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Distribution of Heavy Metal Contents and Chemical Fractions in Anaerobically Digested Manure Slurry

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Abstract Digested slurry samples from twenty-one large-scale anaerobic digestion plants together with intensive pig and dairy farms in Jiangsu Province of China were collected and analyzed for total and dissolved concentrations of Zn, Cu and As, as well as chemical characteristics. The results showed that total concentrations of Zn, Cu and As in digested pig slurries were concentrated to <10, <5 and 0.02–0.1 mg/l, respectively; while <2 and 10–30, <1, and 0.02–0.1 mg/l, respectively, in digested dairy slurries. Lowering the dietary supply of these elements to pig and dairy would be the most effective way to control heavy metal contents in digested manure slurries. Dissolved fractions of Zn, Cu and As accounted for 1–74%, 1–33% and 2–53% of the total concentrations, respectively, in digested pig slurries; and 18–65%, 12–58% and 3–68% in digested dairy slurries. The chemical fractions of heavy metals in digested slurries were not only dependent on the total concentrations of heavy metals in raw manures but also on conditions of digestion and storage. Oxidation pond systems could significantly cripple the total contents of heavy metals in digested slurries, and the removal effect was better in multi-oxidation-pond systems than that in primary-oxidation-pond systems. However, the chemical fractions of heavy metals in digested slurries changed in a complicated manner when stored in oxidation ponds, due to the suspended solid deposition, elements reduction, as well as variations of pH values and oxidation-reduction potential.

Keywords Digested slurry · Large-scale anaerobic digestion plant · Total concentration of heavy metal · Chemical fraction · Oxidation pond system

Introduction

With the development of raising animals, the annual livestock and poultry manure slurry production in China has reached approximately 3 billion tons [1, 2], the residues of which

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became one of the most important pollutants causing water eutrophication [3]. One of the promising solutions to this environmental issue could be anaerobic digestion (AD) of organic wastes [4–7], which has been largely applied in China [8]. By the end of 2007, there had been 3,800 large-scale AD plants (digestion reactor volume >500 m^3) in China [2].

As the main by-product of biogas, more than 1 billion tons of liquid digestate (i.e., digested slurry) have been produced by AD plants. Applying digestate to land is the most attractive and effective option in terms of environmental issues, because it allows nutrients (such as nitrogen and phosphorus) to be recycled and, hence, increases the organic matter in soils [9, 10]. The levels of Zn, Cu and As in pig and dairy manure slurries are elevated due to the use of animal feed supplements for growth-stimulating and antimicrobial effects [11, 12]. Take intensive pig farms in southern China as an example, concentrations of these three metals in manures were 113.6–1,505.6, 35.7–1,726.3 and 4–78 mg/kg, respectively, which were much higher than those in other areas of China [13, 14] and other countries [15, 16]. Furthermore, these metals contents were larger in raw pig manures than those in raw dairy manures, due to their higher dietary supply and lower use efficiency to pigs [13, 15]. The AD plant is conservative for heavy metals, which results in high total concentrations of Zn, Cu and As in digested slurries.

Moreover, AD treatment effectively degrades the organic matter and causes obvious variations in physical and chemical properties, such as water content, pH, oxidation-reduction potential (ORP) and microbial activities. These changes may influence the chemical fraction of heavy metals [10], which is a critical factor in predicting their mobility and eco-toxicity (especially for arsenic) [17, 18]. The heavy metal speciation and phytotoxic effects of sewage sludge and composted pig manure have been widely studied [19, 20]. Only one study has reported that Zn and Cu were mainly present in 3- to 25-μm particles of digested slurries and that their distributions shifted with the degradation of organic matter or small degradable particles, and formation of large microbial filament during the AD treatment [10]. There has been no study about the transfer and distribution of As in digested slurries. Zinc and Cu were found to be associated with the organic matter in raw manures [13, 21], while As was mainly in the form of organic arsenicals, which was excreted unchanged in the feed addition (e.g., roxarsone and *p*-arsanilic acid), and remained stable in fresh dried manures. During AD treatment, heavy metals may be highly leachable and soluble because of their inorganic forms [12]; they can even shift from stable fractions to more bioavailability/toxic fractions after water addition and decomposition [22], especially for variable valence metalloid As [17].

