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Distribution of heavy metals in raw and anaerobically digested pig slurry: a full-scale study in Taihu basin, China

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A B S T R A C T

In China, increasing concerns arise on the agricultural use of anaerobically digested pig slurry due to the heavy metal pollution resulting from abuse of additives. In this study, both the content and speciation distribution of heavy metals in raw and anaerobically digested slurries were investigated in a full-scale pig manure anaerobic digestion plant in Taihu Lake Basin in China. The dissolved and total concentrations of nine heavy metals (macroelements Zn, Cu, Mn, and microelements Ni, Pb, Cr, Cd, Co, As) in liquid phase were determined to identify the heavy metal distribution, and chemical fractions extracted by a modified Tessier method in solid phase were conducted to predict the heavy metal bioavailability. The results indicated that both dissolved and total heavy metal concentrations increased during digestion, most concentrated in the solid digestate. The contents of heavy metals in digested slurry and solid digestate all exceeded the standard limits. Anaerobic digestion reduced the bioavailability of most heavy metals studied. However, the high concentrations of dissolved heavy metals and mobility factors of As prevent agriculture utilisation of digested manure slurry. Overall, heavy metal pollution of the digested slurry and digestate is a major concern, which should be carefully evaluated before they are utilized as biofertilizer.

Keywords: Anaerobic digestion; Digested slurry; Heavy metal; Bioavailability; Chemical fraction

1. Introduction

The safe use of livestock manure slurry in agriculture has been receiving a great deal of attention as it has been reported that the manure slurry led to water eutrophication and soil heavy metals pollution [1,2]. In the intensive and large-scale farms in China, the forgoing issue has been effectively tackled. However, this problem remains unsolved in the small-scale and scattered farms, which account for 80% of the total farms in Taihu Basin, China. Centralized anaerobic digestion is the widely used treatment method due to the advantages of energy and space saving, stabilization and pathogen reduction [3,4]. In this treatment method, manure slurries are uniformly collected from scattered farms and transported to a centralized and industry-scale anaerobic digestion (AD) plant for waste treatment and biogas production. The digested slurries are then subjected to farmland application which is the most cost-effective disposal method considering the high concentrations of organic matter, nitrogen, phosphorus and mineral elements in digested manure slurry [5]. However, heavy metals (Cu, Zn, As and so on) are extensively used as feeding additives to prevent livestock diseases, improve feed efficiency and promote animal growth, especially in the small-scale and scattered farms [6,7]. Moreover, the assimilation rates of heavy metals for animals are extremely low with most of the heavy metals excreted via faeces and urine, which results in high levels of heavy metals in manure slurry [8–10]. Consequently, the

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long-term application of manure slurry to farmlands will cause accumulation of heavy metals in the topsoil and plants, which will ultimately lead to the pollution of the food chain and threaten human health [11–13].

Recently, some studies have focused on the effect of anaerobic digestion on both contents and speciation of heavy metals in the treated sludge or livestock manure [13–15]. During the process of anaerobic digestion, organic matter is effectively degraded while the heavy metals remain, resulting in high concentrations of heavy metals in Digested Slurry (DS) and solid digestate. Two sequential extraction methods (i.e., Tessier and BCR) are commonly used to characterize the chemical fractions of heavy metals in sludge and digestate [16,17]. However, due to variations of extraction methods and samples, the results obtained by the above-mentioned studies are not comparable. Moreover, the BCR extraction method has been reported to be relatively unreliable for anaerobic matrixes due to the transfer of forms during extraction process. For the long-term farmland application of DS, both total contents and chemical forms should be considered to evaluate the bioavailability and toxicity of the heavy metals [18,19]. The previous studies mainly focused on the intensive farms or lab-scale experiments; however, most of them only investigated the solid digestate without a comprehensive characterization of heavy metals in the slurry from an industrial scale centralized digestion plant [20,21].

Environmental conditions and intrinsic properties play an important role in the distribution and bioavailability of heavy metals during anaerobic digestion. Degradation rate and particle size could affect the distribution of heavy metals between liquid DS and solid digestate [22]. The heavy metal speciation greatly depends on reactor conditions, such as pH, oxide redox potential (ORP), concentration of hydrogen sulphide(H_2S). These conditions also significantly affect their bioavailability and toxicity [23–25]. Although some heavy metals are necessary for microorganism metabolism, excess concentrations of the available heavy metals are detrimental to the anaerobic process. As such the mobility and bioavailability of heavy metals are critical for assessment of stability of the anaerobic process and the risk by using DS. Moreover, the toxicity of heavy metals depends on not only the distribution of chemical fraction but also on the amount of biofertilizer that will be used in a specific land.

