

Review

Occurrence, Fate, and Implications of Heavy Metals during Anaerobic Digestion: A Review

Rahul Kadam, Kamonwan Khanthong, Heewon Jang, Jonghwa Lee and Jungyu Park * 

Department of Advanced Energy Engineering, Chosun University, Gwangju 61452, Republic of Korea

* Correspondence: jp@chosun.ac.kr; Tel.: +82-62-230-7119; Fax: +82-62-230-7110

Abstract: Over the years, anaerobic digestion (AD) has evolved as a competent technology to retrieve energy potential from various organic substrates, including wastewater. However, the energy metabolisms of anaerobic microorganisms, biochemical reactions, and biogas production are affected by various parameters, including heavy metals. It is important to understand the interaction of heavy metals with anaerobes and their potential influence on the process to enhance energy potential. This review methodically outlines the occurrence and role of heavy metals in the AD process. Additionally, the repercussions of the most common heavy metals (i.e., Cu, Zn, Cd, Fe, and Ni) on each stage of AD (i.e., hydrolysis, acidogenesis, and methanogenesis) have been discussed. We found that traces of heavy metals can endorse anaerobic digestion, but inhibition increases with increasing concentration. Methanogenic archaea are more susceptible to heavy metal inhibition than hydrolytic and acidogenic archaea. An improved understanding and relevant intuition will help to promote biogas production along with heavy metal management.

Keywords: anaerobic digestion; heavy metals; fate and effect; toxicity; remediation



Citation: 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. <https://doi.org/10.3390/en15228618>

Academic Editor: Xavier Flotats

Received: 28 September 2022

Accepted: 16 November 2022

Published: 17 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Anaerobic digestion (AD) is an engineered ecosystem where organic compounds are anaerobically disintegrated and degraded via a complex cascade of microbe-assisted reactions including hydrolysis, acidogenesis, acetogenesis, and methanogenesis [1]. Being one of the most effective technologies to treat waste, it has been widely implemented to treat industrial, municipal, and agricultural wastes for the last 100 years. It offers various advantages, such as minimal energy requirement and high energy recovery with very low sludge production. AD has the capability to meet 25% of global natural gas demand and 6% of primary energy demand [2]. Regardless of the enormous waste availability and significant potential of AD, its commercialization remains underrated, with a 0.2–0.4% share of global electricity production [3]. For efficient utilization of the potential of biogas generation, existing technological barriers such as sluggish start-ups and process inhibitors such as ammonium, volatile fatty acids (VFAs), and more importantly, heavy metals (HMs), should be addressed. Industrial and economic evolution has resulted in the release of high-concentration HMs, such as nickel (Ni), copper (Cu), and iron (Fe) into the environment via industrial discharge [4]. Similarly, animal manure, which is rich in nutrients such as nitrogen (N) and phosphorous (P), contains significant numbers of HMs including Cu, zinc (Zn), cadmium (Cd), arsenic (As), lead (Pb), chromium (Cr), Ni, and manganese (Mn) [5]. These HMs are characterized as non-biodegradable and toxic in nature, with the majority originating from plants and their addition in animal feed [6]. Despite that, only 10–20% of HMs are utilized by animals, and the rest 80–90% are discharged in solid and liquid forms [7]. Discharged HMs via industrial wastewater and animal manure end up in the reactors of AD-based treatment facilities. Even if AD can treat all sorts of waste associated with HMs, it is reported that they inhibit the AD process [8,9], which largely depends on their type and concentration. HMs in industrial wastewater and

animal manure disrupt the AD balance, which eventually leads to variation in the microbial community [10]. The presence of high concentrations of HM in AD reactors breaks the mass transfer barrier, further reducing the efficacy of AD. In addition, it becomes problematic to retrieve microorganism activity from AD reactors affected by HMs [11]. Most studies have focused on the influence of individual HMs on microorganisms and process stability. Recent reviews have provided information on the effect of HMs along with other contaminants on AD [12], as well as the fate and availability of HMs during AD and composting [13]. However, these reviews mainly focused on performance, bioavailability, environmental risk assessment, inhibition mechanisms, and interaction with the microbial community. In this review, we systematically provide information on the occurrence, interaction, and fate of HMs in the AD process, along with detoxifying techniques especially aimed at managing HMs. Additionally, impending threats and future perspectives are discussed.

2. Presence and Role of HMs in the AD Process

The presence of HMs has been identified as trace elements requisite in the AD process, which is key in biogas generation. HMs such as Cd, Cr, Cu, Fe, Pb, Ni, Zn, and other trace metals are commonly detected in agricultural and industrial wastewaters, and municipal sludge [14]. The sources and levels of HMs in various AD substrates are presented in Table S1 (Supplementary File) [15,16]. The term HM is attributed to metals and metalloids with densities higher than 5 g cm^{-3} and is mostly associated with environmental concerns. In the AD process, HMs may feature as microelements crucial for diverse enzymatic reactions, prohibitors of sulfide toxicity, and binding irrevocable nutrients, inhibitors, and toxicants of microbial biomass, and biomass stimulants [17]. Furthermore, microbial growth in the AD process directly or indirectly depends on the availability of HM. The necessity of different HMs such as Mo, Cu, Fe, Ni, and Zn by methanogenic bacteria has been reported in previous literature [18]. Similarly, it has been reported that AD reactors operated with solid organic matter such as crop residues, municipal solid waste (organic fraction), and animal manure require traces of HMs [19].

HMs from the iron family (i.e., Co, Fe, and Ni) are believed to play a critical role in the AD process owing to their redox properties and participation in electron transport catabolism. Ni is crucial for catalyzing hydrogenase conversion and carbon monoxide dehydrogenase (CODH), which plays a key role in acetogenic bacteria [14]. Ni stimulates the growth of acetoclastic, hydrogenotrophic methanogens, and sulfate-diminishing microorganisms. Ni and Mn are irremediable for phosphodiesterase enzymes in *Methanococcus jannaschii* [20]. HMs such as Ni, Mo, and Co are needed in small concentrations, and the HM contents in methanogenic strains are in the order of $\text{Fe} > \text{Zn}, \text{Ni} > \text{Co} = \text{Mo} > \text{Cu}$ [14]. Fe acts as a cofactor of enzymes, cytochrome, and ferredoxin in methanogens to trigger electron transport metabolism. Cu incites the superoxide dismutase enzyme (SODM) and hydrogenase of methane-producing bacteria (MPB). Zn fosters SODM and hydrogenase in MPB and sulfate-reducing bacteria (SRB). Selenium (Se) plays a critical role in hydrogenase, formate dehydrogenase (FDH), and clostridia. Tungsten (W) and Mo are present in FDH, and W is crucial in acetogenetic clostridia, and Mo inhibits SRB activity [21]. Previous literature depicted that small concentrations of HMs could improve the activity of organisms, which would eventually assist AD. The presence and absence of HMs and their effect on the AD process reported in previous literature are summarized in Table S2 (Supplementary File). One study proved that a limited supply of HMs can significantly advance chemical oxygen demand (COD) solubilization and short-chain fatty acid (SCFA) formation with higher methane turnout [22]. Gonzalez-Gil et al. concluded that an increase in Ni and Co concentrations resulted in higher methane yields by breaking the micronutrient limitations [23]. An inadequate supply of HMs can curb the cellular and metabolic activities of microorganisms. For instance, Pobeheim et al. reported that a lack of Ni and Co dosage can reduce methane production by 10% and 25%, respectively [24]. Xu et al. proved that Cd can augment SCFA production by 10.6%, but the performance deteriorates if the concentration exceeds 10 mg/g of volatile suspended solids (VSS) [25]. Kim et al. indicated that potent

