

Article

Chemical Removal of Cu and Zn from Swine Feces before Soil Application

Moo-Joon Shim and Seung-Mok Lee * 

Department of Biosystems and Convergence Engineering, Catholic Kwandong University, Gangneung 25601, Korea; moojoonshim@gmail.com

* Correspondence: leesm@cku.ac.kr; Tel.: +82-33-649-7535

Abstract: Cu and Zn are known to be abundant in swine feces; hence, concentrations of these metals need to be lowered before swine feces are applied to land in order to prevent potential environmental problems. The main objective of this study was to develop an appropriate chemical process to remove Cu and Zn from swine feces using acid extractions. The removal efficiencies of Cu and Zn decreased in the order of $H_2SO_4 > HNO_3 >$ organic acids (citric and oxalic acids). Owing to the highest removal efficiencies of Cu and Zn by using H_2SO_4 , it was selected for further elimination of Cu and Zn from swine feces. By using H_2SO_4 , the optimal concentration, solid-to-liquid ratio, and reaction time were 2%, 1:50, and 8 h, respectively. At the optimum conditions, Cu concentration was decreased from 198 mg/kg to 40.1 mg/kg and Zn concentration from 474 mg/kg to 80.0 mg/kg, with removal rates of 79.7% and 83.1%, respectively. The low Cu removal efficiency, resulting from the strong complexation between Cu and organic matter of swine feces, was improved by the increase in the reaction time and H_2SO_4 solution concentrations. However, about half of the total nitrogen (TN) was also removed by using H_2SO_4 , indicating that the swine feces treated with H_2SO_4 may have poor value as fertilizer. Additional studies are required to find an optimal method to maintain TN concentrations while simultaneously removing Cu and Zn.



Citation: Shim, M.-J.; Lee, S.-M. Chemical Removal of Cu and Zn from Swine Feces before Soil Application. *Agriculture* **2021**, *11*, 377. <https://doi.org/10.3390/agriculture11050377>

Academic Editors: Markku Yli-Halla

Received: 15 February 2021

Accepted: 19 April 2021

Published: 21 April 2021

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



Copyright: © 2021 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/>).

Keywords: swine feces; metals; fertilizer; removal; sludge

1. Introduction

Owing to the economic profitability and low environmental pollution, swine feces have been frequently used as an alternative to chemical fertilizers [1,2]. Heavy metals, especially Cu and Zn, are abundant in swine feces as they are used in swine feed to prevent anemia and diarrhea and increase weight [3–8]. Most of Cu and Zn (up to ~95%) are excreted in swine feces, and concentrations of Cu and Zn in swine feces are greater than those in cattle and horse feces [7,9,10]. In addition, Cu and Zn concentrations in swine manure were greater than those in cattle, sheep, and poultry manure in England, Wales, and China [11,12]. Thus, compared to other metals, swine manure and feces could be a more important source for Cu and Zn in soil [6].

Land application of swine manure as a fertilizer can lead to the accumulation of heavy metals (e.g., Cu and Zn) in soil [6,11,13,14]; hence, soil and groundwater qualities possibly deteriorate, and the metals accumulated in soil can be transferred to plants in excessive amounts, which finally affect the health of animals and humans through the food chain [15]. Similarly, as swine feces can be a key source for plant nutrients Cu and Zn in soil, swine feces also can lead to too high Cu and Zn concentrations of soil. Therefore, Cu and Zn should be reduced to a moderate level swine feces before the land application of feces.

Several studies have reported the distribution and characteristics of chemical elements in swine feces and manure [1,6–8,16]. For instance, Henja et al. (2019) [6] evaluated concentrations of swine feces, manure, and feed to examine the swine rearing system. The authors concluded that the highest concentrations of Cu and Zn are observed in swine

diet and the metal concentrations of swine manure and feces are affected by feed. Svane and Karring (2019) [7] also reported that concentrations of Cu and Zn in a swine manure slurry were greater than those in cattle manure slurry; hence, cattle manure may be better as a fertilizer. Besides chemical analysis, microbiota in swine feces and manure also were analyzed. For example, the microbial community was affected by heavy metals, such as Zn and Cd [8], and swine feces and manure contained high levels of antibiotic bacteria [17].

Transformation of chemical elements (e.g., nitrogen and carbon), as well as fate of pathogens, have been examined during composting [18–20]. For instance, during the processing of composting, nitrate concentration of swine manure decreased with the depth and anoxic zone [19]. Wang et al. (2014) [14] also reported that the organic carbon content of swine manure was less than that in cow and chicken manure and that the degradability of organic matter in swine manure is faster than that in cow manure. In addition, effects of swine manure and slurry on the microbial community in soil have been examined [21–23]. For example, Suleiman et al. (2016) [23] reported that microbial diversity decreases after slurry application in soil.

However, there is lack of information regarding the appropriate process for decreasing Cu and Zn from swine feces. In contrast, several studies have been conducted to develop processes to remove heavy metals from sludge [19,24–28]. To decrease heavy metals from sludge, optimal conditions such as chemical reagent concentrations, stirring time, solid-to-liquid ratio have been reported. Furthermore, compared to inorganic acids, organic acids were reported to better extract heavy metals [29], and Cu has been one of the heavy metals with a low removal efficiency due to strong complexation with organic matter [26,30,31].

As mentioned above, concentrations of Cu and Zn needed to be decreased from swine feces before composting of swine feces. Thus, method development was conducted to suggest appropriate processes to decrease concentrations of Cu and Zn in swine feces using the reported methods for metal removal from sludge. The objective of this study was: (1) to elucidate a better chemical reagent with higher removal efficiencies of Cu and Zn from swine feces; (2) to find optimal conditions such as selected reagent concentration, reaction time, and solid-to-liquid ratio; and (3) to develop a method to increase the Cu removal efficiency.

2. Materials and Methods

2.1. Source of Swine Feces

Samples of swine feces were collected from a thickening tank of a swine fecal treatment plant in H city in Korea, which separates swine feces into solid and liquid and composts the solid. The samples were scooped into a plastic box using a plastic spoon. After collecting the swine feces from the thickening tank, the samples were transported to the lab, completely homogenized, dried at 105 °C, and maintained at room temperature until Cu and Zn removal experiments were conducted. The feces samples were taken out as needed for each experiment. Before the removal experiment, ignition loss and water content were analyzed. The ignition loss and water content were 73.6 ± 0.80 (n = 4) and 58.4% (n = 1), respectively. Water content was calculated using the weight difference after drying swine feces in an oven at 105 °C, and ignition loss was analyzed by weight difference after burning swine feces at 550 °C for 3 h using dried feces.