Zinc and Cu at lower concentrations are essential micronutrients for plants, but As is a non-essential but toxic element. Higher doses of these metals may cause metabolic disorders and growth inhibitions for most of the plant species, and often lead to death [23, 24]. In addition to environmental concerns, the release of these metals into soils, water and plants through the use of livestock slurries as fertilizer/amendments also poses public health risks through the food chain [25, 26]. Thus, when we apply digested slurries as a soil amendment, heavy metal pollution should bring enough attention. Demonstrating the level and chemical fraction of heavy metals in digested slurries is adequate in understanding the true burden or extent of soil contamination and resulting health risks after its application.

In recent years, rapid development of farm-based AD systems has received strong government support and subsidies from local governments in China. Jiangsu Province is a typical area, which has relatively developed economy system and large numbers of intensive livestock farms, located along the east coast of China. By the end of 2009, there had been twenty-one large-scale farm-based AD plants in Jiangsu Province, including sixteen in intensive pig farms and five in intensive dairy farms. The existing and newly built AD systems tend to operate on intensive pig and dairy farms because the operation of this model (i.e., an AD plant with manure) is fairly well known and the manure handling systems are already in place [27]. However, there was no study dealing with the distribution of heavy metal contents and chemical fractions in digested slurries from farm-based AD plants at local scale.

In this study, we chose Jiangsu Province as a research area, which borders Shanghai to the south. Digested manure slurry samples from these twenty-one large-scale AD plants along with intensive pig and dairy farms were collected, to analyze total and dissolved concentrations of heavy metals (Zn, Cu and As) in samples. The main aim of this study was to demonstrate the levels and chemical fractions of Zn, Cu and As in digested pig and dairy slurries at local scale, and to find an explanation for this distribution.

Methods

Sampling Collection

This study on heavy metals of digested manure slurries was a part of an extensive investigation, involving biogas production, pathogenic microorganisms, nutrients of digested slurries and their land amendment in farm-based AD plants from sixteen cities in Jiangsu Province, southern China. Twenty-one large-scale AD plants (digestion reactor volume >500 m^3) treating livestock manure slurries together with intensive farms were sampled six times from September 2009 to March 2010; these farms consisted of sixteen pig farms and five dairy farms. Eleven pig farms and three dairy farms were located in the southern part of Jiangsu Province, and the rest was in the middle and northern parts (Fig. 1).

There were eight AD plants running for more than 2 years and eight for less than 1 year. Four biogas plants used the continuous stirred-tank reactor (CSTR) and seven used the upflow solid reactor (USR). The anaerobic digesters were run at ambient temperature (10–35 °C), with a hydraulic retention time of approximately 15–20 days. Feeding was processed at the rate ranging from 20 to 40 m^3/day depending on outputs of farms. The biogas was collected for power generation, which was used for lighting and heating in farms. The installed power-generating capacity was between 80 and 200 kW.

AD plants in China are designed to make the digested slurries directly overflow into reception tanks and oxidation ponds without any treatment units. The solid digestate deposited to the bottom of digesters was removed one to two times per year and always used for composting, unlike the process used in Europe, where liquid fraction and solid fraction of digested slurries are frequently separated by sedimentation, mechanical screen techniques or centrifugation before using the spreading injectors, and the liquid fraction was stored in tanks for up to several months before land application [10]. In our study, four types of materials were sampled: raw manure slurry (RMS) from the regulating reservoir, digested slurries from anaerobic digester (DS_{AD}; stored in the reception tank) and oxidation pond (DS_{OP}), solid fraction of digestate (SFD) in the bottom of the digester (Fig. 2).

The sampling method was designed with the following assumptions: (1) The feedstock contained all kind manure slurries from different development stages of pigs and dairies. (2) For CSTR and USR, it is generally accepted that a period of 3–5 hydraulic retention time is necessary to reach a steady state [28]. In our study, the digesters had already been working for several months with regular RMS input. Only one in dairy farm was operating for 2 weeks. (3) DS_{AD} concentration is identical at all points in the reactor and is independent