eradication of propionate at elevated VFA levels needs dosing of Ca, Fe, Ni, and Co in a thermophilic, non-mixed reactor [26]. The co-digestion of poultry manure with potato waste resulted in higher biogas generation with supplementation of HMs at a concentration of 2.5 mg L^{-1} with higher increments in the order of $\text{Cd} > \text{Ni} > \text{Zn}$ [27]. Similarly, Ishaq et al. demonstrated that HM deficiency led to the underperformance of sewage sludge bioreactors. However, Co supplementation enhanced acetate conversion into methane in the range of 9–50% [28]. All these studies proved that HM could improve the performance of AD reactors by affecting microbial communities. Nonetheless, if the concentration of HM exceeds the required limit, it can cause toxicity by interfering with the normal metabolism of anaerobic microorganisms [29]. The coactive and antagonistic outcomes of HM in AD largely depend on their dosing as supplements. The physiochemical form of HM also largely influences anaerobic systems, as they are associated with various physiochemical reactions with the evolution of complexes. It is important to realize that HM supplementation to maximize biogas production is accompanied by their loss in the environment. Furthermore, industrial wastewaters and municipal sludges contain a higher amount of HM, and most research has focused on their deterrent and toxic effects on the AD process, as elaborated in Section 4.

3. Fate of HMs in the AD Process

In the AD process, HM species are mainly generated under desirable reduction conditions. The AD of organic matter adequately curtails redox potential and yields varieties of HM complexes and precipitates. As per the European Community Bureau of Reference, HM speciation is classified into the following four groups: (1) soluble/exchangeable acidic fraction, (2) reducible fraction, (3) oxidizable fraction, and (4) residual fraction, with a movability order as follows: $1 > 2 > 3 > 4$ [30]. Among them, fractions (1) and (2) are the most toxic to nature as they are easily involved with organisms. Fraction (3) remains the most erratic under oxidizing circumstances and is regarded as the most toxic in long-term scenarios. Fraction (4) has the lowest toxicity [31–33]. To understand their mechanisms and toxic aftermath on ecosystems, utmost importance should be given to follow their transformation/migration footprints in the AD process.

With the progression of the AD process, the HM content splits into biogas residue and digestate, largely depending on process parameters. The presence of some HMs in biogas digestates after AD is shown in Table 1. According to Zhu et al., sequential extraction of anaerobic digestate revealed that each HM has different distribution characteristics [34]. Zn content was found to be the maximum, followed by Cu and Mn, depending on their inclusion in the feed. Mesophilic AD of animal manure (pig) resulted in higher Zn, Cu, and As contents in biogas residue, while Cd, Pb, and Cr contents were within permissible limits for biogas fertilizers. The Cu, Zn, Cd, As, Pb, Cr, Ni, and Mn contents in biogas residue were 419, 931, 0.27, 0.49, 1.66, 3.88, 7.71, and 581 mg/kg, respectively [35]. Similarly, the AD of chicken manure showed unequal speciation of HM in biogas residue, with Cu, Zn, Cd, As, Pb, Cr, and Mn concentrations of 152.78, 1101, 0.32, 25.36, 5.32, 27.25, and 1234 mg/kg, respectively [36]. By correlating HM content before and after AD in previous literatures, it was found that the presence of HM biogas residue was greater than that of the substrate, with an increase of 0–100%. This is linked to the potential organic disintegration of substrate and methane production, which plummets its weight and volume [13]. Jin and Chang (2011) reported that HM content was higher in digestate than that in digester influent because most HMs were closely bounded with insoluble solids [37]. In addition, the earliest stage (hydrolysis + acidification) of AD generates acids, which leads to VFA accumulation and a decrease in pH. As a result, HMs could be dissolved in the process, which is the main reason behind the rise in the HM content in the digestate at the beginning [38]. As AD advances further, methanogenic organisms drain up surplus VFAs, which restore alkaline conditions. Under these conditions, HM does not really exist in the form of free ions but produces precipitate and organometallic compounds [34]. Moreover, since the biogas stream is free of HM, the mass flow rate of HM should be the same in both influent and

effluent in steady-state conditions. However, differences in concentration could be due to solubilization and different methods of measurement. In the AD process, the movement and agglomeration of HMs are mainly caused by their precipitation into hydroxides, carbonates, and phosphates, along with their adsorption by extracellular polymers, microbial cell walls, and solid particles [13,39]. The fate and transformation of HMs during AD are summarized in Table S3 (Supplementary File). Distinct processes lead to the movement of biogas slurry, which intentionally or unintentionally ends up in the environment and induces potential environmental risk.

AD causes significant changes in the chemical forms of most HMs, and this transformation could be beneficial to estimating their potential impact on the ecosystem. As stated above, the HM transformation is divided into four fractions. For Cu, various studies have confirmed that Cu mostly goes along with the third fraction, reducing its bioavailability during AD. However, poultry manure may increase the bioavailability of Cu. Hence, potential toxic effects in biogas residue should be considered in the case of Cu [40]. Zn can be transformed from stable to active fractions through AD, which largely improves its bioavailability in the case of animal manure. However, it should be noted that Zn sulfide minerals dissolve in soils, which increases the bioavailable fraction in soil [41]. As far as Cd is concerned, experimental results confirm that its bioavailability improves after AD, even if its concentration is lower in manure. Consequently, the Cd content of biogas residue increases environmental concern [42]. AD can improve or reduce the bioavailability of As, depending on the substrate. For instance, in the case of swine manure, it decreases, while in the case of poultry manure, it increases. Therefore, AD cannot control the toxicity created by As; as such, it remains toxic in all forms [43]. Similarly, AD leads to low mobility and bioavailability of Pb, which can also remain in a stable form in digestate. Likewise, experimental research proves that AD can lower the bioavailability of certain HMs, but this decrease is negligible. Even if HMs remain temporarily stable in reactors, they are potentially toxic to our ecosystem.

In the AD of organic materials and sediments, HMs participate in redox reactions, which are then released into the environment as soil or compost with distinct entry routes. As AD slurry enriched with HM undergoes aerobic conditions for post-treatment, its various chemical and physical changes have detrimental effects on the ecosystem, including humans. This is the main reason why many European Union (EU) countries have barred the application of AD slurry on land and have strictly prohibited any other kind of use [44]. There is a general agreement on various chemical and biochemical reactions that could occur when AD slurry enriched with HMs is exposed to aerobic conditions, with some skepticism about intermediates and the final product. For instance, Vink et al. found that pH automatically drops when anaerobic sediment sludge is introduced into the ground [45]. This may happen owing to the oxidation of sulfides, which increases SO_4^{2-} , ultimately leading to the release of sulfides associated with HMs in the ground. Therefore, anaerobically digested biogas slurry should be properly disposed of, as it can act as a non-point source of pollution. The concern for HM is much higher in the livestock and poultry industry substrates than in others. To meet the growing requirements, the poultry and livestock industries have significantly increased the use of supplements in their feed. The average Zn and Cu contents in livestock and poultry manure are 8.4–1726 and 39.5–11,379 mg/kg, respectively [46]. Continued use of livestock manure and animal manure can expedite the accumulation of HMs in soil, which can further leach into surface and underground waters. As per reports, the total Zn infiltrating into the soil from animal manure is ~78% in France and 51% in China [41]. Similar to soils, the aquatic ecosystem is also severely threatened by HM release. For many decades, several experiments have demonstrated the relevance of water chemistry and the detrimental consequences of HMs on aquatic life. The substantial observations from those experiments can be used to protect our ecosystem and to scrutinize the probability of restating digestate.