2.2. Removal of Cu and Zn

2.2.1. Selection of a Chemical Reagent

To investigate optimal removal conditions of Cu and Zn from swine feces, preliminary experiments were first conducted to select an appropriate chemical reagent for the removal of Cu and Zn. Owing to the lack of studies on heavy metal removal from swine feces, chemical reagents were selected from studies reported previously on the removal of heavy metals from sludge. Inorganic acid (e.g., HNO₃ and H₂SO₄) and organic acid (citric and oxalic acids) solutions were used in this study. The extracting solution concentrations were utilized from previous studies. For instance, citric and oxalic acid concentrations of 0.1 M

were prepared, as reported previously [27]. As described in a previous study [28], HNO₃ and H₂SO₄ concentrations were adjusted to 1.5 M and 1 M, respectively. The solid-to-liquid ratio was 1:50, which was the optimal ratio for the removal of heavy metals from sludge using HNO₂ [26]. That is, 8 g of samples were added into a beaker, and 400 mL of each reagent was added, followed by stirring the samples for 5 h in a jar tester. After stirring, the liquid was separated from feces by centrifuge (3000 rpm for 15 min), and the precipitated feces was put in an electric oven at 105 °C until dried. All removal tests were performed in duplicate in this study.

2.2.2. Impact of Solid-to-Liquid Ratios, Stirring Time, and Reagent Concentrations

After selecting H₂SO₄ as the chemical reagent, the impact of solid-to-liquid ratio was investigated. For this test, various ratios (e.g., 1:10, 1:25, 1:37.5, 1:50, 1:100, and 1:200) were utilized. The samples were stirred for 5 h. After the optimal ratio was selected, the effect of H₂SO₄ concentrations (0.5%, 1%, 2%, 5%, 7%, 10%, and 15%) on the Cu and Zn removal efficiencies was investigated. The stirring time was fixed at 5 h with a solid-to-liquid ratio of 1:50. After this test, the effect of the stirring time on the Cu and Zn removal was investigated using the optimal concentration and solid-to-liquid ratios. The feces samples were stirred for 1 h, 2 h, 3.5 h, 5 h, and 8 h. Regression analysis of Excel was used to obtain a *p* value at 95% confidence level in order to measure statistical significance between removal efficiency and the conditions. There is statistical significance if the *p* value is less than 0.05. If the *p* value is greater than 0.05, however, there is no significance.

2.2.3. Addition of the Ultrasound Process

Ultrasound-assisted H₂SO₄ removal of Cu and Zn was performed to examine the application of the ultrasound process via the variation in the sonication time (1 h, 2 h, and 4 h). An ultrasonic machine (Sonictopia, STH-750S) was operated at a frequency of 20 kHz by using a cylindrical titanium probe (diameter of 10 mm and length of 125 mm) [26]. The same solid-to-liquid ratio (1:50) was utilized in this test with 1% and 10% H₂SO₄ solutions.

2.3. Analyses of Cu and Zn

After stirring the swine feces, the feces were dried in an oven and digested with c-H₂SO₄ and 60% HClO₄ solutions. The detailed digestion process was reported elsewhere [26]. The accuracy of this experiment was investigated using certified reference materials (CRM) (Metals in Sewage Sludge, Sigma–Aldrich, product ID: SQC001S) for the analysis of the feces samples [26]. Flame atomic absorption measurements were carried out to determine the Cu and Zn concentrations (Varian, AA240FS). Table 1 listed the recoveries and method detection limits for Cu and Zn. After removal and analysis, the removal efficiency was estimated using the following equation [26]:

$$\text{Removal efficiency (\%)} = (C_i - C) / C_i \times 100$$

where *C_i* and *C* represent concentrations of Cu and Zn before and after removal experiments, respectively.

Table 1. Recoveries for the CRM and method detection limits for AA analysis.

	Cu	Zn
Recovery (%) (n = 2)	106	112
SD	5.06	8.03
Method detection limit (mg/kg dry solids)	0.027	0.036

2.4. Analysis of Total Nitrogen

Total nitrogen (TN) was measured by Korean Standard Methods of Water Quality Test. First, dried swine feces (0.1 g) was added into a 250 mL glass bottle, followed by the addition of 50 mL of deionized water (DIW) and 50 mL of alkali $K_2S_2O_8$. Second, alkali $K_2S_2O_8$ was prepared by dissolving 15 g of NaOH and 50 g of $K_2S_2O_8$ in 1 L of DIW. Next, the 250 mL bottles containing the samples were autoclaved for 40 min, followed by cooling the bottles at room temperature. The supernatant was filtered, and HCl was added to the filtrate. After measuring the absorbance at 220 nm, TN concentrations were calculated using a calibration curve prepared in advance using a KNO_3 solution. The accuracy of TN analysis was investigated by the same CRM used to examine the accuracy of Cu and Zn analysis in feces samples. The accuracy was $99.0 \pm 4.2\%$ ($n = 2$).

3. Results and Discussion

3.1. Selection of Method

The concentrations of Cu and Zn before and after treatment are indicated in Appendix A. Before the treatment, the initial concentrations of Cu and Zn were 198 and 474 mg/kg, respectively. Based on the previous studies [1,6,7], Cu and Zn concentrations were varied. For instance, Cu concentrations (mg/kg dried) of swine feces were 650 in England, 514 in German, 784 in China, and 131 in Denmark. Zinc concentrations were 470 in England, 93~8239 in German, 1113 in China, and 232.5 in Denmark. Thus, the concentrations of Cu and Zn in the swine feces collected for this study were not generally higher than in other studies. Table 2 lists the removal efficiencies of Cu and Zn from swine feces by using several reagents. It was difficult to select the best chemical reagent due to the different concentrations for each reagent. Despite this, the removal method for Cu and Zn using 10% H_2SO_4 was adopted herein as the highest removal efficiencies of Cu and Zn were observed by using 10% H_2SO_4 . Copper removal efficiency decreased in the order of $H_2SO_4 > HNO_3 > \text{oxalic acid} > \text{citric acid}$ (Table 2). Similarly, Zn removal efficiency using inorganic acids (H_2SO_4 and HNO_3) was greater than that using organic acids (citric and oxalic acids) (Table 2). Studies on the removal of heavy metals from swine feces using both inorganic and organic acids have been rare. Thus, our results were compared with those of studies reported previously on the removal of metal from sludge.

Table 2. Removal efficiency with chemical reagents ($n = 2$).

Reagent	Cu		Zn	
	RE (%)	SD	RE (%)	SD
HNO_3	80.5	1.98	83.6	2.15
H_2SO_4	91.4	0.24	90.1	1.04
Citric acid	12.9	5.82	64.9	1.46
Oxalic acid	50.9	3.01	56.6	3.49

Contrary to the results obtained herein, some studies have a lower removal efficiency of heavy metals by using inorganic acids [29,32]. For instance, Veeken and Hamelers (1999) [29] reported that the removal efficiencies of Cu and Zn from sludge decreased in the order of citric acid (70% and 90% for Cu and Zn) > oxalic acid (60% and 70% for Cu and Zn) > nitric acid (38% and 65% for Cu and Zn). Marchioretto et al. (2002) [33] also reported that the removal efficiency using citric acid was greater than that using HNO_3 and HCl at pH 3 or 4. Heavy metals in sludge are adsorbed onto organic and inorganic sludge phases, as well as inorganic precipitates as in carbonates, phosphates, sulfides, etc. [34,35]. When inorganic acids are reacted with sludge, heavy metals adsorbed on sludge are exchanged by protons, thereby releasing the heavy metals from precipitates [31,35–37].

