Effects of substrate types on the transformation of heavy metal speciation and bioavailability in an anaerobic digestion system

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ARTICLE INFO

ABSTRACT

Chemical speciation can fundamentally affect the potential toxicity and bioavailability of heavy metals. The transformation of heavy metal speciation and change of bioavailability were investigated in an anaerobic digestion (AD) system using four different substrates (pig manure (PM), cattle manure (CAM), chicken manure (CHM) and rice straw (RS)). The results obtained indicated that the total contents of heavy metals in PM, CHM and CAM were higher than in RS and decreased in the order Zn > Cu > Ni > Pb > As > Cd in all substrates. Moreover, the speciation with the largest proportion for each heavy metal was the same both in the different substrates and the biogas residues. Among them, Zn, Ni, Cd and As were mainly in the reducible fraction (F2), while Cu was mainly in the oxidizable fraction (F3) and Pb occurred predominantly in the residual fraction (F4). Our results further indicated that the AD process had a greater effect on the speciation of heavy metals in CHM and PM, but less on CAM and RS. The rates of change in bioavailability followed the order PM > CHM > CAM > RS. Changes in organic matter, humic acid or local metal ion environment as a result of AD were inferred as likely mechanisms leading to the transformation of heavy metal speciation. These results enhanced our understanding of the behavior of heavy metals in AD and provided a new perspective for the treatment and disposal of the substrates.

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Introduction

Anaerobic digestion (AD) is a complex biochemical process in which anaerobic microorganisms convert organic matter into biogas in an oxygen-free environment. Many studies have proven that anaerobic digestion technology can be employed with many types of solid organic wastes, including pig manure (PM), cattle manure (CAM), chicken manure (CHM) and rice straw (RS) (Banks et al., 2011; Ye et al., 2017, 2019). AD is a good way to reduce waste volume and increase energy recovery, as well as minimize environmental impacts. The data showed that approximately 770 million tons of crop straw and 39.7 billion tons of poultry and livestock manure are produced

every year in China (Zhang et al., 2009). Therefore, AD is a suitable method for converting solid organic waste into biogas, biogas residue and biogas slurry. Biogas is the main product of AD systems, and biogas residue and biogas slurry are by-products. The biogas is a form of renewable energy, thereby helping to meet the increasing demand for energy. The biogas residue (solid fraction) and biogas slurry (liquid fraction) can be used as crop culture media and fertilizers (Stefaniuk et al., 2015; Zhu et al., 2016). Nevertheless, ubiquitous heavy metals, such as Zn, Cu, Cd, As, Pb and Ni, can occur in substrates due to human behavior. Repeated manure application can result in heavy metal pollution in edible plants and soils, because of the toxicity, non-degradability and accumulation of heavy metals (Xu et al., 2013). For this reason, it is important to understand the total contents and distribution of heavy metals in substrates and their by-products.

The contents of heavy metals in AD systems with different substrates vary greatly (Doelsch et al., 2008; Weng et al., 2014; Xie et al., 2015). Animal feed is the main source of heavy metals in livestock and poultry manure due to feed additives containing heavy metals, such as Zn and Cu (Ji et al., 2012). Heavy metals in straw crops are mainly derived from the soil in which they grow, or indirectly from water and surrounding factories. The heavy metal contents of the substrates in an AD system will affect the content and distribution of heavy metals in the corresponding biogas residue and slurry. At early stages, due to the higher contents of Zn and Cu in the substrates of livestock and poultry, much of the research focused on Zn and Cu. For instance, Marcato et al. examined the particle size distribution and trace element patterns in a full-scale AD plant treating pig slurry; the results suggested that the contents of Cu and Zn were between 170 and 2600 mg/kg due to pig dietary supplements, and most of the Cu and Zn (86%) was trapped within particles between 3 and 25 μm (Marcato et al., 2008). Meng et al. (2017) showed that composting and pyrolysis processes could convert exchangeable and carbonate-bound Cu and Zn to organic matter and residual fractions, and significantly reduce the potential availability of metals in composts and biochars-. Unfortunately, in addition to Zn and Cu, several heavy metals, such as Cd, As and Pb, have been found in substrates used for AD in excess of environment standards. Although heavy metals like Cd, As and Pb are relatively low in content, their harm to the environment should not be underestimated. At the same time, heavy metals have been less systematically investigated in AD systems, and researchers have often studied just one type of substrate (e.g., PM) in AD systems. In fact, there are many kinds of substrates used in AD systems and the properties of different substrates are quite different, which may influence the behavior of the heavy metals. Hence, it is of great significance to study the heavy metal contents of biogas residue and slurry in AD processes using different substrates.

Bioavailability is the degree to which elements are available for interaction with biological systems (Cai et al., 2018). It is widely known that chemical speciation can influence bioavailability, and bioavailability influences the toxicity of heavy metals. Since the bioavailability, eco-toxicity and metal cycles are largely dependent on the speciation of heavy metals, it is vital to quantify the heavy metal speciation (Gao et al., 2014; Hullebusch et al., 2016).

Sequential extraction is a useful and suitable method for determining the chemical fractions in which these metals are present. It provides important information on the toxicity or potential bioavailability of heavy metals (Singh and Kalamdhad, 2012). The BCR three–step procedure, proposed by the Standards, Measurements and Testing (SM&T) Programme of the European Commission, is viewed as one of most widely applicable extraction procedures for heavy metal speciation (Mossop and Davidson, 2003). Consequently, speciation of heavy metals using the BCR method has been widely studied in soils, substrates and aerobic composting processes in AD systems to determine the bioavailability and eco-toxicity of heavy metals (He et al., 2016; Yang et al., 2017). The AD process may lead to the changes in the speciation and bioavailability of metals, and their relative abundance in biogas residue and slurry. However, much still remains unknown regarding the distribution of heavy metals and the transformation of chemical speciation, which is vital to enhance the understanding of the behavior of heavy metals in AD and provide a new perspective for treatment and disposal of the substrates or by-products after AD.

Herein, this current work was conducted to explore several heavy metals, including Zn, Cu, Ni, Cd, As and Pb, in the following aspects: (1) the distribution of the total contents of six heavy metals in four different substrates (PM, CAM, CHM and RS) and biogas residue and slurry after reaction in an AD system; (2) the effects of substrate types on the transformation of heavy metal speciation and bioavailability in an AD system.

1. Materials and methods

1.1. Substrates and inoculums

Both animal manure and straws were included in the study. PM and CHM were collected from pig and chicken farms located in Putian, Fujian, China. All pigs and chickens were fed on feedstuffs. CAM was obtained from a cattle farm in Xiamen, Fujian, China. The manure was collected as a semisolid and was not mixed with cleaning water from animal houses. The debris (e.g., body hair and stones) was removed and the manure was crushed by a grinder (Hummer 900, Baozhen, China) to an approximate size of 2–3 mm. It was then frozen until use in batch experiments. RS was obtained from villagers in Sanming, Fujian, China. The dried straw was chopped using a grinder (Hummer 900, Baozhen, China) to an approximate size of 2–3 mm. An inoculum from a 6.5 L anaerobic digester with PM was used and maintained at 37°C for 2–3 weeks before experiments to remove most of the remaining methane production. Table 1 summarizes the characterization of the substrates and inoculums.

1.2. Experimental design and set-up

The biochemical methane potential (BMP) of manure comes from digestion of the organic components in the feces and in the straw used as bedding material, which is mainly carbohydrates, proteins and lipids. The BMP of animal manures and straws were determined using a batch technique based

Table 1 – Characterization of the substrates and inoculums.