Table 1. Presence of heavy metals (HMs) in biogas digestates.

Substrate	HM (mg/kg)						Reference
	Ni	Pb	Cr	Cu	Zn	Cd	
SW	13.83	3.96	-	439.40	1215.67	0.16	[30]
SW	7.71	1.66	3.88	419	931	0.27	[35]
PM	-	22.97	61.20	71.72	370.24	0.39	[47]
PM	5.79	0.85	0.01	63.8	534	0.01	[48]
PM	-	4.63	25.78	130.07	947	0.31	[36]
CM	162.5	-	-	43.5	325.2	-	[49]
CM	-	-	-	16.63	57.13	-	[37]

SW: swine manure, PM: poultry manure, CM: cattle manure.

4. Effect of HMs on the AD Process

It is well known that HMs play a significant role in various biochemical reactions involved in AD, mostly related to microbial activity. However, at certain levels, they can be stimulatory and above permissible limits, which induces toxicity in the process. Typical studies regarding the impact of HMs on the AD process (hydrolysis, acidogenesis/acetogenesis, and methanogenesis) are summarized in Table S4 (Supplementary File). The pernicious consequences of HMs on the AD process have been evaluated since the last few decades. The potential impacts of HMs (i.e., the IC₅₀ values of HMs) are shown in Table 2. Most of the research was intended to examine the overall performance of AD reactors. The overall aftermath of each stage of the AD process is discussed below.

Table 2. IC₅₀ value of major heavy metals (HMs) in the anaerobic digestion (AD) process.

HM	IC ₅₀ Values (mg/L)		Reference
	Acidogenesis	Methanogenesis	
Cr	17	14.7	
Zn	3.5	16	
Ni	440	400	
Cd	29	7.7	[50]
Cu	0.9	12.5	
Pb	880	67.2	
Co	-	0.77	[51]

IC: inhibitory concentrations, IC₅₀: the concentration of HM that reduces the corresponding biological reaction rate by 50%.

4.1. Hydrolysis Phase

The hydrolysis phase is commonly known as the rate-limiting stage of AD, in which the solubilization of organic matter along with the biological disintegration of polymers into monomers occurs simultaneously. It is believed that Cu easily interacts with cellulase and heavily alters the hydrolysis process. This is frequently observed in AD reactors treating poultry and animal manure, as they use Cu-added feed to bolster immunity as well as growth [52]. Even though the poultry and livestock industries use huge amounts of Cu, not all of it is utilized, and some ends up in manure. Guo et al. found that cellulase activity in AD operated with the optimum level of Cu (i.e., 150 mg/kg) and was 16.33% higher than that in the control group [53]; this amount of Cu is crucial for maintaining microbial activity because Cu acts as a cofactor to stimulate enzymatic reactions. However, if the concentration exceeds > 300 mg/kg, cellulase activity is curtailed by 5.86% along with complete disruption of microbial activity, possibly because large amounts of Cu affect the spatial structure of enzymes, ultimately lowering cellulase activity in the AD reactor. Wang et al. evaluated the potential impact of Cu on the AD of pig manure (mainly on hydrolysis) [54]. The results showed that Cu at a concentration of 5000 mg/kg worked in favor of the hydrolysis of proteins and carbohydrates in parallel with a lengthened hydrolysis duration. Notwithstanding, higher Cu levels are found to be the most toxic

to microorganisms as they forbid the deterioration of composite organic macromolecules. Ni is designated as the most toxic HM; however, it is essential to retain the activity and metabolism of microorganisms. Tian et al. found that Ni addition to AD reactors caused higher cellulase activity, further increasing biogas production, which was highest on the 13th day [55]. Further supplementation of Ni ceased cellulase activity, which halted biogas generation on the 26th day of operation. Qi et al. concluded that 50–100 mg/g total solid Zn influences the dissolution and hydrolysis of organic compounds with an accumulation of the following two products: soluble protein and polysaccharide. The study showed that low Zn concentrations can step up the dissolution and hydrolysis of organic substrates (protein degradation) [56]. To date, research on the effect of Cu and Ni on hydrolysis remains limited, warranting further research to understand the mechanisms of inhibition, specifically on cellulase activity.

4.2. Acidogenesis/Acetogenesis Phase

In the acidogenesis phase, acidogenic bacteria further break the remaining organic compounds, which results in VFA production with NH_3 and CO_2 . In the acetogenesis phase, simple molecules digested by acetogens result in the generation of CH_3COOH , H_2 , and CO_2 . VFA generation is identified as the most important intermediate, as a slightly higher concentration can lead to a pH imbalance in the AD reactor. Lin evaluated the impacts of HMs such as Cd, Cu, Cr, Zn, Pb, and Ni on VFA disintegration in the AD process [50]. The experimental results revealed that Cd and Cu were the most toxic to VFA-diminishing microorganisms, while Pb and Ni were the least toxic. Yu and Fang investigated the effects of Cr and Cd addition on acidogenesis. Cd of <20 mg/L significantly improved acidogenesis, while dosages over 20 mg/L severely halted acidogenesis [56]. In the case of Cr, a small amount (i.e., 5 mg/L) decreased VFA production, which suggests that Cr is more toxic to acidogenesis than Cd. Similarly, Facchin et al. suggested that a Cu level of 100 mg/L stimulated VFA destruction while simultaneously promoting biogas production [57]. A smaller concentration can augment VFA-decomposing organisms along with a rapid transformation of organic matter [58]. A concentration of >100 mg/L hindered the acidification mechanism, directly affecting biogas production [59]. Wang et al. proved that Cu of 5000 mg/kg facilitated the accumulation of VFAs, which further boosted the acidification process [54]. Many studies have confirmed that Zn is essential in the acidogenesis phase of AD but differed in threshold values for the promotion and inhibition of acidogenesis. One study showed that Zn at 50–70 mg/L can promote palmitic acid removal, while high concentrations inhibit VFA production [60]. Yenigun et al. found that Zn at 5–40 mg/L slowed the production of propionic acid, while 20 mg/L stopped the generation of propionic acid. From this, we can conclude that appropriate levels of Zn facilitate hydrolysis, but it largely depends on many parameters such as pH, operating conditions, and substrate [61]. As most experimental research has focused on the impact of HMs on methanogenesis, limited experimental research is available on their impact on the acidogenesis/acetogenesis phase.