Another mechanism for the removal of heavy metals using organic acids involves their complexation with carboxylic functional groups [19,25,29,35,37] even though organic acids

can also remove heavy metals via proton exchange [37]. Therefore, the higher removal efficiency by using organic acids is explained by their dual roles of an acid and a chelator to remove heavy metals from sludge. As a result, organic acids demonstrate promise as an alternative chemical extracting agent for the removal of metals from sludge [24,27].

The results obtained herein were different from those reported previously indicating that organic acids exhibit a higher removal efficiency for heavy metals. Our results revealed that inorganic acids are better than organic acids for the removal of heavy metals (Cu and Zn) from swine feces. Previous studies reported that the highest removal efficiencies of Cu and Zn from sludge using citric and oxalic acids are observed at pH 3–4 [29,33]. The pH of the citric and oxalic acid solutions used herein was ~1.5. Thus, the lower removal efficiencies of Cu and Zn was possibly related to the lower pH, which was not optimal to remove Cu and Zn. The removal experiment was conducted with swine feces and not sludge; therefore, the difference between sludge and swine feces may be related to the higher removal efficiency using inorganic acids. To determine the reason, additional removal experiments should be performed under the same conditions (e.g., concentrations and pH) of organic and inorganic acids.

Compared to oxalic acid, citric acid was reported to exhibit a higher removal efficiency [29,37]. As mentioned above, carboxylic functional groups serve as a chelator to complex with heavy metals. Different removal efficiencies by using citric and oxalic acids have been explained by the number of carboxylic functional groups [29,37]. A high number of carboxylic functional groups may be more advantageous for complexation with heavy metals. Citric acid has three carboxylic groups, whereas oxalic acid has two carboxylic groups. Furthermore, another mechanism can describe the lower removal efficiency by using oxalic acid. Calcium oxalate may be formed if calcium is present in the sludge, leading to the less efficient removal of heavy metals [37]. However, in this study, the use of citric acid led to a considerably lower Cu removal efficiency, a better Zn removal efficiency (Table 2). Thus, the higher Zn removal efficiency can be explained by the above-explained reason. However, additional studies are required to explain the lower Cu removal efficiency by using citric acid.

3.2. Optimal Solid to-Liquid Ratio

Figure 1 shows the effect of the solid-to-liquid ratio on the removal efficiencies of Cu and Zn from swine feces. The pH was not adjusted when the effect of solid-to-liquid ratios on removal efficiency of heavy metals was conducted. With the increase in the solid-to-liquid ratio, the Cu removal efficiency increased ($p < 0.05$), with the maximum removal efficiency of 91.9% observed at a solid-to-liquid ratio of 1:50 (Figure 1). When the solid-to-liquid ratio was increased above 1:50, removal efficiency of Cu was lowered. This was because the correlation between solid-to-liquid ratio and removal efficiency was not significant ($p > 0.05$). In contrast, Zn removal efficiency increased with solid-to-liquid ratios ($p < 0.05$). The highest Zn removal efficiency (94.4%) was observed at solid-to-liquid ratios of 1:100 and 1:200. The low removal efficiency with a low solid-to-liquid ratio resulted from the incomplete mixing between the metals and H_2SO_4 solution [38]. The decrease in the Cu removal efficiency with the increase in the solid-to-liquid ratio from 1:50 to 1:100 and 1:200 cannot be explained at this moment. Although the best solid-to-liquid ratios for the removal of Cu and Zn were different, the optimal solid-to-liquid ratio was selected as 1:50 due to the decrease in the Cu removal efficiency at a solid-to-liquid ratio of greater than 1:50 (Figure 1). Similarly, the same solid-to-liquid ratio of 1:50 was selected when heavy metals were extracted from sludge using HNO_2 [26]. Cu and Zn removal efficiencies of 50.6% and 83.7%, respectively, were observed by stirring sludge with HNO_2 for 7 h [26].

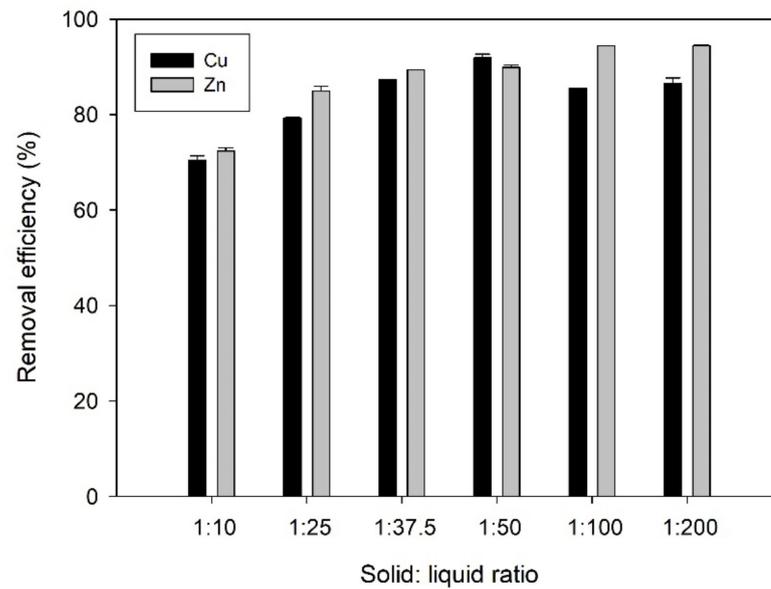


Figure 1. The effect of solid to liquid ratio on removal efficiency of Cu and Zn from swine feces.

3.3. Effect of H_2SO_4 Concentrations

With the increase in the H_2SO_4 concentrations, the removal efficiency of Cu and Zn increased ($p < 0.05$) (Figure 2). This result was similar to that reported previously, indicating that a high acid concentration leads to the high removal efficiency of heavy metals from sludge [39]. At low H_2SO_4 concentrations (0.5 and 1%), the removal efficiency of Cu was less than that of Zn; however, their removal efficiencies were similar at H_2SO_4 concentrations of greater than 1% (Figure 2). The use of 0.5 M H_2SO_4 for heavy metal (Cd, Cu, and Pb) removal led to the lowest removal efficiency of Cu from sludge [30]. The lower Cu removal efficiency was explained by the strong organic Cu bond in sludge. Shim et al. (2020) [26] also reported that a low efficiency was observed by using HNO_2 and citric acid for the removal of Cu from sludge.