Character	PM	CHM	CAM	RS	Inoculum
TS (%)	22.50 ± 0.92	24.15 ± 1.75	20.85 ± 0.58	94.65 ± 4.80	3.98 ± 0.15
VS (%)	15.85 ± 2.01a*	16.74 ± 1.18b	18.45 ± 0.12c	79.58 ± 4.40d	1.73 ± 0.24
TC (g/kg TS)	346.87 ± 36.19	350.82 ± 2.56	387.60 ± 19.30	398.57 ± 14.39	NA
TOC (g/kg TS)	268.44 ± 1.79	287.35 ± 1.74	332.1 ± 12.04	367.4 ± 6.57	NA
COD (g/kg TS)	1209.24 ± 33.37	1435 ± 68.2	1021 ± 76.5	978.2 ± 45.5	598.43 ± 63.22
VFA (g/kg TS)	4.66 ± 0.13a	6.54 ± 0.24b	1.97 ± 0.15c	ND	NA
TAN (g/kg TS)	4.96 ± 0.03	15.02 ± 0.43	1.55 ± 0.13	ND	2.89 ± 0.31
TKN (g/kg TS)	43.28 ± 0.35	65.34 ± 0.91	27.56 ± 1.39	5.02 ± 0.40	NA
Al (g/kg VS)	5.37 ± 0.09	1.49 ± 0.05	12.98 ± 1.50	0.56 ± 0.01	ND
Ca (g/kg VS)	122.55 ± 1.14	369.92 ± 2.19	71.65 ± 3.02	9.82 ± 0.10	ND
Fe (g/kg VS)	12.35 ± 0.34	4.66 ± 0.11	7.68 ± 0.21	2.27 ± 0.04	31.20 ± 0.48
K (g/kg VS)	47.95 ± 2.31	135.77 ± 2.63	30.30 ± 2.83	148.42 ± 7.25	ND
Mg (g/kg VS)	41.92 ± 0.23	24.90 ± 1.28	12.98 ± 0.20	4.44 ± 0.75	ND
Na (g/kg VS)	16.65 ± 1.05	22.42 ± 0.21	6.74 ± 0.78	3.83 ± 0.13	ND
Lignin (%)	4.36 ± 0.15a	2.36 ± 0.21b	20.2 ± 0.34c	9.25 ± 0.55d	ND
Protein (g/kg TS)	217.5 ± 5.15a	195.3 ± 2.30b	165.2 ± 3.15c	35.2 ± 0.25d	ND
Lipid (g/kg TS)	62.38 ± 4.2a	28.75 ± 1.21b	18.25 ± 2.41c	5.21 ± 0.32d	ND

PM: pig manure; CAM: cattle manure; CHM: chicken manure; RS: rice straw; NA: not determined; ND: below the detection limit of the measuring method; TS: total solids; VS: volatile solid; TC: total carbon; TOC: total organic carbon; COD: chemical oxygen demand; VFA: volatile fatty acid; TAN: total ammonium nitrogen; TKN: total Kjeldahl nitrogen.

* Different lowercase letters indicate a significant difference ($P < 0.05$), and there are no significant differences between results with the same letters.

on previously reported methods (Møller et al., 2004). The lipid content was analyzed by measuring the amount of material able to be extracted with diethyl ether in a Soxhlet extraction instrument after hydrolysis with 3 N hydrochloric acid. The lignin content was analyzed by determination of the suspended volatile solids (VS) after boiling with a detergent in 1 mol/L sulfuric acid followed by suspension in 72% sulfuric acid, and the lignin contents were estimated using a VELP FIWE6 fiber analyzer (FIWE6, VELP, Italy). The protein content was determined by multiplying the difference between total nitrogen (TN) and NH_4^+-N by the factor 6.25. Each AD process was tested in triplicate using Erlenmeyer flasks with working volume of 200 mL (the ratio of manure, inoculum and water was 60%, 30% and 10%) kept at a mesophilic temperature (37 ± 0.2 °C). The loadings of PM, CHM, CAM and RS and inoculum in each reactor were all adjusted to a target VS concentration of 20 g/L from the manure and 10 g/L from the inoculum. After the inoculum was mixed with the substrate in the reactors, tap water was added to an effective liquid volume of 200 mL. In PM, CHM, CAM and RS anaerobic digestion systems, the ratio of C/N was 8.0, 5.4, 14.0 and 79.0, respectively. A set of blank reactors with the same amount of inoculum and water was used to determine the operational parameters and heavy metal contents of the inoculum. All the reactors were tightly closed with rubber plugs and screw caps after being flushed with N_2. Digestion continued until no further biogas production was observed (35 days). All reactors were equipped with a magnetic mixer to ensure that the reactor contents were thoroughly mixed. 10 mL of the sample (suspended solids) was taken out from the reactor for centrifugation (6000 r/min for 20 min). After separation, the solid fraction was considered biogas residue and the liquid fraction as biogas slurry. Biogas volume was determined by water displacement.

1.3. Analytical methods

Total Kjeldahl nitrogen (TKN) and total ammonium nitrogen (TAN), chemical oxygen demand (COD), pH, total solids (TS) and volatile solid (VS) analyses of the substrates and inoculums were conducted in accordance with APHA Standard methods (APHA, 1995). The volatile fatty acid (VFA) concentration was determined by gas chromatography (GC-FID, SP-2100A, Beifen, China). Substrates were first diluted and centrifuged. Then 1 mL of the supernatant was added to formic acid (pH < 3) and analyzed by gas chromatography. A PEG-20M column was used (30 m × 0.25 mm × 0.5 μm), nitrogen was the carrier gas and the flow rate was 25 mL/min. The initial, vaporization and FID temperatures were 120°C, 200°C and 220°C, respectively. The ascending rate was 20°C/min to 200°C, held for 10 min, and the injection volume was 1.0 μL. The total carbon (TC) and total organic carbon (TOC) contents were analyzed by a CHN elemental analyzer (Perkin Elmer, USA). The TOC content was determined by adding HCl, following established methods (Schumacher, 2002). The CH_4 content in the produced biogas was determined using gas chromatography (GC-TCD, SP-2100A, Beifen, China). The concentrations of metals present (Ca, Mg, K, Na, Al and Fe) were determined by digestion. In the digestion procedure, 0.1 g of biogas residue was weighed and added into a PTFE vessel, followed by adding 5 mL of hydrofluoric acid and 1 mL of perchloric acid, and reacted at room temperature until the sample was dissolved; then the solution was cooled and diluted to 100 mL and the concentration was determined by inductively coupled plasma-optical emission spectroscopy (Optima 5300DV, Perkin Elmer, USA). The total heavy metal concentration was determined with an Optima 7000DV ICP-MS (7000DV, ThermoScientific, USA) after first digesting the sample using HCl-HNO$_3$-HClO.

Table 2 – BCR sequential extraction procedure*.

Chemical fraction	Extraction procedure
Acid extractable fraction (F1)	0.1 g dehydrated biogas residue + 20 mL 0.11 mol/L CH_3COOH+ shaking for 16 hr at 25°C
Reducible fraction (F2)	Step (I) residue + 20 mL 0.5 mol/L NH_2OH-HCl (pH 1.5) + shaking for 16 hr at 25°C
Oxidizable fraction (F3)	Step (II) residue + 5 mL 30% H_2O_2 solution (pH 2) + shaking for 1 hr at 25°C; then the digested residue + 25 mL 1 mol/L NH_4Ac with 20% (V/V) HNO_3 (pH 2) + shaking for 16 hr at 25°C
Residual fraction (F4)	Step (III) residue + 10 mL HNO_3 + H_2O_2 (V:V = 4:1) + microwave digesting at 180°C for 40 min

 * The residues were centrifuged at 6000 r/min for 20 min and dried at 85°C for 18 hr between each extraction step.

## 1.4. *Determination of heavy metal speciation*

The speciation of heavy metals, divided into an acid extractable fraction (F1), a reducible fraction (F2), an oxidizable fraction (F3) and a residual fraction (F4), was determined using the BCR sequential extraction procedure (Kubova et al., 2008; Yuan et al., 2011). 0.1 g dehydrated biogas residue was used for speciation analysis. The detailed sequential extraction procedure is displayed in Table 2.