4.3. Methanogenesis Phase

The methanogenesis phase is the final and most pre-eminent stage of AD, where biomethane is generated as the stabilization of biomass occurs via methanogenic microorganisms under anaerobic conditions. Similar to hydrolysis and acidogenesis, which require traces of HM, methanogens also require HM to maintain their activity [62]. As per reports, Cu is believed to be the most vital, as it directly affects the growth and activity of methanogenic organisms. According to Wu et al., Cu at a concentration of 5 mg/L had a positive impact on methanogens, while a concentration of 300 mg/kg completely suppressed methanogenic activity [63]. When Cu levels in the solid fermentation reactor were between 30 and 100 mg/L, biogas production increased by 43.62% and 20.77%, respectively. However, when the Cu level was gradually increased to 500 mg/L, biogas generation was reduced by 69.47% compared with the control group [59]. An analogous trend was

observed in solid and wastewater fermentation reactors, with lower levels promoting biogas production and higher levels halting biogas production. Likewise, Ni influences the growth and activity of methanogenic organisms. One study demonstrated that an Ni concentration of <30 mg/L facilitated methanogenic activity; however, concentration levels of >30 mg/L impeded methanogenic activity [64]. In terms of the performance of the AD system, the highest biogas turnout was obtained when the Ni level was 0.8 mg/L, which was 18% higher than the AD system operating without Ni addition [55]. Lo et al. found that dosages of 0.06, 0.6, 3, and 6 mg/L significantly improved cumulative biogas yield by 4.71, 15.29, 14.71, and 14.12%, respectively, while a dose of 100 mg/L inhibited biogas production [65]. The maximum biogas production ever reported was with an Ni supplement rate of 4 mg/L in AD reactors, which was 25% higher than the AD reactor without Ni [64]. Additionally, it was found that Ni is crucial for the coenzyme F430 of methanogenic microorganisms, as it contains an Ni complex that directly or indirectly affects biogas production, but with a concentration limit from 0.8 to 4 mg/L [8]. Fe is another important HM that is related to cellulase activity, eventually influencing the methanogenic stage; in addition, Fe can cut down sulfide intermeddling, improving cellulase activity; methanogenic activity is directly proportional to cellulase activity [66]. In the case of AD, the presence of Fe substantially improved biogas production by 35%, with the highest production at a concentration level of 205 mg/L [67]. When the concentration level of Fe was <12,000 mg/L, there was no impact on methane production; however, when the concentration level reached 20,000 mg/L, methane production completely stopped [68]. Finally, traces of Fe are also important for proteolytic enzymes, triggering cytochromes, and ferredoxin evolution for electron transportation [69].

Cd is a toxic HM that can be easily dissolved in water, and its presence in AD reactors can affect the rise and metabolism of methanogenic archaea. Cd has the highest toxicity towards methanogens; even 1 mg/L of Cd concentration can affect methanogenic activity, with inhibition directly proportional to the concentration of Cd in the AD reactor [64]. The biogas generation rates in AD reactors with Cd levels of 0, 2.5, and 5 ppm were 2360, 5960, and 5040 mL, respectively [27]. Cd equally affects the methane content of biogas, with lower methanogenic activity (concentrations between 0.1 and 0.3 mg/L) enhancing the biogas production rate. The logic behind this is that Cd acts as a cofactor that energizes the enzymatic activity of methanogenic archaea [70]. Eventually, higher Cd levels affect biogas production; the higher the concentration, the stronger the suppression of methanogenic organisms affecting biogas yield. Similar to Ni, Fe, and Cd, Zn also influences the growth and activity of methanogenic archaea. A Zn concentration level of 2 mg/L promotes methanogenic activity; however, if it exceeds 3 mg/L, methanogenic activity declines [64]. In the case of AD reactors, biogas production exceeded 4.44% at a Zn concentration of 5 mg/L but dropped by 11.11% and 33.33% when the Zn concentration in the AD reactor was 50 and 100 mg/L, respectively [65]. It is evident that Zn concentrations of <100 mg/L promote biogas yield but severely inhibit the process when the concentration exceeds this level. From the literature, the ideal Zn concentration required for methanogens is 5 mg/L, which is necessary to retain the cell catabolism of methanogenic organisms [69].

From the literature, it is clear that a low concentration of HMs has a positive impact on the AD process. Appropriate levels of HMs such as Fe and Ni can improve methanogenic activity with higher biogas production; despite this, higher HM concentrations can reduce methane yield [71]. Excessive HM content can affect proteolytic enzymes during the hydrolysis phase, which reduces their activity. Additionally, it can also reduce the microbial population in the AD reactor [53]. Nonetheless, Zn is the most essential HM for methanogens, as it reduces the accumulation of SCFAs and promotes methane yield. However, high concentrations lead to the inhibition of methanogens, which also largely depends on particle size. Many researchers have found that long-term and continuous feeding experiments result in bio-accumulative toxicity. Certain methanogens can tolerate low concentrations of HMs. Hence, long-term experiments are required to obtain more conclusive results. The differences demonstrate that the hormesis effect of HMs largely

depends on the composition of the substrate, operating conditions, metals, and their particle size. The promoting and inhibiting concentration levels of HMs on AD are shown in Table 3. AD can be an efficient path of treating waste along with optimal concentrations of major HMs such as Cd, Zn, Fe, Cu, and Ni present in AD reactors.

Table 3. Promoting and inhibiting concentrations of heavy metals (HMs) on anaerobic digestion (AD).

HM	Promoting Concentration (mg/L)	Inhibiting Concentration (mg/L)	Reference
Zn	0.03 < Zn < 2	7.5 < Zn < 1500	[72]
Pb	<0.2	67.2 < Pb < 8000	[64]
Ni	0.03 < Ni < 27	35 < Ni < 1600	[72]
Fe	<0.3	-	[73]
Cu	0 < Cu < 100	Cu < 500	[59]
Cd	0.1 < Cd < 0.3	Cd < 1.2	[74]

5. HM Mitigation

The commonly used techniques to alleviate HMs are chelation, sorption, and precipitation [17], which largely depend on various parameters such as temperature, pH, and type of substrate. HM remediation is key to reducing HM levels within permissible limits for further use as a biofertilizer. The optimum values of HM for efficient AD and permissible values of HM for the use of fertilizer in the EU are mentioned in Table 4. Among the three, precipitation is the most used method as it offers advantages to treating high HM concentrated wastewater. Sulfide was found to be the most used agent to expedite HM removal, with 20 mg/L Cu being removed with less removal time [75]. However, a higher sulfide application rate could inhibit methanogenic activity, which can be curtailed by adding ferrous sulfate. The only disadvantage of precipitation is that the effluent of a large volume of sludge still contains traces of HM, which thus requires further treatment prior to discharge. Therefore, there has been increased interest over the years in developing low-cost adsorbent materials for use in the sorption process.

The degree of sorption is directly proportional to the surface area of the sludge and is commonly known as chemisorption [76]. Many naturally occurring materials, such as zeolite, bentonite, and diatomite, have been used to eliminate HMs from wastewater. The use of waste products such as red mud, fly ash, and blast furnace slag to remove HMs has also been reported in previous literature [77]. Currently, activated carbon is most commonly used since it offers a large surface area; however, its high cost is a limitation. The use of nanomaterials in the adsorption process could be an effective strategy for the removal of HMs owing to their distinct physiochemical properties. For instance, polyaniline nanocomposites are simple, effective, and economical for the removal of HMs. However, performance largely depends on factors such as the physiochemical nature of the material and experimental conditions. Despite this, the application of polyaniline nanocomposites has not attained optimal performance for full-scale operation [78]. Phytoremediation offers a sustainable alternative as it utilizes plants and their associated microorganisms to remove toxicants such as HM. However, despite successful lab-scale experiments, its practical application requires further optimization as it is affected by several environmental parameters [79]. Similarly, the use of biowaste from the wood, agricultural, and fisheries industries to remove HMs has the potential to complement the circular economy concept. However, important issues related to the sorption capacities of biosorbents, desorption, regeneration, and recycling need to be resolved [80].