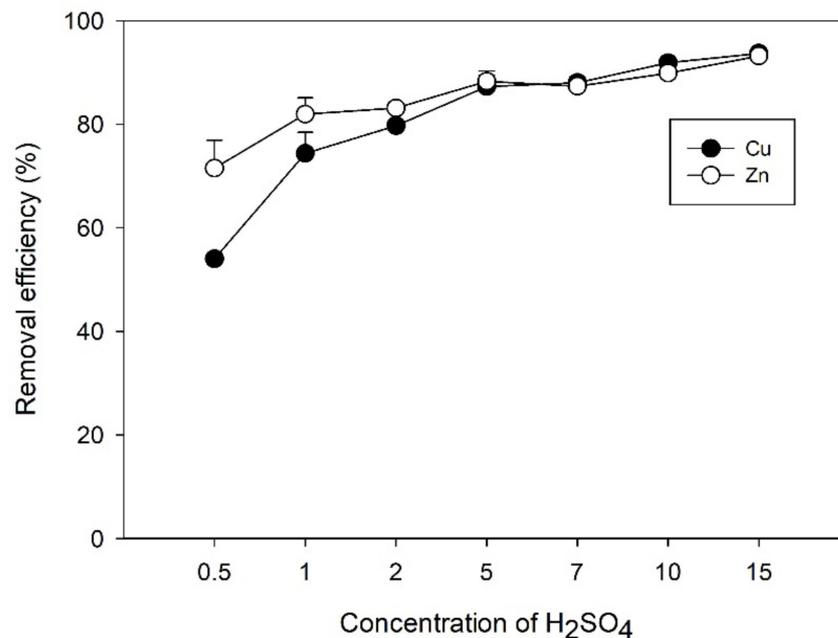


Figure 2. The influence of concentrations of H_2SO_4 (%) on removal efficiency of Cu and Zn from swine feces (reaction time: 5 h, solid to liquid ratio: 1:50).

Several studies have removal efficiencies of Cu and Zn from sludge using H₂SO₄ as the chemical reagent [31,40–43]. However, it was difficult to directly compare the Cu and Zn removal efficiencies with previous studies because each study utilized different conditions, such as H₂SO₄ solution concentrations, reaction time, solid-to-liquid ratio, and temperature. Table 3 lists the removal efficiencies of Cu and Zn from sludge reported previously. As mentioned above, few studies for the removal of metals from swine feces are available; hence, sludge studies have been compared. For instance, Stylianou et al. (2007) [31] conducted experiments for the removal of heavy metals from sludge using 10% H₂SO₄ and reported Cu and Zn removal efficiencies of 38% and 72%, respectively, which were less than those observed in this study. Cu and Zn removal efficiencies of ~90% were observed by using 10% H₂SO₄ in this study. Different removal efficiencies between two studies were possibly related to the difference in the other conditions, such as solid-to-liquid ratio and reaction time. The solid-to-liquid ratio and stirring time were 1:5 and 30 min in the previous study [31], whereas those values in this study were 1:50 and 5 h, respectively. Thus, a low solid-to-liquid ratio and reaction time could lead to the low removal efficiency.

Table 3. Comparison of Cu and Zn removal from sludge by H₂SO₄ extraction.

Concentration	Solid to Liquid Ratio	Reaction Time	Metal Removal Efficiency (%)		Referecnce
			Cu	Zn	
10%	1:5	30 min	54	70	Stylianou et al., 2007
20%	1:5	60 min	86	72	Stylianou et al., 2007
100 g/L	1:5	25 h	88.6	99.2	Silva et al., 2002
0.5 M		1 h	20	78	Yoshizaki and Tomida, 2000
	0.5 g/250 mL	12 h	57.8	82.3	Mingot et al., 1995
0.5 M		24 h	1	72	Jenkins and Scheybeler, 1981
10%	1:50	5 h	91.4	90.1	This study

Copper removal efficiency has increased with the increase in H₂SO₄ concentration from 10% to 20% and prolongation of stirring time from 30 min to 60 min [31] (Table 3). However, although Stylianou et al. (2007) [31] used 20% H₂SO₄, the removal efficiency was still less than that observed in this study possibly due to low solid-to-liquid ratio and reaction time (Table 3). Despite different conditions, previous studies have reported that the removal efficiency of Cu has been less than that of Zn (Table 3). As mentioned above, these results have been explained by the complexation of Cu with sludge. The most important form of Cu was reported to be organic complexes in swine manure [1]. Therefore, organic Cu complexes in swine feces can also lead to a lower removal efficiency. However, at a H₂SO₄ concentration of greater than 2%, similar Cu and Zn removal efficiencies were obtained (Figure 2). Therefore, the effect of the organic Cu complexation seems to have disappeared at H₂SO₄ concentration of greater than 2% for the removal of Cu from swine feces.

3.4. Effect of Reaction Time

Figure 3 shows the effect of reaction time on the removal of Cu and Zn using 10% H₂SO₄ and a solid-to-liquid ratio of 1:50. With the increase in the stirring time, the Zn removal efficiency did not increase noticeably ($p > 0.05$) (Figure 3). The removal efficiency of Zn from swine feces reached up to ~90% in 1 h, and the removal efficiency was maintained until 8 h of reaction. In contrast, there seems to be a slight increase in Cu removal efficiency, but it was not statistically significant ($p > 0.05$). The removal efficiency of Cu was 72% after 1 h of reaction, but increased to 94% in 8 h of reaction. Notably, the Cu and Zn removal efficiencies were remarkably different after a stirring time of 1 h. The removal efficiency

of Cu was considerably less than that of Zn at a stirring time of 1 h (Figure 3), suggesting that a 1 h reaction is not sufficient to break the complex between Cu and organic matter in swine feces. Our results revealed that a minimum time of 2 h is required to achieve similar removal efficiencies of Cu and Zn (Figure 3) and to break the Cu organic complex by using 10% H_2SO_4 and a solid-to-liquid ratio of 1:50. In addition, the Cu removal efficiency was greater than the Zn removal efficiency when stirring was conducted for 5 h and 8 h (Figure 3). However, considering treatment cost, there was no need to extend the reaction time from 2 h to 5 h.