The F1 fraction of metals is likely to be influenced by changes in water composition and released by ion-exchange processes; this fraction is considered to be mobile and easily bioavailable to organisms. F2 is thermodynamically unstable under anoxic conditions, while F3 is unstable under oxidizing conditions and can result in the release of metals into solution, and thus often identified as a potentially toxic fraction. F4 of the metals is considered to be associated with stable minerals and makes little contribution to heavy metal bioavailability. Therefore, the toxicity and bioavailability mostly depend on the easily available fractions, such as F1 and F2. In fact, accurate bioavailability is difficult to obtain because of the complexity of the AD system; therefore, the measured bioavailability according to sequential extraction means "potential bioavailability". The bioavailability of metals decreases approximately in the order F1 > F2 > F3 > F4. Recoveries of 94.12%–100.26% were obtained in most instances in this work, which are consistent with other works (Xiao et al., 2015), and showed that BCR sequential extraction method is a reliable method for determining the speciation of heavy metals.

The bioavailability of heavy metals is the key factor in the application of substrates or biogas residue. According to a modified method (Zeng et al., 2018), the sum of F1and F2 for a heavy metal presents its total concentration of bioavailable fractions (F_A), and F_T is defined the sum of the total fractions (F1+F2+F3+F4) of heavy metals. Therefore, the bioavailability factor can be defined as the ratio of F_A to F_T.

1.5. *Data analysis*

Data was analyzed with Originpro8. All results were presented as means of three replicates ± SD (standard deviations were computed to determine the variability of the average values between replicates). SPSS was also used to carry out ANOVA and the post hoc test / Tukey's t-test was applied. Different lowercase letters indicate a significant difference ($P < 0.05$) and there is no significant difference between results with the same letters.
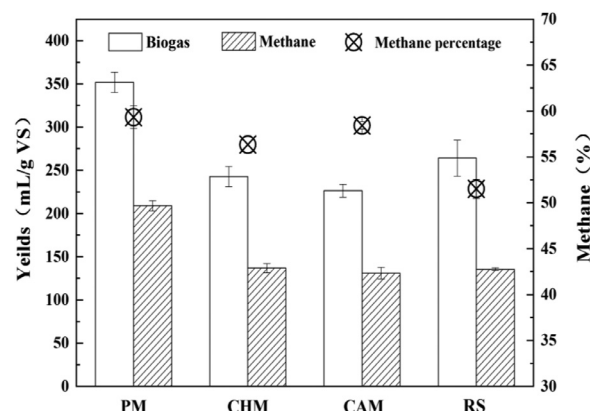
Fig. 1 – Biogas yield, methane yield and methane percentage for four commonly used substrates.

2. Results and discussion

2.1. *Anaerobic digestion properties*

A properly functioning anaerobic digestion system is an important basis for investigating the distribution and transformation of heavy metal speciation during the anaerobic digestion process. Therefore, in this study, biogas yield, methane yield, methane percentage, TS, VS, VFAs and other parameters were investigated to monitor whether the anaerobic system was working properly. The biogas yield, methane yield and methane percentages of four commonly used raw material substrates are shown in Fig. 1. The biogas yields of PM, CHM, CAM and RS were 351.89 mL/g VS, 242.75 mL/g VS, 226.45 mL/g VS and 264.09 mL/g VS, respectively (VS in the unit mL/g VS refers to the initial VS added to the reactor). The methane yields were 208.90 mL/g VS, 136.89 mL/g VS, 131.04 mL/g VS and 135.57 mL/g VS, respectively, which was consistent with the results of other studies (Gu et al., 2014; Wang et al., 2012). As can be seen from Fig. 1, PM had the highest methane gas production rate and CAM had the lowest. As expected, this may be because the cattle were fed roughage, which contains greater amounts of lignin. The average proportion of lignin was larger in CAM than in PM, i.e., (20.2 ± 0.34)% and (4.36 ± 0.15)%, respectively (Table 1). Moreover, the amounts of proteins shown in Table 1 were higher in PM rather than in CAM, which also contributed to higher methane production. From Table 1, we discovered that the lipid content, which was closely related to the methane potential, was the highest in

Table 3 – Anaerobic digestion performance.

Parameter	PM	CHM	CAM	RS
pH	7.30 ± 0.04	7.21 ± 0.04	6.98 ± 0.10	6.95 ± 0.15
VFAs (mg/L)	422.53 ± 27.24a*	160.56 ± 3.58b	235.07 ± 3.96c	267.45 ± 31.17d
CODs (mg/L)	1209.24 ± 33.37	1688.97 ± 12.11	1187.35 ± 7.18	1243.67 ± 124.66
TS initial (g/L)	31.98 ± 2.32	30.11 ± 2.17	24.87 ± 0.34	23.78 ± 1.5
TS final (g/L)	17.64 ± 0.75	15.43 ± 0.53	13.24 ± 0.84	13.89 ± 1.0
TS change (%)	44.75 ± 2.04a	48.59 ± 3.89b	46.74 ± 3.83c	41.36 ± 0.13d
VS initial (g/L)	21.45 ± 1.57	21.44 ± 1.08	20.37 ± 2.94	19.95 ± 0.65
VS final (g/L)	9.87 ± 0.56	10.67 ± 2.19	11.01 ± 0.33	9.91 ± 1.12
VS change (%)	53.70 ± 6.05a	50.47 ± 8.27b	45.00 ± 9.96c	50.22 ± 4.23d
NH_4^+-N (mg/L)	389.33 ± 21.47	1789.56 ± 29.40	90.22 ± 3.83	20.56 ± 2.55

* Different lowercase letters indicate a significant difference (P < 0.05), and there is no significant difference between results with the same letters.

PM (62.38 ± 4.2 g/kg TS); this was also the reason why PM had the highest methane gas production rate.

Table 3 shows the AD performance of the four substrates. The VS concentration is a very important anaerobic performance parameter, which directly reflects the substrate treatment capacity of each AD reaction system (Elango et al., 2007). It can be seen from Table 3 that the VS change rates of the AD reactors with PM, CHM and RS as the substrate was high, while relatively low for CAM. The pH and total VFA are important indicators used for monitoring AD operation, and the VFA concentration is a critical parameter in the operation and regulation of the AD process (Zhao et al., 2012). The pH values of all the AD reactors were within reasonable ranges (6.9–7.5), indicating that all reactors were operating normally. Another study showed that excess production of volatile fatty acids (> 4000 mg/L) may result in an inhibitory effect on the anaerobic digestion process (Siegert and Banks, 2005). However, the VFAs of the reactors in this study were between 160.56 and 422.53 mg/L, which indicated that no inhibition occurred during the AD. The general characteristics of the AD in terms of COD and NH_4^+-N were also detected. We discovered that the content of COD and NH_4^+-N in CHM was higher than the others; this may be due to the short digestive tract of the chicken, where the food can only stay for about 4 hr in the body and a large amount of nutrients are not excreted and absorbed, but all the values were in the reasonable range.

In general, the AD reactor functioned properly and was a suitable system for the study of the distribution and speciation of heavy metals.

2.2. Total concentration and speciation of heavy metals in four commonly used substrates

Different substrates used in AD systems may have an influence on the behavior of heavy metals, including the total contents and speciation. Therefore, it is vital to investigate the effects of substrate types on heavy metal contents and speciation. The total concentrations of six heavy metals in different substrates are shown in Fig. 2. It was apparent that the concentration of heavy metals decreased in the order Zn > Cu > Ni > Pb > As > Cd in the four substrates. The heavy metal contents in different substrates were significantly different ($F = 835$, $p < 0.001$ for Zn, $F = 3675$, $p < 0.001$ for Cu,
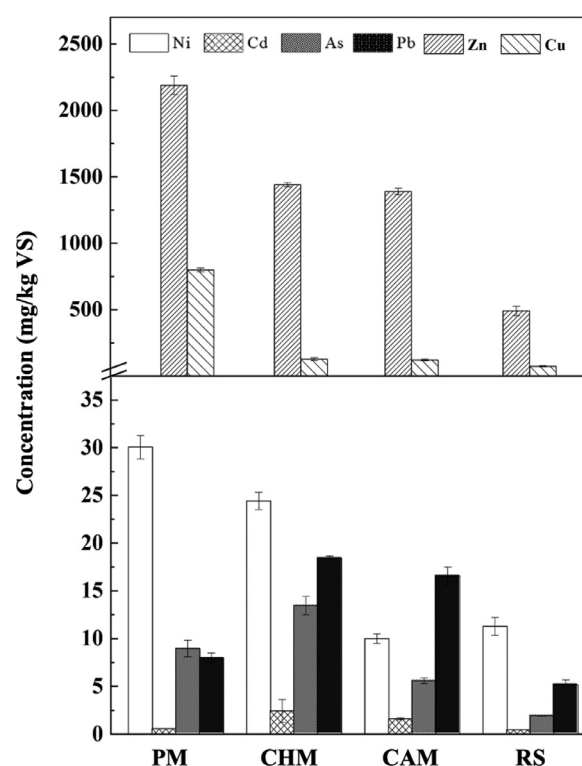
Fig. 2 – Total concentration of six heavy metals in substrates.