In this study, bioavailability and toxicity of heavy metals were evaluated for both DS and digestate by measuring total contents and chemical fraction of heavy metals. Effects of anaerobic digestion on the heavy metal bioavailability was also evaluated. In the end, discussions were made on the factors that influence the distribution of heavy metal contents and chemical fractions.

2. Materials and methods

2.1. Anaerobic digestion unit and sample collection

The present work was conducted at a full-scale biogas plant where manure slurries from 74 small-scale and scattered pig farms are treated. The faeces and urine were not separated in these farms. Manure slurries were uniformly collected and transported to the regulating tank, and then pumped into the 1,500 m^3 continuously stirred tank reactor (CSTR) for anaerobic digestion. The anaerobic digester ran at 35°C ± 2°C with a hydraulic retention time (HRT) of 17 d. The input included 87 t/d pig manure slurry and 1 t/d straw. The output was 84.5 t/d DS, 3.5 t/d digestate and 1,480 m^3/d biogas. In China, the DS was directly discharged into a sedimentation tank and stored for up to several months before sent to farmlands. This process is in contrast to those used in European countries where the DS is often treated by precipitation, mechanical sieving or centrifugation to separate the solid and liquid components [15].

Before samples were collected, the digester has been operated for several months with regular input. Raw manure slurry (RMS) was collected by taking 5 sub-samples from different positions within the regulating tank and mixing them to form one 50 L sample. For the regulating tank, normal mechanical stirring was necessary to ensure that the sample was adequately representative. DS was collected by taking 5 sub-samples at different times at the outlet of the digester, which were then mixed uniformly to constitute a 50 L sample. All of the samples were stored at 4°C for subsequent analysis.

2.2. Sample preparation and heavy metal analysis

Analyses were performed right after sampling. The total solids (TS) and suspended solid (SS) were measured in dry mass after the samples were dried at 105°C for 24 h. The pH and oxidation-reduction potential (ORP) were determined using a portable multi-parameter digitised analyser (USA Hash HQd series). Volatile solids (VS) were determined by heating the samples at 200°C for 2 h and then at 550°C for 10 h (SRJX-4-13-type high-temperature muffle furnace, China). The total nitrogen (TN) was analysed using the alkaline potassium persulphate digestion-UV spectrophotometric method (HJ 636-2012). The total phosphorus (TP) was measured using ammonium molybdate spectrophotometry (UV 1801). The chemical oxygen demand (COD) was analysed using the potassium dichromate oxidation method. All of the above analyses were in accordance with the Water and Wastewater Monitoring Method in China (4th edition).

Prior to heavy metal analyses, RMS and DS were centrifuged at 10,000 rpm for 20 min to obtain the solid raw manure (RM) and solid fraction of digestate (SFD), respectively. The supernatant after centrifugation was filtered through a 0.45 μm filter membrane, then digested with aqua regia for ICP-MS analysis. The resulting heavy metals are considered as dissolved fraction of heavy metals. The dissolved fraction and total contents of heavy metals in the RMS and DS (liquid sample, expressed as mg/L), and chemical fractions and total contents in RM and SFD (dry sample, expressed as mg/kg, dry mass) were determined in this paper. The samples were digested with a 50/50 hydrochloric/nitric acid solution instead of aqua regia because of the high organic matter content in the samples. The samples were kept overnight at 20°C then heated to 160°C for 2 h until the solution turned transparent. After cooled to the room temperature, the solutions were filtered at 0.45 μm and diluted with deionized (UHQ) water.

To determine the chemical fraction of heavy metals in the RM and SFD, they were dried at 105°C in a forced air oven for 24 h. Dried samples were ground in an agate mortar, homogenized and stored at 4°C for subsequent extraction.