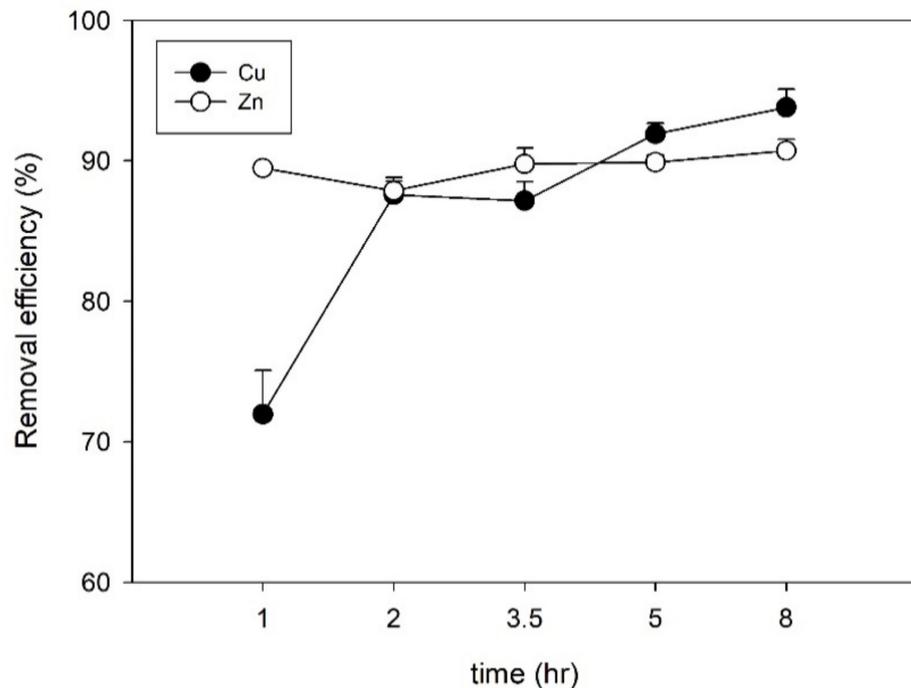


Figure 3. The influence of reaction time on removal efficiency of Cu and Zn from swine feces (concentration of H_2SO_4 : 10%, solid to liquid ratio: 1:50).

3.5. Effect of the Addition of the Ultrasound Process

The addition of the ultrasound process to chemical extraction can improve the removal efficiencies of metals from sludge and decrease the reaction time [26,44,45]. Ultrasound spreads through a medium with strong energy dissipation, producing vapor bubbles that collapse and subsequently lead to acoustic cavitation [19,46]. The collapse of the bubbles instantaneously produces a local high temperature, pressure, and shear stress, which result from the disintegration of the sludge floc and cell wall; hence, heavy metals that are attached to sludge particles are released [44,47–49]. In addition, the effect of addition of the ultrasound process for the extraction of Cu and Zn from swine feces was examined.

Removal efficiencies with and without the ultrasound process by using 10% H_2SO_4 were compared (Figure 4). The application of the ultrasound process led to the slight increase in the Zn removal efficiency. However, after removal was conducted by the application of the ultrasound process, the Cu removal efficiency clearly increased with stirring times of 1 h and 2 h (Figure 4). This result suggested that the ultrasound process can aid in breaking the complex between Cu and organic matter in swine feces. This result was similar to that reported previously, indicating it takes a shorter reaction time to achieve the maximum efficiency by treating the sludge with free nitrous acid and applying the ultrasound process [26].

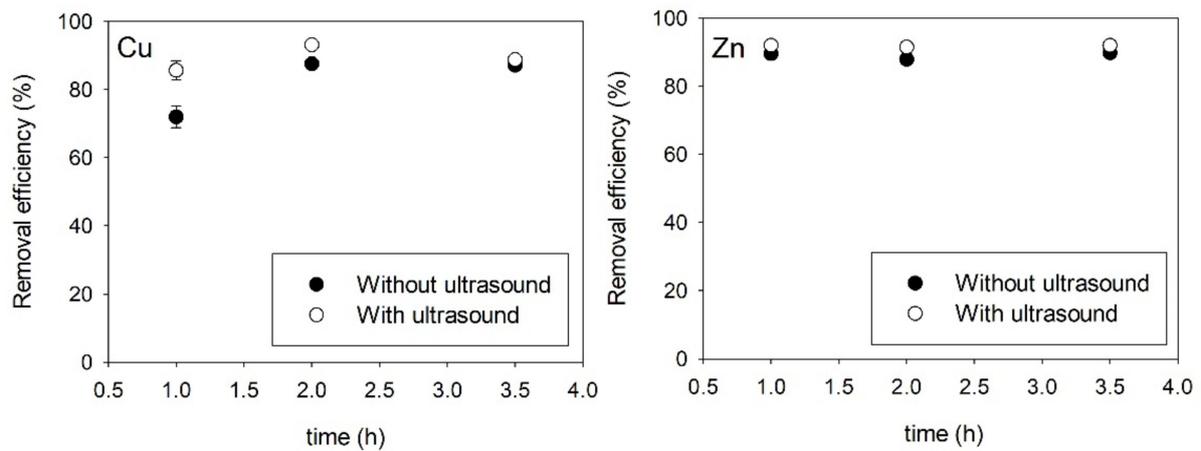


Figure 4. Comparison of removal efficiencies with and without ultrasound process when 10% H₂SO₄ was applied.

With the application of the ultrasound process the removal efficiency by using 10% H₂SO₄ was greater than that by using 1% H₂SO₄ (Table 4), indicating that high H₂SO₄ concentrations were favorable for the removal of Cu and Zn from swine feces. By conducting the removal experiment for 1 h using 10% H₂SO₄ with the application of the ultrasound process, the removal efficiency of Zn (92.0%) was greater than that of Cu (85.6%) (Table 4). However, for a 2 h reaction, the Cu and Zn removal efficiencies were not different, which was similar to the result obtained for the effect of the reaction time, suggesting that 2 h stirring was required to break the complex between Cu and the organic matter in swine feces. Meanwhile, when the removal experiment was conducted with 1% H₂SO₄ with the application of the ultrasound process, the removal efficiency of Zn was greater than that of Cu at every interval (Table 4), indicating that 1% H₂SO₄ was not sufficient to extract the Cu-organic complex even with the assistance of ultrasound.

Table 4. The influence of addition of ultrasound process on removal efficiency of Cu and Zn from swine feces.

Reaction Time (Hour)	Cu		Zn	
	1% H ₂ SO ₄	10% H ₂ SO ₄	1% H ₂ SO ₄	10% H ₂ SO ₄
1	68.3 ± 1.01	85.6 ± 2.90	83.2 ± 1.40	92.0 ± 0.21
2	75.8 ± 0.58	93.1 ± 0.29	83.4 ± 1.93	91.4 ± 0.16
4	69.8 ± 0.11	88.8 ± 1.36	79.2 ± 4.35	92.0 ± 0.13

3.6. Effect of H₂SO₄ on TN Concentrations

The concentration of TN in swine feces was estimated to be 13,000 mg/kg (Table 5). According to the Korean Standards of Fertilizer, the TN concentration was equivalent to class 1 compost. As stated, the highest Cu and Zn removal efficiencies were obtained using H₂SO₄ as the chemical reagent. However, the highest TN removal efficiency was observed by using H₂SO₄ as the extractant in this study (Table 5). That is, the removal of TN by using H₂SO₄ was greater than that by using citric and oxalic acids. About half of TN was removed by using 10% H₂SO₄ at a stirring time of 5 h. In addition, the TN removal increased with the increase in the H₂SO₄ concentrations. For example, ~70% of TN was removed during the removal process using 15% H₂SO₄ (Table 5). These results suggested that high H₂SO₄ concentrations to remove Cu and Zn are not always optimal to re-use swine feces as a fertilizer because the TN levels of swine feces should be maintained TN levels for recycling as a fertilizer. By using a constant concentration of 10% H₂SO₄ to remove Cu and Zn from swine feces, the TN removal did not increase with the reaction time (Table 5). Clearly, the TN concentration increased when swine feces were treated with

10% HNO₃, possibly because of the addition of nitric acid in swine feces. The addition of NO₃ in swine feces rendered extra good quality to the compost as per Korean standards.