For a long time, chelation with organic ligands has been used to detoxify HMs. In addition to these methods, anaerobic microorganisms are believed to possess diverse internal HM mitigation mechanisms such as biomethylation, exocytosis, chelation of metal ions at the cell surface, and plasmid-mediated resistance [31]. For instance, *paracoccus* has the ability to soak up to 40% of Cd [81]. A possible future scenario would be isolating HM-defiant microbes such as proteolytic bacteria to enhance biogas production. Con-

siderable research has been conducted to employ probiotics as a defense mechanism for the AD microbiome [82]. However, research on HM mitigation is limited; hence, future research should be focused on various feasible and low-cost HM mitigation techniques for AD reactors.

Table 4. Optimum values of heavy metals (HMs) for efficient anaerobic digestion (AD) and permissible values of HMs for use of fertilizer in EU.

HM	Optimum Value (mg/L)	Permissible Value (mg/kg) in EU	Reference
Zn	5	200	
Cd	0.1	1	
Fe	0–1000	-	[44,83]
Ni	0.8–4	50	
Cu	5–30	100	

6. Future Perspective

Based on our brief discussion, the challenge of HMs in the AD process and their toxicity effect remains unsolved. The management of HMs in AD reactors should receive more attention to improve the efficacy of the process. In recent years, more emphasis has been given to examining the effects of HMs on AD; however, chemical speciation is most crucial in terms of toxicity. Hence, future research should attempt to understand the synergy between anaerobes and the chemical speciation of HM. The results will help to anticipate possible unfavorable impacts of HM on AD. In addition, attention should be paid to evaluating the effect of HM particle size, as recent studies have demonstrated the hormesis of micro- and nano-sized HMs on AD. Similarly, future studies should determine key substances that play prime roles in various biochemical reactions of AD as well as the role of extracellular polymeric substances (EPS) in transmission pathways. Furthermore, the fate of HM on the environment, particularly agricultural land, is mostly unclarified or ambiguous. Therefore, joint efforts and tools are necessary to understand the role and possible impact of HMs on the AD process. Finally, reports regarding HM remediation from AD are currently inadequate. In addition to traditional techniques, more advanced and sustainable approaches should be explored and implemented. Phytoremediation could be a potential approach as it curtails HMs pollution from soils. Therefore, setting up energy crop cultivation and its digestion to produce biogas could naturally remediate HMs [84]. Consequently, the sustainable path can simultaneously enhance biogas production with HM management.

7. Conclusions

The AD process mainly consists of the following three phases: hydrolysis, acidogenesis, and methanogenesis; in each stage, a series of biochemical reactions occur, catalyzed by anaerobes, producing biogas, which is used as fuel along with beneficial fertilizers such as residue and slurry from AD reactors. As the process is affected by several parameters, the fate and effect of HMs have been discussed in this review. The leading interpretation from our discussions is that HM may promote or suppress AD, which largely depends on their concentration. This review primarily summarized the fates and effects of five common HMs (Cu, Ni, Fe, Cd, and Zn) and their ideal concentrations that have been recommended, and their possible role in the AD process as a function of concentration. In addition, the transformation of HMs during the AD process, as reported in previous literature, has been critically discussed. Most importantly, this review discusses the impact of HMs on each stage of AD as a function of concentration, allowing us to quantify HM levels in the substrate in order to enhance biogas production. Distinct HM species have a negligible impact on VFA but strongly affect the microbial community during AD. Similarly, methanogenic microorganisms are more sensitive toward HM concentration than are hydrolytic and acidogenic microorganisms. This review discusses various detoxifying techniques as well

as an economic and sustainable approach. Phytoremediation offers a sustainable approach to HM mitigation; however, the practical approach needs further evaluation. Moreover, more research efforts are required to develop new mitigation techniques.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/en15228618/s1>, Table S1: Source and level of each HM in various AD substrates; Table S2: Presence and outcome of HM on AD reported in previous studies included in this manuscript; Table S3: Transformation of HM during AD reported in the previous literature included in this manuscript; Table S4: Typical studies regarding the impact of HM on AD process included in this manuscript.

Author Contributions: R.K., conceptualization, writing—original draft preparation, writing—review, and editing; K.K., H.J. and J.L., writing—review and editing; J.P., review and supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Chosun University 2021 (grant number K208702001) and was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2021R111A3044486).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript.

AD	Anaerobic digestion
HM	Heavy metals
VFAs	Volatile fatty acid
Fe	Iron
Cu	Copper
Zn	Zinc
Cd	Cadmium
As	Arsenic
Pb	Lead
Cr	Chromium
Ni	Nickel
Mn	Manganese
Co	Cobalt
Ca	Calcium
SODM	Superoxide dismutase
FDH	Formate dehydrogenase
MPB	Methane-producing bacteria
SRB	Sulfate-reducing bacteria
COD	Chemical oxygen demand
VSS	Volatile suspended solids
CODH	Carbon monoxide dehydrogenase
SCFAs	Short-chain fatty acids
EU	European Union
IC50	50% inhibition value towards biochemical utility
EPS	Extracellular polymeric substances

References

1. Amha, Y.M.; Anwar, M.Z.; Brower, A.; Jacobsen, C.S.; Stadler, L.B.; Webster, T.M.; Smith, A.L. Inhibition of anaerobic digestion processes: Applications of molecular tools. *Bioresour. Technol.* **2018**, *247*, 999–1014. [[CrossRef](#)] [[PubMed](#)]
2. Guo, M.; Song, W.; Buhain, J. Bioenergy and biofuels: History, status, and perspective. *Renew. Sustain. Energy Rev.* **2015**, *42*, 712–725. [[CrossRef](#)]
3. Enerdata. Global Energy Transition Statistics. Available online: <https://yearbook.enerdata.net/> (accessed on 14 September 2022).
4. He, K.; Sun, Z.; Hu, Y.; Zeng, X.; Yu, Z.; Cheng, H. Comparison of soil heavy metal pollution caused by e-waste recycling activities and traditional industrial operations. *Environ. Sci. Pollut. Res.* **2017**, *24*, 9387–9398. [[CrossRef](#)] [[PubMed](#)]