The heavy metal fractions were extracted and measured using a five-step Tessier method (exchangeable, bound to carbonates, bound to iron and manganese oxides, bound to organic matter and residual). In brief, 1 g of sample was mixed with extractant and shaken for 1 h, then centrifuged. The extract was filtrated through a 0.45 μm filter paper and the solid residue was subjected to the next extraction step. For the residual fraction, the extraction step was the same as the total content method. Each aqueous extract sample was digested with aqua regia at 120°C in order to destroy organic materials completely for ICP–OES analysis. The acidity of the test solution was maintained within approximately 1%–2%. The heavy metals (Cu, Zn, Pb, Cd, Cr, Ni, Mn, As and Co) in all the samples were measured by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies 7700 Series). Each test sample and blank sample was performed in triplicate for reproducibility.

2.3. Statistical analysis

A certified reference material (CRM) was analysed to assess the accuracy and precision of the analytical method. The accuracy of the heavy metal fractions extraction was also confirmed by the ratio of the sum of five fractions to the total concentration, which had a recovery rate of 100% ± 10%. Correlation analysis was performed to analyse the correlation between variations of physicochemical properties and percentages of heavy metal fractions before and after anaerobic digestion. All statistical analyses were performed using SPSS Statistics 19.0.

3. Results and discussion

3.1. Physicochemical properties of raw manure slurry and digested slurry

The basic physicochemical characteristics of RMS and DS involved in this study are presented in Table 1. Others' studies indicated that pH and ORP play important role in not only the microbial activity but also the distribution of heavy metal fractions [26,27]. From the results in this study, the pH increased slightly, while the ORP decreased during anaerobic digestion, both of which were in the optimal range. Dry matter content of RMS was low because the excrement and urine were not subjected to solid-liquid separation. During anaerobic digestion, COD, TS and VS were significantly decreased with VS decreasing by 63.9%. In contrast, the soluble COD increased from 1,068 to 2,326 mg/L. TN and TP decreased slightly with anaerobic digestion probably owing to the formation of struvite, which is consistent with that found in DS in other reports [22].

Table 1
Physicochemical properties of raw manure slurry (RMS) and digested slurry (DS) (mean ± standard deviation)

	RMS	DS
pH	7.20 ± 0.28	7.67 ± 0.23
ORP, mV	–170 ± 10	–300 ± 15
TS, g/L	22.11 ± 1.70	12.38 ± 1.05
SS, g/L	20.32 ± 1.04	8.56 ± 0.89
VS, g/L	16.69 ± 0.31	6.02 ± 0.37
TCOD, mg/L	20,645 ± 618	12,717 ± 357
SCOD, mg/L	1,068 ± 115	2,326 ± 150
TN, mg/L	1,062.2 ± 56.01	909.0 ± 48.49
TP, mg/L	142.45 ± 3.89	103.1 ± 10.89

pH, potential hydrogen; ORP, oxidation-reduction potential; TS, total solid; SS, suspended solid; VS, volatile solid; TCOD, total chemical oxygen demand; SCOD, soluble COD; TN, total nitrogen; TP, total phosphorus.

3.2. Heavy metal contents in raw manure slurry and digested slurry

Due to the abuse of additive in the pig feeds, increasing levels of heavy metals were excreted through faeces, resulting in high concentration of heavy metals in the manure slurry. Both dissolved fraction and total heavy metal contents in RMS and DS were shown in Table 2. Theoretically, the total heavy metal contents should be consistent before and after digestion as a result of non-degradation of the heavy metals. However, our test results revealed that both dissolved fraction and total heavy metal contents in DS were slightly higher than those in the RMS. The reason may be that some of the easily degradable organic matter was converted to biogas, which led to a minor decrease of the total amount of slurry but a slight increase of total heavy metal concentrations. During anaerobic digestion, particulate organic matter is decomposed to smaller molecules. However, these small molecules cannot be fully utilized by methanogen, which therefore increases SCOD. Meanwhile, heavy metals bound organic matters are transformed to dissolved fraction, resulting in the rise of dissolved concentration. The total contents of heavy metals were in different levels, with macro-elements Zn, Cu, Mn in 10^1 ppm level, microelements Ni, Pb in 10^0 ppm level, and microelements Cr, Cd, Co, As in 10^{-1} ppm level.