Table 5. The removal efficiency of TN from swine feces.

		RE(%)	SD
	HNO ₃	–	–
	H ₂ SO ₄	51.1	9.6
	Citric acid	16.8	5.5
	Oxalic acid	14.9	10.0
H ₂ SO ₄ concentrations (%)	0.5	–	–
	1	–	–
	2	–	–
	5	26.7	0.48
	7	41.0	1.84
	10	54.9	3.83
	15	71.7	7.47
reaction time (hour)	1	53.3	0.68
	2	51.5	0.03
	3.5	52.1	1.73
	8	56.4	1.19

–: negative efficiency.

After removal processes, we should consider disposal of H₂SO₄ solution including Cu, Zn, nitrogen, and possibly pathogens extracted from swine feces. There is a monetary cost to discard the acid used for removal processes. In addition, the acid deposited in the swine feces can be harmful to the environment if the swine feces as fertilizer enters soil. Therefore, research should be conducted on the development of alternatives that do not require disposal costs and are harmless to the environment. Our group is working on the recycling of chemical reagents to keep the costs as low as possible. In addition, biosurfactants and bioleaching methods are also being conducted elsewhere [38,50]. However, studies on acid disposal and replacement are outside the scope of this study and should be carried out in future studies.

4. Conclusions

Removal efficiencies of Cu and Zn from swine feces decreased in the order of H₂SO₄ > HNO₃ > organic acids. Our results can be interpreted by sludge studies as there have been insufficient studies on the removal of Cu and Zn from swine feces. However, results of sludge studies reported previously were contrary to those obtained herein. Therefore, our results may be interpreted differently even though the removal of Cu and Zn by using organic acids was not performed with the optimal pH conditions reported in the previous study, suggesting that additional studies should be conducted to determine whether the substances containing Cu and Zn affect the removal efficiency.

Based on the removal efficiency, H₂SO₄ was adopted to remove Cu and Zn from swine feces. The optimal concentration, solid-to-liquid ratio, and reaction time were 2%, 1:50, and 8 h, respectively, even though 10% H₂SO₄ solutions showed the highest removal efficiency. Despite the high removal efficiency by using 10% H₂SO₄, a problem may arise by using 10% H₂SO₄ as the chemical reagent because about half of the TN was also removed along with Cu and Zn, implying that swine feces treated with H₂SO₄ may have poor value as ingredients of a fertilizer. Therefore, the 2% H₂SO₄ concentration without a reduction in TN was determined as the optimum concentration. The application of the ultrasound

process led to the improved removal efficiency of Cu by using 10% H₂SO₄. The removal efficiency of Cu was less than that of Zn possibly due to the strong complexation between Cu and organic matter of swine feces. However, the removal efficiencies became similar with the increase in the reaction time and H₂SO₄ concentrations. This result suggested that the strong organic Cu bond may have broken with the increase in H₂SO₄ concentrations and reaction time. The addition of ultrasound process also can help break the complex between Cu and organic matter in swine feces, which can save the treatment cost.

Follow-up studies are required to elucidate influence of fertilizer made from the treated swine feces on environmental gas emissions and accumulation of metals in soil and plants. In addition, economic feasibility for the fertilizer production after metal removal should be also investigated.

Author Contributions: Conceptualization, M.-J.S.; methodology, M.-J.S.; validation, M.-J.S. and S.-M.L.; formal analysis, M.-J.S.; writing—original draft preparation, M.-J.S. and S.-M.L.; writing—review and editing, M.-J.S. and S.-M.L.; supervision, S.-M.L.; project administration, S.-M.L.; funding acquisition, S.-M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Basic Science Research Program through the National Research Foundation of Korea (NRF-2019R111A3A0106242412).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author (leesm@cku.ac.kr).

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

Appendix A

Concentrations of Cu and Zn before and after Treatment (Unit: mg/kg dried). All Removal Experiments Were Conducted in Duplicate, and the Initial Concentrations Were Analyzed in Three Replicates

		Cu		Zn		
		Conc.	SD	Conc.	SD	
Before treatment		198	7.38	474	15.8	
	Reagents	HNO ₃	38.6	3.91	77.7	10.2
		H ₂ SO ₄	17.0	0.48	46.9	4.93
		Citric acid	172	11.5	167	6.91
		Oxalic acid	97.1	5.96	206	16.6
After treatment	0.5	90.9	1.90	135	25.7	
	1	50.7	8.07	85.6	15.0	
	2	40.1	1.02	80.0	3.03	
	H ₂ SO ₄ concentration (%)	5	25.1	4.35	55.7	9.46
		7	23.7	0.36	59.9	8.22
		10	16.0	1.56	48.0	2.44
		15	12.5	1.63	32.3	0.57

		Cu		Zn		
		Conc.	SD	Conc.	SD	
After treatment	solid-to-liquid ratio	1:10	58.5	1.76	131	2.94
		1:25	41.2	0.31	71.4	4.83
		1:37.5	25.2	0.00	50.5	0.00
		1:50	16.0	1.56	48.0	2.44
		1:100	28.6	0.00	26.3	0.00
		1:200	26.6	2.21	26.4	0.53
	Reaction time (h)	1	55.4	6.13	50.0	1.85
		2	24.6	1.93	57.6	4.49
		3.5	25.4	2.67	48.6	5.40
		5	16.0	1.56	48.0	2.44
		8	12.3	2.59	44.1	3.87
		Ultrasound	1	62.7	2.00	79.6
1% H ₂ SO ₄	2	47.9	1.15	78.9	9.16	
Reaction time (h)	4	59.8	0.23	98.6	20.6	
Ultrasound	1	28.5	5.73	38.0	1.00	
10% H ₂ SO ₄	2	13.6	0.57	40.6	0.75	
Reaction time (h)	4	22.2	2.68	38.1	0.64	