5. Li, S.; Zou, D.; Li, L.; Wu, L.; Liu, F.; Zeng, X.; Wang, H.; Zhu, Y.; Xiao, Z. Evolution of heavy metals during thermal treatment of manure: A critical review and outlooks. *Chemosphere* **2020**, *247*, 125962. [[CrossRef](#)]
6. Chen, X.; Zhao, Y.; Zhang, C.; Zhang, D.; Yao, C.; Meng, Q.; Zhao, R.; Wei, Z. Speciation, toxicity mechanism and remediation ways of heavy metals during composting: A novel theoretical microbial remediation method is proposed. *J. Environ. Manag.* **2020**, *272*, 111109. [[CrossRef](#)] [[PubMed](#)]
7. Zeng, X.; Xiao, Z.; Zhang, G.; Wang, A.; Li, Z.; Liu, Y.; Wang, H.; Zeng, Q.; Liang, Y.; Zou, D. Speciation and bioavailability of heavy metals in pyrolytic biochar of swine and goat manures. *J. Anal. Appl. Pyrolysis* **2018**, *132*, 82–93. [[CrossRef](#)]
8. Zhang, R.; Wang, X.; Gu, J.; Zhang, Y. Influence of zinc on biogas production and antibiotic resistance gene profiles during anaerobic digestion of swine manure. *Bioresour. Technol.* **2017**, *244*, 63–70. [[CrossRef](#)]
9. Zhang, W.; Zhang, L.; Li, A. Enhanced anaerobic digestion of food waste by trace metal elements supplementation and reduced metals dosage by green chelating agent [S, S]-EDDS via improving metals bioavailability. *Water Res.* **2015**, *84*, 266–277. [[CrossRef](#)]
10. Zhao, L.; Ji, Y.; Sun, P.; Deng, J.; Wang, H.; Yang, Y. Effects of individual and combined zinc oxide nanoparticle, norfloxacin, and sulfamethazine contamination on sludge anaerobic digestion. *Bioresour. Technol.* **2019**, *273*, 454–461. [[CrossRef](#)]
11. Qi, L.; Liu, X.; Miao, Y.; Chatzisyneon, E.; Yang, P.; Lu, H.; Pang, L. Response of cattle manure anaerobic digestion to zinc oxide nanoparticles: Methane production, microbial community, and functions. *J. Environ. Chem. Eng.* **2021**, *9*, 106704. [[CrossRef](#)]
12. Huang, Z.; Niu, Q.; Nie, W.; Li, X.; Yang, C. Effects of heavy metals and antibiotics on performances and mechanisms of anaerobic digestion. *Bioresour. Technol.* **2022**, *361*, 127683. [[CrossRef](#)]
13. Zheng, X.; Zou, D.; Wu, Q.; Wang, H.; Li, S.; Liu, F.; Xiao, Z. Review on fate and bioavailability of heavy metals during anaerobic digestion and composting of animal manure. *Waste Manag.* **2022**, *150*, 75–89. [[CrossRef](#)] [[PubMed](#)]
14. Mudhoo, A.; Kumar, S. Effects of heavy metals as stress factors on anaerobic digestion processes and biogas production from biomass. *Int. J. Environ. Sci. Technol.* **2013**, *10*, 1383–1398. [[CrossRef](#)]
15. Seo, J.-Y. Heavy Metal Contents of Compost from Household Food Waste. *J. Korea Org. Resour. Recycl. Assoc.* **1996**, *4*, 23–31.
16. Zhang, F.; Li, Y.; Yang, M.; Li, W. Content of heavy metals in animal feeds and manures from farms of different scales in northeast China. *Int. J. Environ. Res. Public Health* **2012**, *9*, 2658–2668. [[CrossRef](#)] [[PubMed](#)]
17. Oleszkiewicz, J.; Sharma, V. Stimulation and inhibition of anaerobic processes by heavy metals—A review. *Biol. Wastes* **1990**, *31*, 45–67. [[CrossRef](#)]
18. Glass, J.B.; Orphan, V.J. Trace metal requirements for microbial enzymes involved in the production and consumption of methane and nitrous oxide. *Front. Microbiol.* **2012**, *3*, 61. [[CrossRef](#)]
19. Demirel, B.; Scherer, P. Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass Bioenergy* **2011**, *35*, 992–998. [[CrossRef](#)]
20. Chen, S.; Yakunin, A.F.; Kuznetsova, E.; Busso, D.; Pufan, R.; Proudfoot, M.; Kim, R.; Kim, S.-H. Structural and functional characterization of a novel phosphodiesterase from *Methanococcus jannaschii*. *J. Biol. Chem.* **2004**, *279*, 31854–31862. [[CrossRef](#)]
21. Luo, J.; Zhang, Q.; Zhao, J.; Wu, Y.; Wu, L.; Li, H.; Tang, M.; Sun, Y.; Guo, W.; Feng, Q. Potential influences of exogenous pollutants occurred in waste activated sludge on anaerobic digestion: A review. *J. Hazard. Mater.* **2020**, *383*, 121176. [[CrossRef](#)]
22. Jagadabhi, P.S.; Kaparaju, P.; Väisänen, A.; Rintala, J. Effect of macro- and micro-nutrients addition during anaerobic mono-digestion of grass silage in leach-bed reactors. *Environ. Technol.* **2019**, *40*, 418–429. [[CrossRef](#)] [[PubMed](#)]
23. Gonzalez-Gil, G.; Kleerebezem, R.; Lettinga, G. Effects of nickel and cobalt on kinetics of methanol conversion by methanogenic sludge as assessed by on-line CH₄ monitoring. *Appl. Environ. Microbiol.* **1999**, *65*, 1789–1793. [[CrossRef](#)] [[PubMed](#)]
24. Pobeheim, H.; Munk, B.; Johansson, J.; Guebitz, G.M. Influence of trace elements on methane formation from a synthetic model substrate for maize silage. *Bioresour. Technol.* **2010**, *101*, 836–839. [[CrossRef](#)] [[PubMed](#)]
25. Xu, Q.; Li, X.; Ding, R.; Wang, D.; Liu, Y.; Wang, Q.; Zhao, J.; Chen, F.; Zeng, G.; Yang, Q. Understanding and mitigating the toxicity of cadmium to the anaerobic fermentation of waste activated sludge. *Water Res.* **2017**, *124*, 269–279. [[CrossRef](#)] [[PubMed](#)]
26. Kim, M.; Ahn, Y.-H.; Speece, R. Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. *Water Res.* **2002**, *36*, 4369–4385. [[CrossRef](#)]
27. Kumar, A.; Miglani, P.; Gupta, R.; Bhattacharya, T. Impact of Ni (II), Zn (II) and Cd (II) on biogassification of potato waste. *J. Environ. Biol.* **2006**, *27*, 61–66.
28. Ishaq, F.; Bridgeman, J.; Carliell-Marquet, C. Site energy performance as an indicator for trace element deficiency in full-scale digesters. In Proceedings of the 13th World Congress on Anaerobic Digestion (IWA Specialist Conference), Santiago de Compostela, Spain, 25–28 June 2013.
29. Zhang, L.; Zhang, Z.; He, X.; Zheng, L.; Cheng, S.; Li, Z. Diminished inhibitory impact of ZnO nanoparticles on anaerobic fermentation by the presence of TiO₂ nanoparticles: Phenomenon and mechanism. *Sci. Total Environ.* **2019**, *647*, 313–322. [[CrossRef](#)]
30. Zheng, X.; Wu, K.; Sun, P.; Zhouyang, S.; Wang, Y.; Wang, H.; Zheng, Y.; Li, Q. Effects of substrate types on the transformation of heavy metal speciation and bioavailability in an anaerobic digestion system. *J. Environ. Sci.* **2021**, *101*, 361–372. [[CrossRef](#)]
31. Chen, M.; Li, X.-M.; Yang, Q.; Zeng, G.-M.; Zhang, Y.; Liao, D.-X.; Liu, J.-J.; Hu, J.-M.; Guo, L. Total concentrations and speciation of heavy metals in municipal sludge from Changsha, Zhuzhou and Xiangtan in middle-south region of China. *J. Hazard. Mater.* **2008**, *160*, 324–329. [[CrossRef](#)]