Dissolved fractions of heavy metals are easily absorbed by organisms. They can also cause water pollution through surface runoffs. The release of the heavy metals bound extracellular polymeric substances can give rise to the redistribution between soluble and insoluble fractions. Although, both dissolved fraction and total concentration of heavy metals increased with digestion, the percentage of dissolved fraction with respect to the total concentration which can be expressed as the increase of SCOD/TCOD also increased slightly (Fig. 1). In other words, the direct environmental risk of agricultural use of DS increased. This phenomenon may be related to the production and accumulation of soluble microbial products during digestion. The dissolved fraction of most heavy metals was less than 10%, which agrees with those reported by Zhu [17]. But for As, the percentage of dissolved fraction was higher than 10%, indicating a much higher level of toxicity. Results of dissolved fraction alone are inadequate to fully characterize bioavailability of heavy metals. Additional parameter such as the chemical fraction of heavy metals in the SFD should be needed in conjunction with dissolved fraction.

The previous study on DS from large-scale anaerobic digestion in Jiangsu province revealed that concentrations of Cu, Zn and As contents were 0.21–73.40, 1.02–93.20 and 0.01–2.08 mg/L, respectively [16]. The measurement of Zn, Cu,

Table 2
Concentrations (mg/L) of dissolved fraction and total heavy metals in raw manure slurry (RMS) and digested slurry (DS)

		Cu	Zn	Pb	Cd	Cr	Ni	Mn	As	Co
Dissolved Concentration	RMS	0.28	1.46	0.05	0.02	0.01	0.32	0.30	0.03	0.01
	DS	0.32	2.13	0.10	0.03	0.02	0.44	0.36	0.05	0.02
Total Concentration	RMS	10.42	25.06	2.96	0.50	0.45	4.64	10.90	0.25	0.33
	DS	11.37	27.22	3.06	0.63	0.68	5.07	12.00	0.41	0.35
Standard[a]		1	2	0.2	0.01	0.1			0.05	

[a]Standards for irrigation water quality in China (GB 5084-2005).

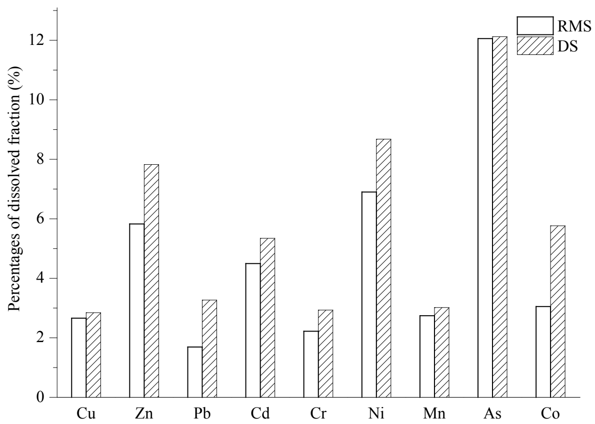

Fig.1. Percentages of dissolved fraction in total concentrations of heavy metals in raw manure slurry (RMS) and digested slurry (DS), respectively.

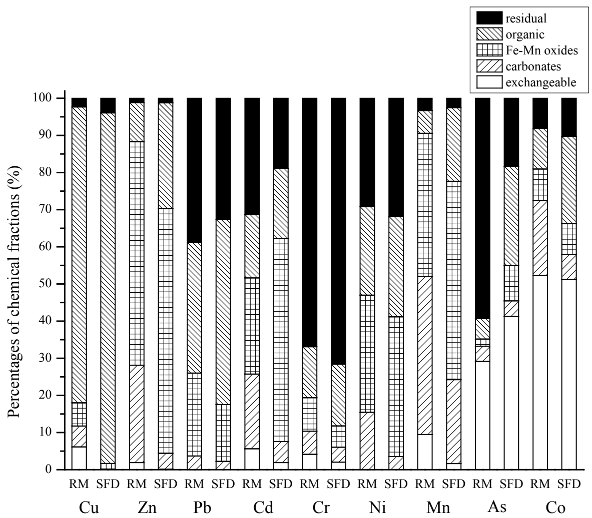

Fig. 2. Distribution and transformation of chemical fractions of heavy metals before and after anaerobic digestion.

and As in this study all fell in the above ranges. In reference to *Standards for irrigation water quality* in China (GB 5084-2005), all the heavy metals in the DS exceeded the standard limits. Even the dissolved fractions of Zn, Cd and As in the DS were higher than the standard values. Therefore, the DS should not be directly applied in the agriculture land without any treatment.