References

- Bao, Q.; Lin, Q.; Tian, G.; Wang, G.; Yu, J.; Peng, G. Copper distribution in water-dispersible colloids of swine manure and its transport through quartz sand. *J. Hazard. Mater.* **2011**, *186*, 1660–1666. [\[CrossRef\]](#)
- Gao, F.-Z.; He, L.-Y.; He, L.-X.; Zou, H.-Y.; Zhang, M.; Wu, D.-L.; Liu, Y.-S.; Shi, Y.-J.; Bai, H.; Ying, G.-G. Untreated swine wastes changed antibiotic resistance and microbial community in the soils and impacted abundances of antibiotic resistance genes in the vegetables. *Sci. Total Environ.* **2020**, *741*, 140482. [\[CrossRef\]](#) [\[PubMed\]](#)
- Burton, C.H.; Turner, C.H. *Manure Management: Treatment Strategies for Sustainable Agriculture*, 2nd ed.; Silsoe Research Institute: Wrest Park, Silsoe, UK, 2003.
- Ciesinski, L.; Guenther, S.; Pieper, R.; Kalisch, M.; Bednorz, C.; Wieler, L.H. High dietary zinc feeding promotes persistence of multi-resistant *E. coli* in the swine gut. *PLoS ONE* **2018**, *13*, e0191660. [\[CrossRef\]](#) [\[PubMed\]](#)
- Poulsen, H.D. Zinc and copper as feed additives, growth factors or unwanted environmental factors. *J. Anim. Feed Sci.* **1998**, *7* (Suppl. S1), 135–142. [\[CrossRef\]](#)
- Hejna, M.; Moscatelli, A.; Onelli, E.; Baldi, A.; Pilu, S.; Rossi, L. Evaluation of concentration of heavy metals in animal rearing system. *Ital. J. Anim. Sci.* **2019**, *18*, 1372–1384. [\[CrossRef\]](#)
- Svane, S.; Karring, H. A comparison of the transition metal concentrations in the faeces, urine, and manure slurry from different livestock animals related to environmentally relevant microbial processes. *Cogent Chem.* **2019**, *5*, 1644702. [\[CrossRef\]](#)
- Peng, Z.; Zhang, J.; Fanning, S.; Wang, L.; Li, M.; Maheshwari, N.; Sun, J.; Li, F. Effects of metal and metalloid pollutants on the microbiota composition of feces obtained from twelve commercial pig farms across China. *Sci. Total Environ.* **2019**, *647*, 577–586. [\[CrossRef\]](#)
- Ding, F.; He, Z.; Liu, S.; Zhang, S.; Zhao, F.; Li, Q.; Stoffella, P.J. Heavy metals in composts of China: Historical changes, regional variation, and potential impact on soil quality. *Environ. Sci. Pollut. Res.* **2017**, *24*, 3194–3209. [\[CrossRef\]](#)
- Yin, Y.; Gu, J.; Wang, X.; Song, W.; Zhang, K.; Sun, W.; Zhang, X.; Zhang, Y.; Li, H. Effects of Copper Addition on Copper Resistance, Antibiotic Resistance Genes, and intl1 during Swine Manure Composting. *Front. Microbiol.* **2017**, *8*. [\[CrossRef\]](#)
- Nicholson, F.A.; Smith, S.R.; Alloway, B.J.; Carlton-Smith, C.; Chambers, B.J. An inventory of heavy metals inputs to agricultural soils in England and Wales. *Sci. Total Environ.* **2003**, *311*, 205–219. [\[CrossRef\]](#)
- Shi, T.; Ma, J.; Wu, F.; Ju, T.; Gong, Y.; Zhang, Y.; Wu, X.; Hou, H.; Zhao, L.; Shi, H. Mass balance-based inventory of heavy metals inputs to and outputs from agricultural soils in Zhejiang Province, China. *Sci. Total Environ.* **2019**, *649*, 1269–1280. [\[CrossRef\]](#) [\[PubMed\]](#)
- Jakubus, M.; Dach, J.; Starmans, D. Bioavailability of copper and zinc in pig and cattle slurries. *Fresen. Environ. Bull.* **2013**, *22*, 995–1002.