32. Xiao, Z.; Yuan, X.; Li, H.; Jiang, L.; Leng, L.; Chen, X.; Zeng, G.; Li, F.; Cao, L. Chemical speciation, mobility and phyto-accessibility of heavy metals in fly ash and slag from combustion of pelletized municipal sewage sludge. *Sci. Total Environ.* **2015**, *536*, 774–783. [[CrossRef](#)]
33. Fuentes, A.; Lloréns, M.; Sáez, J.; Aguilar, M.I.; Ortuño, J.F.; Meseguer, V.F. Comparative study of six different sludges by sequential speciation of heavy metals. *Bioresour. Technol.* **2008**, *99*, 517–525. [[CrossRef](#)] [[PubMed](#)]
34. 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](#)]
35. Wang, J.; Hao, X.; Liu, Z.; Guo, Z.; Zhu, L.; Xiong, B.; Jiang, D.; Shen, L.; Li, M.; Kang, B. Biochar improves heavy metal passivation during wet anaerobic digestion of pig manure. *Environ. Sci. Pollut. Res.* **2021**, *28*, 635–644. [[CrossRef](#)] [[PubMed](#)]
36. Yan, Y.; Zhang, L.; Feng, L.; Sun, D.; Dang, Y. Comparison of varying operating parameters on heavy metals ecological risk during anaerobic co-digestion of chicken manure and corn stover. *Bioresour. Technol.* **2018**, *247*, 660–668. [[CrossRef](#)] [[PubMed](#)]
37. 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](#)] [[PubMed](#)]
38. Pan, J.; Ma, J.; Liu, X.; Zhai, L.; Ouyang, X.; Liu, H. Effects of different types of biochar on the anaerobic digestion of chicken manure. *Bioresour. Technol.* **2019**, *275*, 258–265. [[CrossRef](#)] [[PubMed](#)]
39. 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](#)]
40. Legros, S.; Levard, C.; Marcato-Romain, C.-E.; Guisresse, M.; Doelsch, E. Anaerobic digestion alters copper and zinc speciation. *Environ. Sci. Technol.* **2017**, *51*, 10326–10334. [[CrossRef](#)]
41. Formentini, T.A.; Legros, S.; Fernandes, C.V.S.; Pinheiro, A.; Le Bars, M.; Levard, C.; Mallmann, F.J.K.; Da Veiga, M.; Doelsch, E. Radical change of Zn speciation in pig slurry amended soil: Key role of nano-sized sulfide particles. *Environ. Pollut.* **2017**, *222*, 495–503. [[CrossRef](#)]
42. Cui, X.; Zhang, J.; Wang, X.; Pan, M.; Lin, Q.; Khan, K.Y.; Yan, B.; Li, T.; He, Z.; Yang, X. A review on the thermal treatment of heavy metal hyperaccumulator: Fates of heavy metals and generation of products. *J. Hazard. Mater.* **2021**, *405*, 123832. [[CrossRef](#)]
43. Pous, N.; Casentini, B.; Rossetti, S.; Fazi, S.; Puig, S.; Aulenta, F. Anaerobic arsenite oxidation with an electrode serving as the sole electron acceptor: A novel approach to the bioremediation of arsenic-polluted groundwater. *J. Hazard. Mater.* **2015**, *283*, 617–622. [[CrossRef](#)] [[PubMed](#)]
44. Tóth, G.; Hermann, T.; Da Silva, M.; Montanarella, L. Heavy metals in agricultural soils of the European Union with implications for food safety. *Environ. Int.* **2016**, *88*, 299–309. [[CrossRef](#)] [[PubMed](#)]
45. Vink, J.P.; Harmsen, J.; Rijnaarts, H. Delayed immobilization of heavy metals in soils and sediments under reducing and anaerobic conditions; consequences for flooding and storage. *J. Soils Sediments* **2010**, *10*, 1633–1645. [[CrossRef](#)]
46. Wang, H.; Dong, Y.; Yang, Y.; Toor, G.S.; Zhang, X. Changes in heavy metal contents in animal feeds and manures in an intensive animal production region of China. *J. Environ. Sci.* **2013**, *25*, 2435–2442. [[CrossRef](#)]
47. 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](#)]
48. Anjum, R.; Grohmann, E.; Krakat, N. Anaerobic digestion of nitrogen rich poultry manure: Impact of thermophilic biogas process on metal release and microbial resistances. *Chemosphere* **2017**, *168*, 1637–1647. [[CrossRef](#)]
49. 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](#)]
50. Lin, C.-Y. Effect of heavy metals on volatile fatty acid degradation in anaerobic digestion. *Water Res.* **1992**, *26*, 177–183. [[CrossRef](#)]
51. Bartacek, J.; Feroso, F.G.; Baldó-Urrutia, A.M.; van Hullebusch, E.D.; Lens, P.N. Cobalt toxicity in anaerobic granular sludge: Influence of chemical speciation. *J. Ind. Microbiol. Biotechnol.* **2008**, *35*, 1465–1474. [[CrossRef](#)]
52. Huang, G.; Wang, X.; Han, L. Rapid estimation of nutrients in chicken manure during plant-field composting using physicochemical properties. *Bioresour. Technol.* **2011**, *102*, 1455–1461. [[CrossRef](#)]
53. Guo, X.; Gu, J.; Gao, H.; Qin, Q.; Chen, Z.; Shao, L.; Chen, L.; Li, H.; Zhang, W.; Chen, S. Effects of Cu on metabolisms and enzyme activities of microbial communities in the process of composting. *Bioresour. Technol.* **2012**, *108*, 140–148. [[CrossRef](#)] [[PubMed](#)]
54. Wang, R.; Zhang, J.; Liu, J.; Yu, D.; Zhong, H.; Wang, Y.; Chen, M.; Tong, J.; Wei, Y. Effects of chlortetracycline, Cu and their combination on the performance and microbial community dynamics in swine manure anaerobic digestion. *J. Environ. Sci.* **2018**, *67*, 206–215. [[CrossRef](#)] [[PubMed](#)]
55. Tian, Y.; Zhang, H.; Chai, Y.; Wang, L.; Mi, X.; Zhang, L.; Ware, M.A. Biogas properties and enzymatic analysis during anaerobic fermentation of *Phragmites australis* straw and cow dung: Influence of nickel chloride supplement. *Biodegradation* **2017**, *28*, 15–25. [[CrossRef](#)]
56. Yu, H.; Fang, H.H. Inhibition by chromium and cadmium of anaerobic acidogenesis. *Water Sci. Technol.* **2001**, *43*, 267–274. [[CrossRef](#)] [[PubMed](#)]
57. Facchin, V.; Cavinato, C.; Fatone, F.; Pavan, P.; Cecchi, F.; Bolzonella, D. Effect of trace element supplementation on the mesophilic anaerobic digestion of foodwaste in batch trials: The influence of inoculum origin. *Biochem. Eng. J.* **2013**, *70*, 71–77. [[CrossRef](#)]

