ. Sci. Pollut. Res.* **2024**. [CrossRef] [PubMed]
127. Doyeni, M.O.; Stulpinaite, U.; Baksinskaite, A.; Suproniene, S.; Tilvikiene, V. The Effectiveness of Digestate Use for Fertilization in an Agricultural Cropping System. *Plants* **2021**, *10*, 1734. [CrossRef]
128. Fu, Y.; Zhu, Y.; Dong, H.; Li, J.; Zhang, W.; Shao, Y.; Shao, Y. Mechanisms of the effects of humic acid on antibiotic resistance genes and microbial communities in Cd-contaminated soils. *Process Saf. Environ. Prot.* **2022**, *160*, 62–69. [CrossRef]
129. Guo, H.; Xue, S.; Nasir, M.; Gu, J.; Lv, J. Impacts of cadmium addition on the alteration of microbial community and transport of antibiotic resistance genes in oxytetracycline contaminated soil. *J. Environ. Sci.* **2021**, *99*, 51–58. [CrossRef] [PubMed]
130. Fu, Y.; Zhu, Y.; Dong, H.; Li, J.; Zhang, W.; Shao, Y.; Shao, Y. Effects of heavy metals and antibiotics on antibiotic resistance genes and microbial communities in soil. *Process Saf. Environ. Prot.* **2023**, *169*, 418–427. [CrossRef]
131. Xiao, Y.; Raheem, A.; Ding, L.; Chen, W.-H.; Chen, X.; Wang, F.; Lin, S.-L. Pretreatment, modification and applications of sewage sludge-derived biochar for resource recovery—A review. *Chemosphere* **2022**, *287*, 131969. [CrossRef] [PubMed]