$F = 343$, $p < 0.001$ for Ni, $F = 7.16$, $p < 0.01$ for Cd, $F = 161$, $p < 0.001$ for As, $F = 95.1$, $p < 0.001$ for Pb), which meant that the heavy metal content was greatly affected by the type of substrate. We found that the contents of heavy metals in PM, CHM and CAM were higher than in RS. This may be because the heavy metals in RS were mainly derived from soil migration and cultivation methods, so that the results also reflected the fact that there were low levels of heavy metals in the cultivation soil of the RS. In addition, the concentrations of Zn and Cu (PM: 2188.92 mg/kg VS, 798.49 mg/kg VS; CHM: 1440.88 mg/kg VS, 127.39 mg/kg VS; CAM: 1390.30 mg/kg VS, 121.29 mg/kg VS; RS: 490.36 mg/kg VS, 73.58 mg/kg VS), especially in PM, CHM and CAM, were much higher than the other four heavy met-

Table 4 – The speciation of six heavy metals in four commonly used substrates[*].

Raw material	Heavy metal	Speciation (mg/kg VS)			
		F1	F2	F3	F4
PM	Zn	76.48 ± 0.91	1223.86 ± 13.01	502.65 ± 5.46	382.42 ± 3.70
	Cu	49.31 ± 1.84	25.90 ± 0.13	656.04 ± 5.66	68.01 ± 5.67
	Ni	11.10 ± 0.40	18.91 ± 0.63	2.37 ± 0.10	2.51 ± 0.21
	Cd	0.03 ± 0.01	0.24 ± 0.07	0.14 ± 0.01	0.14 ± 0.01
	As	2.82 ± 0.06	3.78 ± 0.12	0.62 ± 0.03	1.41 ± 0.04
	Pb	0.47 ± 0.03	0.76 ± 0.04	0.70 ± 0.05	5.86 ± 0.18
CHM	Zn	40.99 ± 8.62	788.47 ± 19.25	109.85 ± 2.90	498.96 ± 27.36
	Cu	5.52 ± 0.94	5.47 ± 0.52	78.88 ± 7.19	35.57 ± 2.73
	Ni	5.19 ± 0.32	13.49 ± 1.29	4.14 ± 0.87	1.50 ± 0.50
	Cd	0.16 ± 0.01	1.16 ± 0.06	0.61 ± 0.03	0.46 ± 0.06
	As	0.98 ± 0.07	5.57 ± 0.18	2.96 ± 0.10	2.96 ± 0.23
	Pb	0.49 ± 0.05	1.85 ± 0.14	3.06 ± 0.21	11.71 ± 0.27
CAM	Zn	72.71 ± 7.12	807.13 ± 69.65	288.22 ± 12.19	223.62 ± 59.40
	Cu	4.63 ± 0.29	1.87 ± 0.13	89.78 ± 5.85	24.47 ± 4.23
	Ni	1.88 ± 0.10	4.97 ± 0.24	2.06 ± 0.06	0.16 ± 0.04
	Cd	0.06 ± 0.01	0.78 ± 0.10	0.40 ± 0.07	0.37 ± 0.02
	As	0.86 ± 0.08	2.92 ± 0.08	1.15 ± 0.12	0.51 ± 0.15
	Pb	0.35 ± 0.01	1.60 ± 0.36	2.10 ± 0.38	11.05 ± 0.42
RS	Zn	38.99 ± 2.54	312.98 ± 2.49	80.87 ± 5.17	33.76 ± 7.81
	Cu	8.42 ± 0.84	13.63 ± 0.74	27.70 ± 1.58	23.37 ± 0.42
	Ni	0.95 ± 0.10	7.62 ± 0.34	1.96 ± 0.06	0.81 ± 0.10
	Cd	$5.70 \times 10^{-3} \pm 0.00$	0.28 ± 0.03	0.08 ± 0.01	0.06 ± 0.01
	As	0.24 ± 0.01	0.71 ± 0.01	0.31 ± 0.01	0.48 ± 0.01
	Pb	0.48 ± 0.02	1.30 ± 0.04	0.83 ± 0.02	2.40 ± 0.13

[*] Data are given by mean ± standard deviation based on 3 samples.

als. This was due to manufacturers often adding Zn, Cu and Se to feed to promote animal growth. However, most of these heavy metals and trace elements will be excreted in the manure of animals due to their poor absorption and utilization (Mantovi et al., 2003). Compared with the contents of Zn and Cu in the four raw substrates, the contents of Ni, As, Pb and Cd were relatively low. Among them, the content of Pb in CHM and CAM and the content of Ni in PM and CHM were relatively high.

The speciation of the six heavy metals in PM, CHM, CAM and RS is illustrated in Table 4. From the analysis results, it can be seen that the heavy metal speciation was different in the four raw substrates, but the speciation with the largest proportion for each heavy metal was the same in the different substrates, which was due to the distribution being closely related to the nature of the heavy metals. For instance, Zn, Ni, Cd and As were mainly in F2, while Cu was mainly in F3 and Pb was mainly in F4. Overall, these results indicated that different substrates contained variable contents and speciation of heavy metals, reflecting the nature and source of substrates collected from different livestock and straw.

2.3. The distribution of heavy metals in biogas residue and biogas slurry

The distribution characteristics of heavy metals in biogas residue and biogas slurry are closely related to the substrate. Studying the distribution of heavy metals in by-products of different substrates after AD is of great significance for assessing heavy metal pollution in by-products and the utiliza-

tion of by-products. The distribution of heavy metals in biogas residue and slurry are illustrated in Fig. 3. After the AD reaction of the four different substrates, the vast majority of the heavy metals in the substrates was in the biogas residue and only a small amount flowed into the biogas slurry. This was likely because about 60% of the solid matter will enter the biogas residue after anaerobic digestion of substrates, and the biogas residue contains a large amount of carbonate minerals, iron and manganese oxides, organic matter and some humic acids, therefore, heavy metals would easily form complexes and precipitate with these substances through exchange or coordination. This also indicated that the heavy metal content in biogas residue depended largely on that in the substrates. The results showed that the contents of heavy metals in the biogas residue after the digestion of four commonly used substrates was quite different, and the heavy metal content of the biogas residue from AD using RS as the raw material was the lowest. The difference in heavy metal content in the biogas slurry was smaller than that in the biogas residue. Among them, Zn, Pb and Cd were the highest in CHM, PM and CAM, respectively.