58. Karlsson, A.; Einarsson, P.; Schnürer, A.; Sundberg, C.; Ejlertsson, J.; Svensson, B.H. Impact of trace element addition on degradation efficiency of volatile fatty acids, oleic acid and phenyl acetate and on microbial populations in a biogas digester. *J. Biosci. Bioeng.* **2012**, *114*, 446–452. [[CrossRef](#)] [[PubMed](#)]
59. 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](#)]
60. Chan, P.C.; Lu, Q.; de Toledo, R.A.; Gu, J.-D.; Shim, H. Improved anaerobic co-digestion of food waste and domestic wastewater by copper supplementation—Microbial community change and enhanced effluent quality. *Sci. Total Environ.* **2019**, *670*, 337–344. [[CrossRef](#)]
61. Yenigün, O.; Kizilgün, F.; Yilmazer, G. Inhibition effects of zinc and copper on volatile fatty acid production during anaerobic digestion. *Environ. Technol.* **1996**, *17*, 1269–1274. [[CrossRef](#)]
62. Jiang, L.; Hu, Z.; Wang, Y.; Ru, D.; Li, J.; Fan, J. Effect of trace elements on the development of co-cultured nitrite-dependent anaerobic methane oxidation and methanogenic bacteria consortium. *Bioresour. Technol.* **2018**, *268*, 190–196. [[CrossRef](#)]
63. Wu, D.; Yang, Z.; Tian, G. Inhibitory effects of Cu (II) on fermentative methane production using bamboo wastewater as substrate. *J. Hazard. Mater.* **2011**, *195*, 170–174. [[CrossRef](#)] [[PubMed](#)]
64. Altaş, L. Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge. *J. Hazard. Mater.* **2009**, *162*, 1551–1556. [[CrossRef](#)] [[PubMed](#)]
65. Lo, H.; Chiang, C.; Tsao, H.; Pai, T.; Liu, M.; Kurniawan, T.; Chao, K.; Liou, C.; Lin, K.; Chang, C. Effects of spiked metals on the MSW anaerobic digestion. *Waste Manag. Res.* **2012**, *30*, 32–48. [[CrossRef](#)] [[PubMed](#)]
66. Nordell, E.; Nilsson, B.; Pålédal, S.N.; Karisalmi, K.; Moestedt, J. Co-digestion of manure and industrial waste—The effects of trace element addition. *Waste Manag.* **2016**, *47*, 21–27. [[CrossRef](#)]
67. Khatri, S.; Wu, S.; Kizito, S.; Zhang, W.; Li, J.; Dong, R. Synergistic effect of alkaline pretreatment and Fe dosing on batch anaerobic digestion of maize straw. *Appl. Energy* **2015**, *158*, 55–64. [[CrossRef](#)]
68. Andriamanohiarisoamanana, F.J.; Shirai, T.; Yamashiro, T.; Yasui, S.; Iwasaki, M.; Ihara, I.; Nishida, T.; Tangtaweewipat, S.; Umetsu, K. Valorizing waste iron powder in biogas production: Hydrogen sulfide control and process performances. *J. Environ. Manag.* **2018**, *208*, 134–141. [[CrossRef](#)]
69. 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](#)]
70. Bourven, I.; Casellas, M.; Buzier, R.; Lesieur, J.; Lenain, J.-F.; Faix, A.; Bressolier, P.; Maftah, C.; Guibaud, G. Potential of DGT in a new fractionation approach for studying trace metal element impact on anaerobic digestion: The example of cadmium. *Int. Biodeterior. Biodegrad.* **2017**, *119*, 188–195. [[CrossRef](#)]
71. Luna-delRisco, M.; Orupöld, K.; Dubourgui, H.-C. Particle-size effect of CuO and ZnO on biogas and methane production during anaerobic digestion. *J. Hazard. Mater.* **2011**, *189*, 603–608. [[CrossRef](#)]
72. Romero-Güiza, M.; Vila, J.; Mata-Alvarez, J.; Chimenos, J.; Astals, S. The role of additives on anaerobic digestion: A review. *Renew. Sustain. Energy Rev.* **2016**, *58*, 1486–1499. [[CrossRef](#)]
73. Worm, P.; Feroso, F.G.; Lens, P.N.; Plugge, C.M. Decreased activity of a propionate degrading community in a UASB reactor fed with synthetic medium without molybdenum, tungsten and selenium. *Enzym. Microb. Technol.* **2009**, *45*, 139–145. [[CrossRef](#)]
74. Zhang, H.; Tian, Y.; Wang, L.; Zhang, L.; Dai, L. Ecophysiological characteristics and biogas production of cadmium-contaminated crops. *Bioresour. Technol.* **2013**, *146*, 628–636. [[CrossRef](#)] [[PubMed](#)]
75. Zayed, G.; Winter, J. Inhibition of methane production from whey by heavy metals—protective effect of sulfide. *Appl. Microbiol. Biotechnol.* **2000**, *53*, 726–731. [[CrossRef](#)] [[PubMed](#)]
76. Alibhai, K.; Mehrotra, I.; Forster, C. Heavy metal binding to digested sludge. *Water Res.* **1985**, *19*, 1483–1488. [[CrossRef](#)]
77. Ulmanu, M.; Marañón, E.; Fernández, Y.; Castrillón, L.; Anger, I.; Dumitriu, D. Removal of copper and cadmium ions from diluted aqueous solutions by low cost and waste material adsorbents. *Water Air Soil Pollut.* **2003**, *142*, 357–373. [[CrossRef](#)]
78. Hajjaoui, H.; Soufi, A.; Boumya, W.; Abdennouri, M.; Barka, N. Polyaniline/Nanomaterial Composites for the Removal of Heavy Metals by Adsorption: A Review. *J. Compos. Sci.* **2021**, *5*, 233. [[CrossRef](#)]
79. Grzegórska, A.; Rybarczyk, P.; Rogala, A.; Zabrocki, D. Phytoremediation—From environment cleaning to energy generation—Current status and future perspectives. *Energies* **2020**, *13*, 2905. [[CrossRef](#)]
80. Madeła, M.; Skuza, M. Towards a circular economy: Analysis of the use of biowaste as biosorbent for the removal of heavy metals. *Energies* **2021**, *14*, 5427. [[CrossRef](#)]
81. Babu, A.G.; Shea, P.J.; Sudhakar, D.; Jung, I.-B.; Oh, B.-T. Potential use of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to remediate heavy metal (loid)-contaminated mining site soil. *J. Environ. Manag.* **2015**, *151*, 160–166. [[CrossRef](#)]
82. Raghuvanshi, R.; Chaudhari, A.; Kumar, G.N. 2-Ketogluconic acid and pyrroloquinoline quinone secreting probiotic *Escherichia coli* Nissle 1917 as a dietary strategy against heavy metal induced damage in rats. *J. Funct. Foods* **2017**, *37*, 541–552. [[CrossRef](#)]
83. Guo, Q.; Majeed, S.; Xu, R.; Zhang, K.; Kakade, A.; Khan, A.; Hafeez, F.Y.; Mao, C.; Liu, P.; Li, X. Heavy metals interact with the microbial community and affect biogas production in anaerobic digestion: A review. *J. Environ. Manag.* **2019**, *240*, 266–272. [[CrossRef](#)] [[PubMed](#)]
84. Ma, Y.; Rajkumar, M.; Zhang, C.; Freitas, H. Beneficial role of bacterial endophytes in heavy metal phytoremediation. *J. Environ. Manag.* **2016**, *174*, 14–25. [[CrossRef](#)] [[PubMed](#)]