3.3. Distribution of heavy metal forms before and after anaerobic digestion

With the decomposition of organic matters by microorganisms during anaerobic digestion, contents of the heavy metals combined with particulate solids in SFD are varied. Results of chemical fractions and total contents of heavy metals in RM and SFD are showed in (Table 3). The average values of Cu and Zn in the solid digestate were 1354.78 and 2861.80 mg/kg dry mass (DM), respectively. These values were apparently higher than those measured in the raw manure. This result indicated that the heavy metals in solid digestate were relatively concentrated, which is consistent with other studies [17,20]. Most studies of heavy metals in solid digestate in the literature focused on Cu and Zn. The Zn and Cu contents in solid digestate from anaerobic digestion of pig manure were as high as 2,060 and 780 mg/kg DM, respectively [28]. Marcato [20] reported that the Zn and Cu contents in solid digestate were measured to be 2,628 and 1,016 mg/kg DM, respectively. The forgoing values are lower than those measured in this study. There are no relevant standards for solid digestate utilisation for agricultural purposes. When compared with relevant standards, most of the heavy metal contents were excessive.

The toxicity of heavy metals is not only determined by the total amount of the heavy metals but to a large extent by the distributions of the heavy metal fractions [29]. Heavy metal speciation is influenced by many factors, such as pH, ORP, humus, H_2S and particle size during anaerobic digestion. Organic matters in the RMS typically undergoes humification and gasification. This process produces humus with different functional groups that can chelate with heavy metals. The H_2S in the system can react with the heavy metals to generate sulphide [13]. It has been reported that as the small and biodegradable particles are decomposed during anaerobic digestion, larger particles will be left. However, according to the previous studies, most of the heavy metals were distributed on the particles of 3–25 μm in size [15,25]. Since small particles tend to have large specific surface area, the particle size is a major factor affecting the distribution of heavy metals between liquid and solid phases.

Sequential extraction was conducted to identify the main fraction and assess the bioavailability of different heavy metals. The recovery rates were in the range of 94%–105%. The test results showed that heavy metals had different main fractions and their transformation varied substantially before

Table 3
Chemical fraction and total concentration (mg/kg dry matter) of heavy metals in raw manure (RM) and solid fraction of digestate (SFD)

		Cu	Zn	Pb	Cd	Cr	Ni	Mn	As	Co
RM	Exchangeable	54.19	43.19	0.03	1.90	3.05	0.00	80.62	6.94	54.19
	Carbonates	49.78	596.69	10.09	6.82	4.61	6.87	363.28	0.98	49.78
	Fe-Mn oxides	55.04	1,369.22	61.31	8.78	6.64	14.03	328.40	0.46	55.04
	Organic	703.66	239.38	96.67	5.77	10.14	10.59	52.17	1.32	703.66
	Residual	20.72	26.52	106.46	10.61	49.44	13.00	28.34	14.13	20.72
	Sum[a]	883.4	2,275.0	274.6	33.9	73.9	44.5	852.8	23.8	8.3
	Total concentration	857.67	2,420.21	289.01	34.50	76.17	46.4	820.02	23.60	8.69
	Recovery (%)[b]	103	94	95	98	97	96	104	101	95
SFD	Exchangeable	2.56	4.23	0.00	0.76	1.78	0.00	24.41	17.21	4.56
	Carbonates	0.05	119.33	7.62	2.28	3.55	2.53	337.31	1.75	0.60
	Fe-Mn oxides	20.26	1,844.23	52.58	22.09	5.07	26.95	797.81	3.98	0.75
	Organic	1,291.39	795.90	171.46	7.62	14.63	19.36	296.07	11.14	2.09
	Residual	54.07	34.48	112.02	7.62	63.18	22.78	37.56	7.66	0.91
	Sum	1,368.3	2,798.2	343.7	40.4	88.2	71.6	1,493.2	41.7	8.9
	Total concentration	1,354.78	2,861.80	355.54	40.37	93.27	68.22	1,531.97	40.52	9.09
	Recovery (%)	101	98	97	100	95	105	97	103	98

[a]Sum of five fractions (exchangeable, carbonates, Fe-Mn oxides, organic, residual).
[b]Recovery rate of heavy metals (sum/total concentration).

and after digestion. Cu that is mainly bound to organic fraction in the raw manure and solid digestate increased from 79.65% to 94.4%. Some studies have also pointed out that Cu showed high affinity for organic matter and sulphides in anaerobically digested sludge [17,30]. Although the organic fraction is considered relatively stable, it can release into the soil in the long run.