2.4. Distribution and transformation of heavy metal speciation in biogas residue after digestion

To simultaneously investigate the effects of substrate types on the transformation of heavy metal speciation and bioavailability in an anaerobic digestion system with different substrates can enhance our understanding of the behavior of heavy metals in the anaerobic digestion process. Therefore, it
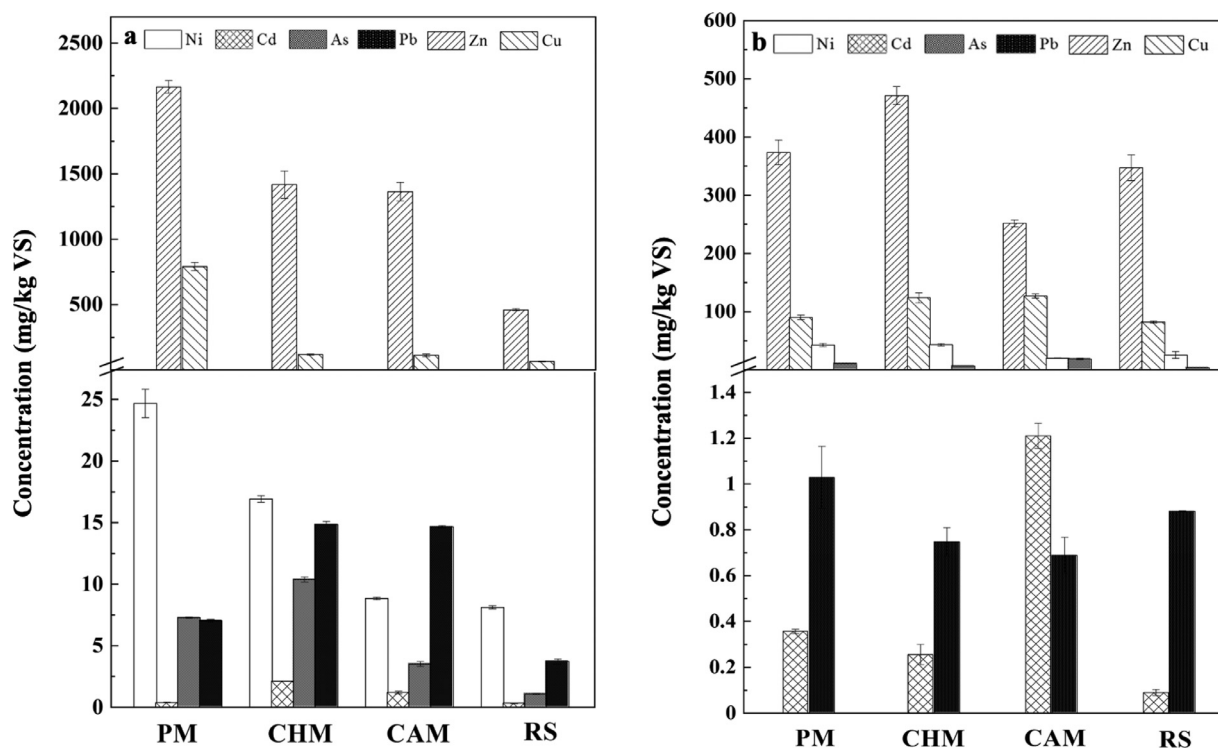
Fig. 3 – Total concentration of six heavy metals in biogas residue (a) and biogas slurry (b).
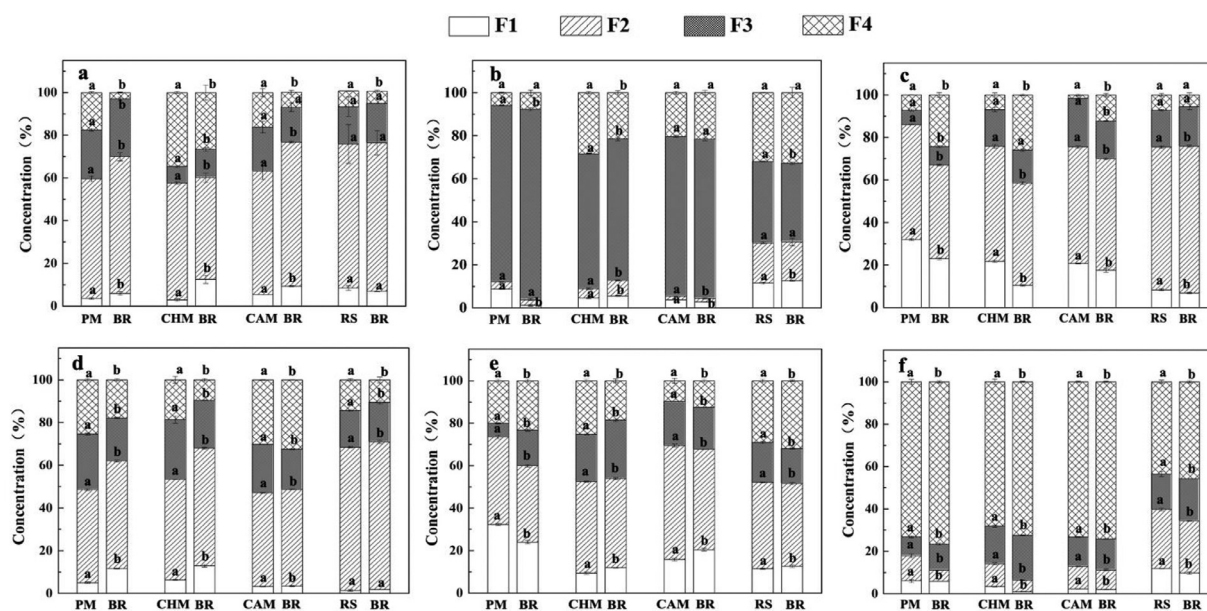

Fig. 4 – The distribution and transformation of (a) Zn, (b) Cu, (c) Ni, (d) Cd, (e) As, (f) Pb speciation in biogas residue after anaerobic digestion of four substrates (BR: biogas residue after digestion; Different lowercase letters indicate a significant difference (P<0.05), and there is no significant difference between the same letters.)

is necessary to study the transformation of heavy metal speciation and bioavailability for the six studied heavy metals in biogas residue after digestion of the four substrates. Based on the nature of the heavy metals and the substrates, the distribution and transformation of the heavy metals were quite different. Fig. 4 unfolds the distribution and transformation of

heavy metal speciation in biogas residue after digestion of the four substrates.

2.4.1. Zinc (Zn)

The proportional distribution of the four speciation fractions of Zn in the biogas residue of the four substrates can be seen

in Fig. 4a. The F2 of Zn in four different biogas residues (PM: 1385.31 mg/kg VS; CHM: 674.59 mg/kg VS; CAM: 919.50 mg/kg VS; RS: 320.02 mg/kg VS) was higher than the other three fractions; the percentages of F2 in PM, CHM, CAM and RS were 63.98%, 47.59%, 67.45% and 69.51%, respectively. This indicated that Zn was mainly in F2, which was the same as the distribution in the substrates. The reason was that Zn appeared to be preferentially bound to Fe oxides. Meng et al. (2017) reached the same conclusion, that Zn was mainly bound to the reducible iron and manganese fractions, accounting for over half of the total concentration of Zn. In addition, the F1 proportions in the biogas residue of PM, CHM and CAM were similar, and higher than that of RS. The F3 of PM was the highest compared with the other three biogas residues. F4 of CHM was the highest compared with the others, at 8.86, 3.82 and 4.75 times that of PM, CAM and RS, respectively. This suggested that Zn in CHM was relatively stable.

The transformation of Zn speciation in biogas residue after anaerobic digestion of the four substrates was explored (Fig. 4a). We found that all speciations of Zn in the four biogas residues were changed after AD. F1, F2 and F3 of Zn in PM, CHM, and CAM all increased, while F4 decreased. Among them, the F1 fraction in CHM increased the most (4.4 times the F1 in CHM substrate), which could be caused by the TAN content in CHM being higher than in other substrates, thus affecting the pH value of the AD process and other reaction parameters. Moreover, F1 was the most vulnerable to environmental impact, so the change trend was the largest. These results indicated that after AD, the bioavailability of Zn in PM, CHM and CAM was significantly enhanced by 10.51%, 2.41% and 13.70%, respectively, which meant that the toxicity was increased. Compared to the other three substrates, the transformation of heavy metal speciation in RS was smaller. F1 and F4 had a slight decrease, while F2 and F3 had a slight increase in RS.