14. Moral, R.; Moreno-Caselles, J.; Perez-Murcia, M.D.; Perez-Espinosa, A.; Rufete, B.; Paredes, C. Characterisation of the organic matter pool in manures. *Bioresour. Technol.* **2005**, *96*, 153–158. [[CrossRef](#)]
15. Lu, Y.; Song, S.; Wang, R.; Liu, Z.; Meng, J.; Sweetman, A.J.; Jenkins, A.; Ferrier, R.C.; Li, H.; Luo, W.; et al. Impacts of soil and water pollution on food safety and health risks in China. *Environ. Int.* **2015**, *77*, 5–15. [[CrossRef](#)]
16. Gagnon, B.; Ziadi, N.; Chantigny, M.H.; Bélanger, G.; Massé, D.I. Biosolids from Treated Swine Manure and Papermill Residues Affect Corn Fertilizer Value. *Agron. J.* **2012**, *104*, 483–492. [[CrossRef](#)]
17. Whitehead, T.R.; Cotta, M.A. Examination of the Aerobic Microflora of Swine Feces and Stored Swine Manure. *J. Environ. Qual.* **2016**, *45*, 604–608. [[CrossRef](#)] [[PubMed](#)]
18. Wang, K.; Li, X.; He, C.; Chen, C.-L.; Bai, J.; Ren, N.; Wang, J.-Y. Transformation of dissolved organic matters in swine, cow and chicken manures during composting. *Bioresour. Technol.* **2014**, *168*, 222–228. [[CrossRef](#)]
19. Wang, K.; Li, W.; Li, X.; Ren, N. Spatial nitrifications of microbial processes during composting of swine, cow and chicken manure. *Sci. Rep.* **2015**, *5*, 14932. [[CrossRef](#)] [[PubMed](#)]
20. Ziemer, C.J.; Bonner, J.M.; Cole, D.; Vinjé, J.; Constantini, V.; Goyal, S.; Gramer, M.; Mackie, R.; Meng, X.J.; Myers, G.; et al. Fate and transport of zoonotic, bacterial, viral, and parasitic pathogens during swine manure treatment, storage, and land application. *J. Anim. Sci.* **2010**, *88* (Suppl. S13), E84–E94. [[CrossRef](#)]
21. Moura, A.C.D.; Sampaio, S.C.; Remor, M.B.; Silva, A.P.D.; Pereira, P.A.M. Long-term effects of swine wastewater and mineral fertilizer association on soil microbiota. *Eng. Agric.* **2016**, *36*, 318–328. [[CrossRef](#)]
22. Sui, Q.; Zhang, J.; Chen, M.; Wang, R.; Wang, Y.; Wei, Y. Fate of microbial pollutants and evolution of antibiotic resistance in three types of soil amended with swine slurry. *Environ. Pollut.* **2019**, *245*, 353–362. [[CrossRef](#)]
23. Suleiman, A.K.A.; Gonzatto, R.; Aita, C.; Lupatini, M.; Jacques, R.J.S.; Kuramae, E.E.; Antoniolli, A.I.; Roesch, L.F.W. Temporal variability of soil microbial communities after application of dicyandiamide-treated swine slurry and mineral fertilizers. *Soil Biol. Biochem.* **2016**, *97*, 71–82. [[CrossRef](#)]
24. Geng, H.; Xu, Y.; Zheng, L.; Gong, H.; Dai, L.; Dai, X. An overview of removing heavy metals from sewage sludge: Achievements and perspectives. *Environ. Pollut.* **2020**, *266*, 115375. [[CrossRef](#)]
25. Ma, D.; Su, M.; Qian, J.; Wang, Q.; Meng, F.; Ge, X.; Ye, Y.; Song, C. Heavy metal removal from sewage sludge under citric acid and electroosmotic leaching processes. *Sep. Purif. Technol.* **2020**, *242*, 116822. [[CrossRef](#)]
26. Shim, M.J.; Jung, T.Y.; Yoon, D.H.; Yang, Y.M.; Rumky, J.; Yoon, Y.Y. HNO₂ treatment of sludge: An alternative way of sludge usage as fertilizer. *J. Environ. Manag.* **2020**, *258*, 110016. [[CrossRef](#)]
27. Suanon, F.; Sun, Q.; Dimon, B.; Mama, D.; Yu, C.-P. Heavy metal removal from sludge with organic chelators: Comparative study of N, N-bis(carboxymethyl) glutamic acid and citric acid. *J. Environ. Manag.* **2016**, *166*, 341–347. [[CrossRef](#)]
28. Zhu, Y.; Zeng, G.; Zhang, P.; Zhang, C.; Ren, M.; Zhang, J.; Chen, M. Feasibility of bioleaching combined with Fenton-like reaction to remove heavy metals from sewage sludge. *Bioresour. Technol.* **2013**, *142*, 530–534. [[CrossRef](#)]
29. Veeken, A.H.M.; Hamelers, H.V.M. Removal of heavy metals from sewage sludge by extraction with organic acids. *Water Sci. Technol.* **1999**, *40*, 129–136. [[CrossRef](#)]
30. Jenkins, R.L.; Scheybeler, B.J. Metals removal and recovery from municipal sludge. *J. Water Pollut. Control Fed.* **1981**, *5*, 25–31.
31. Stylianou, M.A.; Kollia, D.; Haralambous, K.-J.; Inglezakis, V.J.; Moustakas, K.G.; Loizidou, M.D. Effect of acid treatment on the removal of heavy metals from sewage sludge. *Desalination* **2007**, *215*, 73–81. [[CrossRef](#)]
32. Gaber, S.E.; Rizk, M.S.; Yehia, M.M. Extraction of certain heavy metals from sewage sludge using different types of acids. *Biokemistri* **2011**, *23*, 41–48.
33. Marchioretto, M.M.; Bruning, H.; Loan, N.T.P.; Rulkens, W.H. Heavy metals extraction from anaerobically digested sludge. *Water Sci. Technol.* **2002**, *46*, 1–8. [[CrossRef](#)]
34. Veeken, A. Remediation technologies for conversion of heavy metal polluted organic wastes into compost. In *Resource Recovery and Reuse in Organic Solid Waste Management*; Lens, P., Hamelers, B., Hoitink, H., Bidlingmaier, W., Eds.; IWA Publishing: London, UK, 2004; pp. 503–521.
35. Wu, C.-H.; Kuo, C.-Y.; Lo, S.-L. Removal of Metals from Industrial Sludge by Extraction with Different Acids. *J. Environ. Sci. Health Part A* **2004**, *39*, 2205–2219. [[CrossRef](#)] [[PubMed](#)]
36. Babel, S.; del Mundo Dacera, D. Heavy metal removal from contaminated sludge for land application: A review. *Waste Manag.* **2006**, *26*, 988–1004. [[CrossRef](#)] [[PubMed](#)]
37. Gheju, M.; Pode, R.; Manea, F. Comparative heavy metal chemical extraction from anaerobically digested biosolids. *Hydrometallurgy* **2011**, *108*, 115–121. [[CrossRef](#)]
38. Tang, J.; He, J.; Liu, T.; Xin, X.; Hu, H. Removal of heavy metal from sludge by the combined application of a biodegradable biosurfactant and complexing agent in enhanced electrokinetic treatment. *Chemosphere* **2017**, *189*, 599–608. [[CrossRef](#)]
39. Kazi, F.K.M.; Cooper, P.A. Rapid-extraction oxidation process to recover and reuse copper chromium and arsenic from industrial wood preservative sludge. *Waste Manag.* **2002**, *22*, 293–301. [[CrossRef](#)]
40. Mingot, J.I.; Obrador, A.; Alvarez, J.M.; Rico, M.I. Acid Extraction and Sequential Fractionation of Heavy Metals in Water Treatment Sludges. *Environ. Technol.* **1995**, *16*, 869–876. [[CrossRef](#)]
41. Naoum, C.; Fatta, D.; Haralambous, K.J.; Loizidou, M. Removal of heavy metals from sewage sludge by acid treatment. *J. Environ. Sci. Health Part A* **2001**, *36*, 873–881. [[CrossRef](#)] [[PubMed](#)]

42. Silva, J.E.; Soares, D.; Paiva, A.P.; Labrincha, J.A.; Castro, F. Leaching behaviour of a galvanic sludge in sulphuric acid and ammoniacal media. *J. Hazard. Mater.* **2005**, *121*, 195–202. [[CrossRef](#)]
43. Yoshizaki, S.; Tomida, T. Principle and Process of Heavy Metal Removal from Sewage Sludge. *Environ. Sci. Technol.* **2000**, *34*, 1572–1575. [[CrossRef](#)]
44. Deng, J.; Feng, X.; Qiu, X. Extraction of heavy metal from sewage sludge using ultrasound-assisted nitric acid. *Chem. Eng. J.* **2009**, *152*, 177–182. [[CrossRef](#)]
45. Marafi, M.; Stanislaus, A. Waste Catalyst Utilization: Extraction of Valuable Metals from Spent Hydroprocessing Catalysts by Ultrasonic-Assisted Leaching with Acids. *Ind. Eng. Chem. Res.* **2011**, *50*, 9495–9501. [[CrossRef](#)]
46. Petrier, C.; Jiang, Y.; Lamy, M.-F. Ultrasound and Environment: Sonochemical Destruction of Chloroaromatic Derivatives. *Environ. Sci. Technol.* **1998**, *32*, 1316–1318. [[CrossRef](#)]
47. Chu, C.P.; Chang, B.-V.; Liao, G.S.; Jean, D.S.; Lee, D.J. Observations on changes in ultrasonically treated waste-activated sludge. *Water Res.* **2001**, *35*, 1038–1046. [[CrossRef](#)]
48. Feng, X.; Lei, H.; Deng, J.; Yu, Q.; Li, H. Physical and chemical characteristics of waste activated sludge treated ultrasonically. *Chem. Eng. Process. Process Intensif.* **2009**, *48*, 187–194. [[CrossRef](#)]
49. Wang, F.; Lu, S.; Ji, M. Components of released liquid from ultrasonic waste activated sludge disintegration. *Ultrason. Sonochem.* **2006**, *13*, 334–338. [[CrossRef](#)] [[PubMed](#)]
50. Yang, W.; Song, W.; Li, J.; Zhang, X. Bioleaching of heavy metals from wastewater sludge with the aim of land application. *Chemosphere* **2020**, *249*, 126134. [[CrossRef](#)]