Zn that is mainly present as Fe-Mn oxide increased from 60.19% to 65.91% after digestion, which is consistent with other studies [17,30]. The Fe-Mn oxide fraction is a reducible fraction with high potential toxicity that can be easily reduced to exchangeable fraction. The percentage of organic fraction also increased from 10.52% to 28.44%. On the contrary, the carbonate fraction decreased from 26.23% to 4.26%. The transformation of chemical fraction of Zn before and after digestion proved that the bioavailability and toxicity of Zn decreased.

The highest concentrations of Pb and Cr in raw manure and digestate were found in organic and residual fraction. The residual fraction of Cr increased from 66.91% to 71.62%, however, that for Pb decreased from 38.77% to 32.59%. The main fractions of Cd and Ni, were Fe-Mn oxide, organic and residual fraction. Although the sum of the three fractions was more than 90% of the total contents in digestate, the Fe-Mn oxide fraction accounted for nearly 50% of the total contents.

Mn mainly existed in carbonate form while Fe-Mn in oxide form. During digestion, the former was measured to decrease from 42.60% to 22.59% while the latter increased from 38.51% to 53.43%. These two fractions reached 76.02%, which matches the result of Zhu [17]. In contrast, the highest fractions of As and Co in the digestate were both exchangeable, accounting for 41.24% and 51.18%, respectively (Fig. 2). The exchangeable fraction is considered as the most available fraction, which can be easily transferred to the soluble fraction when conditions are changed.

3.4. Bioavailability of heavy metals before and after anaerobic digestion

The bioavailability of heavy metals has been widely used for risk assessment in soil and soil-like materials. The mobility of heavy metals is always used to represent their bioavailability and biotoxicity. The mobility factor (MF) is calculated using the chemical fractions of heavy metals in the following equation [17]:

$$\mathrm{MF} = \frac{F1 + F2}{F1 + F2 + F3 + F4 + F5} \times 100 \tag{1}$$

where *F1, F2, F3, F4,* and *F5* are the exchangeable fraction, the fraction bound to carbonates, the fraction bound to Fe–Mn oxides, the fraction bound to organic matter and the residual fraction, respectively.

The calculated MFs for the heavy metals in the present study are shown in Fig. 3. From the figure, the mobility of Mn, As and Co was higher than that of the other metals. In particular, MF for As and Co reached 45.44 and 57.91, respectively. It can also be observed that except for As, the bioavailability and mobility of the other heavy metals all decreased, especially for Zn and Mn. This may be ascribed to the special characteristic and condition. Overall, the results showed that the bioavailability of heavy metals should be assessed before the agricultural application of the digestate.

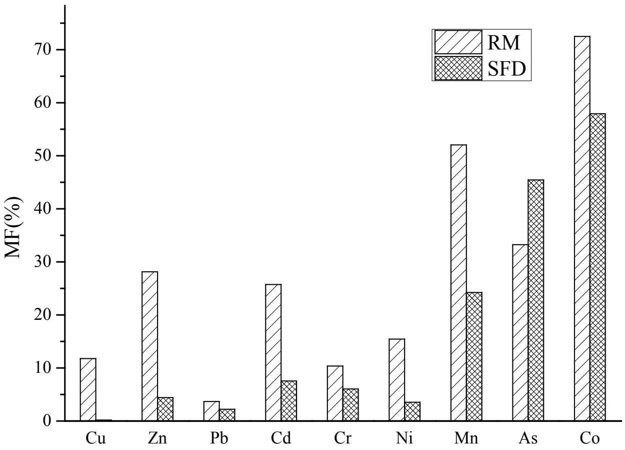

Fig. 3. Mobility factors (MF) of heavy metals in raw manure (RM) and solid fraction of digestate (SFD).

4. Conclusions

During anaerobic digestion, the heavy metal contents were relatively concentrated, mostly bound with solid digestate. However, the agricultural application of DS without solid-liquid separation can cause serious environmental risk. The heavy metal contents in DS and solid digestate all exceeded the standard limits.

Except for As, the dissolved fraction of the heavy metals were less than 10%, but all increased after digestion. The distribution of heavy metal forms varied considerably before and after digestion featuring reduced bioavailability by the digestion. The high mobility of As and Co restricted the long-term application of DS if no treatment is imposed. In this case, further measures should be taken to alleviate the environmental impact.

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