2.4.2. Copper (Cu)

As displayed in Fig. 4b, the results indicated that, different from Zn, F3 of Cu was predominant in all materials (PM: 699.99 mg/kg VS; CHM: 77.45 mg/kg VS; CAM: 83.05 mg/kg VS; RS: 23.97 mg/kg VS). The percentages of F3 in PM, CHM, CAM and RS were 88.54%, 65.67%, 73.99% and 36.55%, respectively. This was because Cu is typically associated strongly with organic matters, forming stable chelated substances (Hsu and Lo, 2001; Li et al., 2019), which may be due to the humic substances in substrates generally having higher complexing affinity for Cu than Zn. Moreover, humic substances always have high contents of carboxyl groups that readily complex with Cu (Shuai et al., 2008). The result was consistent with other works, in which the results of sequential extraction showed that Cu was mainly bound to the organic fraction, contributing 40%-73% to the total amount when pig manure and sawdust were employed for composting (Meng et al., 2017). The content of F3 of Cu in PM was 22.87%, 14.55% and 51.99% higher than in CHM, CAM and RS, respectively, which was caused by the higher content of substrates. Moreover, the contents of F1 and F2 of Cu in RS were much higher than for other substrates. We speculated that this was because the composition of RS was simpler and the mineral content was lower than that of manure, making it difficult to immobilize heavy metals.

Fig. 4b demonstrates that in PM and CHM, F3 of Cu increased by 6.46% and 2.9% after AD, which may be due to the strong affinity of Cu for organic matter particles and their coatings. In contrast, F3 of Cu in CAM and RS decreased slightly. F1 of Cu in PM and CAM decreased by 7.58% and 1.09% respectively, while it increased in CHM and RS. As for F4, we found that it decreased by 6.78% in CHM, but increased slightly in PM, CAM and RS, which meant that the stability of Cu in CHM decreased, while the stability of Cu in PM, CAM and RS increased. Compared with PM and CHM, the speciation change of Cu in CAM and RS was smaller, which may be because the compositions of CAM and RS were relatively simple, and the lipid and protein contents in CAM and RS were lower than in PM and CAM, while the lignin content was higher.

2.4.3. Nickel (Ni)

Fig. 4c reveals that Ni occurred predominantly in F2 in the four different biogas residues (PM: 10.87 mg/kg VS; CHM: 8.13 mg/kg VS; CAM: 4.63 mg/kg VS; RS: 5.60 mg/kg VS). The percentages of F2 in PM, CHM, CAM and RS were 44.01%, 48.01%, 52.33% and 69.01%, respectively. The former two fractions (F1+F2) in PM, CHM, CAM and RS, having direct toxicity to environment, accounted for 67.0%, 55.8%, 69.8% and 75.9%, respectively. In addition, the F3 percentages of RS and CAM were similar. F1 and F4 of PM were the most abundant, at 31.99% and 24.30%, respectively.

It can be seen from Fig. 4c that before and after the reaction, F1 and F2 were the main fractions of Ni in the four materials, but the content of F3 was small, because the Ni cation has less ability to form complexes with organic ligands than Cu^{2+} among the transition metal cations (Qiao and Ho, 1997). This also indicated that Ni may cause environmental toxicity as a result of its mobility. We found that F1 decreased in all materials, by 8.99%, 11.22%, 3.21% and 1.47% in PM, CHM, CAM and RS, respectively. F4 increased in PM, CHM and CAM, while it decreased in RS. In other words, the stability of Ni in PM, CHM and CAM increased, while the stability of Ni in RS decreased. The speciation change for Ni in RS was the smallest, and it was speculated that the reason was that the protein and lipid contents of the RS were relatively low, which are closely related to biochemical methane potential (BMP), while the lignin content was relatively high; therefore the AD process had a relatively small impact.

2.4.4. Cadmium (Cd)

The comparison of Cd speciation in the biogas residue digestion of the four substrates is manifested in Fig. 4d. It is clear that the F2 of Cd in the four different biogas residues (PM: 0.20 mg/kg VS; CHM: 1.17 mg/kg VS; CAM: 0.55 mg/kg VS; RS: 0.23 mg/kg VS) was higher than the other three fractions. The percentages of F2 in PM, CHM, CAM and RS were 50.35%, 55.08%, 45.27% and 69.03%, respectively, showing high bioavailability to the environment despite its low content, which was consistent with another study (Xiao et al., 2015). The reason may be that Cd mainly exists in the form of divalent organic complexes, chelates and complex ions (CdCl⁻, CdOH⁺), and organic matter and humic acid have strong adsorptive capacity for Cd, which could easily cause the accumulation of Cd. The F1 of Cd in CHM was 1.3%, 8.24% and 9.82% higher than in PM, CAM and RS, respectively. However, the fraction (F3+F4) of Cd

in CAM was higher than that of the other three kinds of biogas residue; therefore, it had relatively good stability.

As shown in Fig. 4d, after the AD of the four substrates, F1 in Cd was increased by 6.68%. At the same time, we could find that F3 was reduced by 5.86%, 5.59% and 3.9% in PM, CHM and CAM, which was caused by the decrease in the organic matter content in the raw material with the progress of the AD reaction. Comparing the changes in the F4 fraction for Cd, it was found that except for the slight increase in CAM, the other three kinds of biogas residue saw reductions; that is, the stability of Cd in PM, CHM and RS was weakened after the AD.

2.4.5. Arsenic (As)

The results in Fig. 4e indicate that F2 of As (PM: 2.62 mg/kg VS; CHM: 4.36 mg/kg VS; CAM: 1.67 mg/kg VS; RS: 0.42 mg/kg VS) was higher than other fractions. The percentages of F2 in PM, CHM, CAM and RS were 35.97%, 42.01%, 47.33% and 38.93%, respectively. In addition, F3 of As in CHM was the highest compared with the other three kinds of biogas residue. The F4 fractions in PM and RS were relatively higher than in CHM and CAM.

It can be seen from Fig. 4e that the relative percentages of As speciation in the four different materials all changed after AD. F2 of As in the four substrates decreased in varying degrees after AD, among which CAM decreased the most. In addition, F3 of As in PM and CHM increased by 10.3% and 5.24%, while that of RS and CAM decreased by 1% and 2.27%, which was mainly related to the changes in the organic matter and humic acid content of the substrates. Except for PM, the percentage of F1 increased after anaerobic digestion in the substrates. The figures also indicated that the stability of As in PM, CAM and RS increased as their percentages in the (F3+F4) fraction increased by 3.65%, 6.75% and 2.85% respectively, which was opposite to CHM.

2.4.6. Lead (Pb)

From Fig. 4f, we found that Pb occurred predominantly in F4 in all biogas residues, with 76.5%, 72.4%, 74.1% and 42.6% in PM, CHM, CAM and RS, respectively. This provided evidence of the low mobility and bioavailability of Pb to the environment. This was likely due to the chemical properties of Pb and its insoluble inorganic compounds (Weng et al., 2014). There was no significant difference in the F1 fraction of Pb in the four biogas residues.

Fig. 4f shows the distribution and transformation of Pb speciation in biogas residue after digestion of the four substrates. The variations of all speciations of Pb in the four substrates were significant ($P < 0.05$). It is clear that reduction of the F2 fraction of Pb were observed while F3 increased in all substrates after reaction. Moreover, after anaerobic digestion, F4 of Pb was still the dominant fraction in all biogas residues. A surprising find was that F4 of Pb increased after AD in all materials; the increases for PM, CHM, CAM and RS were 3.41%, 4.42%, 0.9% and 2.2%, respectively. The speciation change of CAM was the smallest of the four substrates.

In summary, consistent with our understanding, these results implied that different substrates contained variable speciations of heavy metals, reflecting the nature and source of the materials. The surprising find was that the speciation with the largest proportion of each heavy metal was the same in the four different substrates. Among them, F2 of Zn, Ni, Cd and As was higher than other forms, F3 of Cu was highest, while Pb occurred predominantly in F4. What's more, transformation of the heavy metal speciation for the six metals occurred after AD in all substrates, because the anaerobic digestion process would change substrate properties that can affect metal speciation and metal association with substrates. It was found that the AD process had a greater effect on the speciation and distribution of heavy metals in CHM and PM, but less on CAM and RS.

2.5. The bioavailability of heavy metals

Bioavailability can reflect the toxicity and migration ability of heavy metals in the environment (Xiao et al., 2017). As we know, if the bioavailability of heavy metals in biogas residue is still high after AD, direct composting or disposal will cause harm to the environment, even if the total concentration of heavy metals is low. Table 5 shows the bioavailability of six heavy metals in four commonly used substrates and biogas residue, and their change rates after AD. The variation in the bioavailability of all heavy metals in the four substrates was significant ($P < 0.05$). It can be seen from the table that the bioavailability of Zn, Ni, Cd and As was much higher than that of Cu and Pb on the whole, which meant that Cu and Pb had the lowest mobility and highest stability in the four substrates. The results manifested that the bioavailability of Zn in the four substrates increased after AD, and the change rates of Zn in PM, CHM, CAM and RS were 10.51%, 2.41%, 13.7% and 0.50%, respectively. This indicated that AD increased the mobility, toxicity and potential hazard of Zn. After AD, the bioavailability of Cu in CHM increased by 3.79%, but decreased in PM, CAM and RS, by 8.94%, 0.98% and 0.53%, respectively. At the same time, we found that As showed the same trend as Cu in the four substrates; that is, the bioavailability decreased in PM, CAM and RS, but increased in CHM. This reflected the enhanced bioavailability and potential harm of Cu and As in CHM. The bioavailability of Ni in PM, CHM and CAM was weakened, while it was enhanced slightly in RS. As for Cd, the bioavailability increased in all substrates: the change rates of PM, CHM, CAM and RS were 13.49%, 14.65%, 1.66% and 2.42%, respectively, indicating that Cd had a high potential for migration despite its low content in the substrates (Weng et al., 2014). It was worth noting that Pb had low bioavailability in the four substrates and biogas residues, and its bioavailability weakened in all materials after AD. The result provided evidence of the low direct and long-term availability of Pb in biogas residue to the environment.

Based on the above results, it can be seen that after AD, the bioavailability of the four substrates changed to different degrees, and the order of change rates was PM > CHM > CAM > RS. This was due to the fact that the contents of organic matter, humic acid, microorganism and local metal ion environment in the four materials were quite different after AD; then these factors directly affected the speciation of heavy metals, thereby influencing the bioavailability, stability and toxicity of the heavy metals (Clemente and Bernal, 2006; Ingelmo et al., 2012; Kalbitz and Wennrich, 1998). Hence, changes in metal speciation will need to be carefully evaluated in the treatment

Table 5 – The bioavailability of six heavy metals in four commonly used substrates, biogas residue, and the change rates*.

Heavy metal	Bioavailability (%)	Substrates			
		PM	CHM	CAM	RS
Zn	Initial	59.48 ± 0.36	57.66 ± 1.50	63.03 ± 1.20	75.90 ± 0.90
	Biogas residue	69.99 ± 1.12	60.07 ± 0.89	76.73 ± 0.95	76.40 ± 1.03
	Change rates	10.51	2.41	13.70	0.50
Cu	Initial	12.00 ± 0.31	8.99 ± 0.54	5.38 ± 0.70	31.14 ± 0.13
	Biogas residue	3.86 ± 0.25	12.78 ± 0.89	4.40 ± 1.04	30.61 ± 2.51
	Change rates	−8.94	3.79	−0.98	−0.53
Ni	Initial	85.97 ± 0.06	75.79 ± 1.00	75.54 ± 0.36	75.47 ± 0.68
	Biogas residue	67.01 ± 1.12	58.46 ± 0.34	69.89 ± 0.78	75.90 ± 1.03
	Change rates	−18.96	−17.33	−5.65	0.43
Cd	Initial	48.54 ± 0.71	53.39 ± 1.57	47.06 ± 0.16	68.48 ± 0.56
	Biogas residue	62.03 ± 0.62	68.04 ± 0.71	48.72 ± 0.07	70.90 ± 1.42
	Change rates	13.49	14.65	1.66	2.42
As	Initial	73.53 ± 0.43	52.47 ± 0.69	69.54 ± 1.13	52.14 ± 0.67
	Biogas residue	59.97 ± 0.67	53.98 ± 0.85	67.76 ± 0.63	51.54 ± 0.39
	Change rates	−13.56	1.51	−1.78	−0.60
Pb	Initial	17.89 ± 1.18	14.12 ± 1.22	12.88 ± 0.39	39.81 ± 0.84
	Biogas residue	11.21 ± 0.50	6.28 ± 0.29	11.24 ± 0.38	34.35 ± 0.48
	Change rates	−6.68	−7.84	−1.64	−5.46

* Data are given by mean ± standard deviation based on 3 samples.

and disposal of the substrates or biogas residues, to decrease the bioavailability and potential toxicity of heavy metals.

Moreover, the risk assessment code (RAC) based on the percentage of metals in (F1+F2) was proposed to assess the bioavailability of heavy metals (Zhai et al., 2014). According to the RAC guideline, there is no risk when the proportion of the bioavailable fraction (F1+F2) is lower than 1%, low risk for a range of 1%–10%, medium risk for a range of 11%–30%, high risk from 31% to 50% and very high risk at more than 50%. Therefore, the bioavailability of substrates and biogas residue displayed in Table 5 also reflects the degree of metal risk before and after anaerobic digestion. The results suggest that except for Cu and Pb in PM, CHM and CAM, which posed low or medium risk, all others posed high or very high risk to the environment. This is a new way to assess whether substrates or biogas residues can be directly used as fertilizer in practical applications.

3. Conclusions

The distribution and transformation of heavy metal speciation were studied in the anaerobic digestion of four different substrates. The contents of heavy metals in pig manure (PM), chicken manure (CHM) and cattle manure (CAM) were higher than in rice straw (RS) and decreased in the order of Zn > Cu > Ni > Pb > As > Cd both in the four substrates and the biogas residues. The results in this study demonstrated that the speciation with the largest proportion of each heavy metal was the same in the different substrates. Among them, Zn, Ni, Cd and As were mainly in the reducible fraction (F2), while the oxidizable fraction (F3) of Cu was highest, and Pb occurred predominantly in the residual fraction (F4). Our results also indicated that the speciation and transformation of different heavy metals varied for the different substrates.

The bioavailability of Zn, Ni, Cd and As was much higher than that of Cu and Pb on the whole, which meant that Cu and Pb had lower mobility and higher stability in the four substrates and biogas residues. We also found that the AD process had a greater effect on the speciation of heavy metals in CHM and PM, but less on CAM and RS. The order of change rates in bioavailability was PM > CHM > CAM > RS. Moreover, the results suggested that except for Cu and Pb in PM, CHM and CAM, which were of low or medium risk, all the others posed high or very high risk to the environment. Changes in organic matter, humic acid or local metal ion environments as a result of AD were inferred as likely mechanisms leading to the transformation of heavy metal speciation. Hence, changes in metal speciation will need to be carefully evaluated in the treatment and disposal of the substrates or biogas residues, to decrease the bioavailability and potential toxicity of heavy metals.

Acknowledgments

REFERENCES

APHA, 1995. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association and Environment Federation, Washington DC, USA, pp. 4–91.

Banks, C.J., Chesshire, M., Heaven, S., Arnold, R., 2011. Anaerobic digestion of source-segregated domestic food waste: performance assessment by mass and energy balance. Bioresour. Technol. 102, 612–620.

Cai, Y., Wang, J., Zhao, Y., Zhao, X., Zheng, Z., Wen, B., et al., 2018. A new perspective of using sequential extraction: To predict the deficiency of trace elements during anaerobic digestion. Water Res. 140, 335–343.

Clemente, R., Bernal, M.P., 2006. Fractionation of heavy metals and distribution of organic carbon in two contaminated soils amended with humic acids. Chemosphere 64, 1264–1273.

Doelsch, E., Moussard, G., Macary, H.S., 2008. Fractionation of tropical soilborne heavy metals—Comparison of two sequential extraction procedures. Geoderma 143, 168–179.

Elango, D., Pulikesi, M., Baskaralingam, P., Ramamurthi, V., Sivanesan, S., 2007. Production of biogas from municipal solid waste with domestic sewage. J. Hazard. Mater. 141, 301–304.

Gao, J., Tian, H., Cheng, K., Lu, L., Wang, Y., Wu, Y., et al., 2014. Seasonal and spatial variation of trace elements in multi-size airborne particulate matters of Beijing, China: mass concentration, enrichment characteristics, source apportionment, chemical speciation and bioavailability. Atmos. Environ. 99, 257–265.

Gu, Y., Chen, X., Liu, Z., Zhou, X., Zhang, Y., 2014. Effect of inoculum sources on the anaerobic digestion of rice straw. Bioresour. Technol. 158, 149–155.

He, X., Zhang, Y., Shen, M., Zeng, G., Zhou, M., Li, M., 2016. Effect of vermicomposting on concentration and speciation of heavy metals in sewage sludge with additive materials. Bioresour. Technol. 218, 867–873.

Hsu, J.H., Lo, S.L., 2001. Effect of composting on characterization and leaching of copper, manganese, and zinc from swine manure. Environ. Pollut. 114, 119–127.

Hullebusch, E.D.V., Guibaud, G., Simon, S.P., Lenz, M., Yekta, S.S., Fermoso, F.G., et al., 2016. Methodological approaches for fractionation and speciation to estimate trace element bioavailability in engineered anaerobic digestion ecosystems: An overview. Crit. Rev. Environ. Control 46, 1324–1366.

Ingelmo, F., Molina, M.J., Soriano, M.D., Gallardo, A, Lapena, L., 2012. Influence of organic matter transformations on the bioavailability of heavy metals in a sludge based compost. J. Environ. Manage. 95, S104–S109.

Ji, X., Shen, Q., Liu, F., Ma, J., Xu, G., Wang, Y., et al., 2012. Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; China. J. Hazard. Mater. 235-236, 178–185.

Kalbitz, K., Wennrich, R., 1998. Mobilization of heavy metals and arsenic in polluted wetland soils and its dependence on dissolved organic matter. Sci. Total Environ. 209, 27–39.

Kubova, J., Matus, P., Bujdos, M., Hagarova, I., Medved, J., 2008. Utilization of optimized BCR three-step sequential and dilute HCl single extraction procedures for soil-plant metal transfer predictions in contaminated lands. Talanta 75, 1110–1122.

Li, J., Zhang, M., Ye, Z., Yang, C., 2019. Effect of manganese oxide-modified biochar addition on methane production and heavy metal speciation during the anaerobic digestion of sewage sludge. J. Environ. Sci. 76, 267–277.

Møller, H.B., Sommer, S.G., Ahring, B.K., 2004. Methane productivity of manure, straw and solid fractions of manure. Biomass Bioenergy 26, 485–495.

Mantovi, P., Bonazzi, G., Maestri, E., Marmiroli, N., 2003. Accumulation of copper and zinc from liquid manure in agricultural soils and crop plants. Plant Soil 250, 249–257.

Marcato, C.E, Pinelli, E., Pouech, P., Winterton, P., Guiresse, M., 2008. Particle size and metal distributions in anaerobically digested pig slurry. Bioresour. Technol. 99, 2340–2348.

Meng, J., Wang, L., Zhong, L., Liu, X., Brookes, P.C., Xu, J., et al., 2017. Contrasting effects of composting and pyrolysis on bioavailability and speciation of Cu and Zn in pig manure. Chemosphere 180, 93–99.

Mossop, K.F., Davidson, C.M., 2003. Comparison of original and modified BCR sequential extraction procedures for the fractionation of copper, iron, lead, manganese and zinc in soils and sediments. Anal. Chim. Acta 478, 111–118.

Qiao, L., Ho, G., 1997. The effects of clay amendment and composting on metal speciation in digested sludge. Water Res 31, 951–964.

Schumacher, B.A., 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments. Ecological risk assessment support center. Office of research and development US. Environment. Protect. Agency 1–34.

Shuai, L., Wang, X.D., Li-Lan, L.U., Diao, S.R., Zhang, J.F., 2008. Competitive complexation of copper and zinc by sequentially extracted humic substances from manure compost. Agric. Sci. China 007, 1253–1259.

Siegert, I., Banks, C., 2005. The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. Process Biochem. 40, 3412–3418.

Singh, J., Kalamdhad, A.S., 2012. Concentration and speciation of heavy metals during water hyacinth composting. Bioresour. Technol. 124, 169–179.

Stefaniuk, M., Bartmiński, P., Różyło, K., Dębicki, R., Oleszczuk, P., 2015. Ecotoxicological assessment of residues from different biogas production plants used as fertilizer for soil. J. Hazard. Mater. 298, 195–202.

Wang, X., Yang, G., Feng, Y., Ren, G., Han, X., 2012. Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. Bioresour. Technol. 120, 78–83.

Weng, H.X., Ma, X.W., Fu, F.X., Zhang, J.J., Liu, Z., Tian, L.X., et al., 2014. Transformation of heavy metal speciation during sludge drying: mechanistic insights. J. Hazard. Mater. 265, 96–103.

Xiao, L., Guan, D., Peart, M.R., Chen, Y., Li, Q., Dai, J., 2017. The influence of bioavailable heavy metals and microbial parameters of soil on the metal accumulation in rice grain. Chemosphere 185, 868–878.

Xiao, Z., Yuan, X., Li, H., Jiang, L., Leng, L., Chen, X., et al., 2015. Chemical speciation, mobility and phyto-accessibility of heavy metals in fly ash and slag from combustion of pelletized municipal sewage sludge. Sci. Total Environ. 536, 774–783.

Xie, S., Ma, Y., Strong, P.J., Clarke, W.P., 2015. Fluctuation of dissolved heavy metal concentrations in the leachate from anaerobic digestion of municipal solid waste in commercial scale landfill bioreactors: The effect of pH and associated mechanisms. J. Hazard. Mater. 299, 577–583.

Xu, Y., Yu, W., Ma, Q., Zhou, H., 2013. Accumulation of copper and zinc in soil and plant within ten-year application of different pig manure rates. Plant Soil Environ 59, 492–499.

Yang, K., Zhu, Y., Shan, R., Shao, Y., Tian, C., 2017. Heavy metals in sludge during anaerobic sanitary landfill: Speciation transformation and phytotoxicity. J. Environ. Manage. 189, 58–66.

Ye, S., Zeng, G., Wu, H., Liang, J., Zhang, C., Dai, J., et al., 2019. The effects of activated biochar addition on remediation efficiency of co-composting with contaminated wetland soil. Resour., Conserv. Recycl. 140, 278–285.

Ye, S., Zeng, G., Wu, H., Zhang, C., Dai, J., Liang, J., et al., 2017. Biological technologies for the remediation of co-contaminated soil. Crit. Rev. Biotechnol. 37, 1062–1076.

Yuan, X., Huang, H., Zeng, G., Li, H., Wang, J., Zhou, C., et al., 2011. Total concentrations and chemical speciation of heavy metals in liquefaction residues of sewage sludge. Bioresour. Technol. 102, 4104–4110.

Zeng, X., Xiao, Z., Zhang, G., Wang, A., Li, Z., Liu, Y., et al., 2018. Speciation and bioavailability of heavy metals in pyrolytic biochar of swine and goat manures. J. Anal. Appl. Pyrolysis 132, 82–93.

Zhai, Y., Chen, H., Xu, B., Xiang, B., Chen, Z., Li, C., et al., 2014. Influence of sewage sludge-based activated carbon and temperature on the liquefaction of sewage sludge: yield and composition of bio-oil, immobilization and risk assessment of heavy metals. Bioresour. Technol. 159, 72–79.

Zhang, P., Yang, Y., Tian, Y., Yang, X., Zhang, Y., Zheng, Y., et al., 2009. Bioenergy industries development in China: dilemma and solution. Renew. Sust. Energ. Rev. 13, 2571–2579.

Zhao, G., Ma, F., Wei, L., Chua, H., 2012. Using rice straw fermentation liquor to produce bioflocculants during an anaerobic dry fermentation process. Bioresour. Technol. 113, 83–88.

Zhu, L., Yan, C., Li, Z., 2016. Microalgal cultivation with biogas slurry for biofuel production. Bioresour. Technol. 220, 629–636.