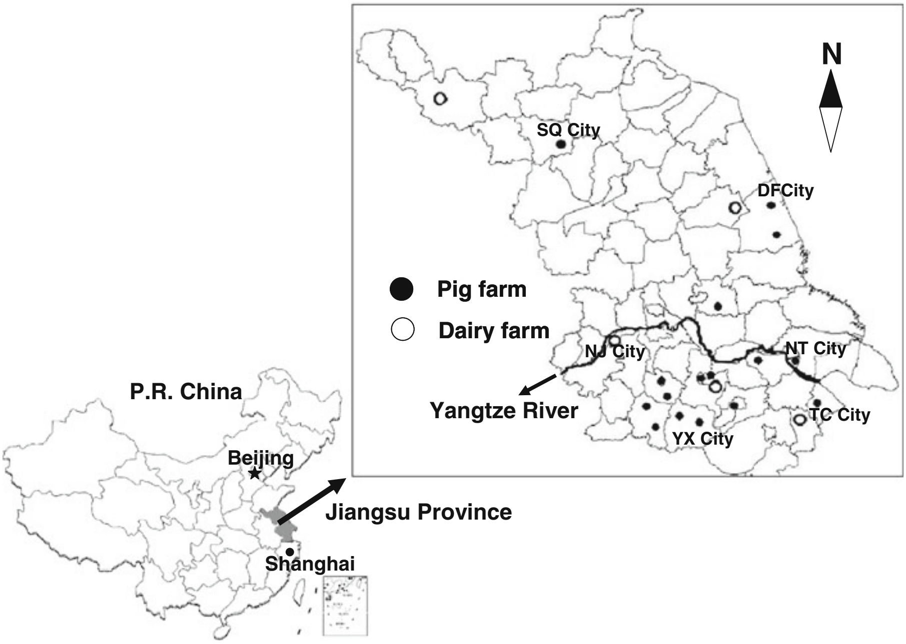

Fig. 1 Schematic illustration of the twenty-one investigated anaerobic digestion (AD) plants' layout in Jiangsu Province, China. There were sixteen AD plants together with pig farms and five with dairy farms. Eleven pig farms and three dairy farms were located in the southern part of Jiangsu Province (mainly based on the Yangtze River); the rest were in the middle and northern parts

of time at any point. (4) DS_{OP} was collected in well-designed and operating AD plants. In this study, we chose two multi-oxidation-pond systems in pig farms from southern part of Jiangsu Province and four primary-oxidation-pond systems, consisting of three in pig farms from the northern part and one in dairy farms from the southern part. All samples were collected at ~1 m from the edge of oxidation ponds and ~0.5 m depth from the surface. For each type of sample, two replicates were taken.

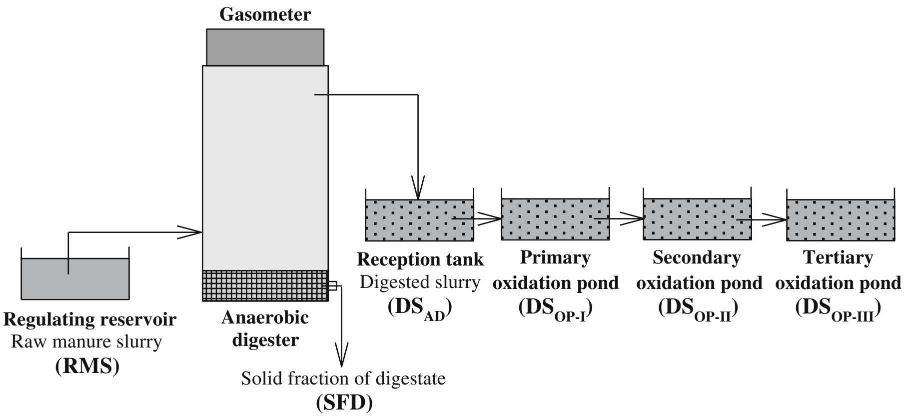

Fig. 2 Flow sheet of the AD plant with oxidation pond system and identification of samples

Analysis and Statistics

Analyses were performed right after sampling. Total solid (TS) was the weight of dry matter of each sample after drying at 105 °C for 24 h. Volatile solids (VS) were determined by heating to 200 °C for 2 h and then at 550 °C for 10 h. Variables in DS and its filtrate (filtered through 0.45-μm millipore filter paper) were represented as total amount and dissolved fraction, respectively. Hydrogen potential (pH) and ORP were determined with the combination glass electrode of PHS-2F (Shanghai Precision & Scientific Instrument Ltd., China) and ORP sensor (GF+Signet 2720, USA), respectively. Chemical oxygen demand (COD) and soluble COD (SCOD) were analyzed using potassium dichromate oxidation method. Biological oxygen demand (BOD_5) was analyzed using standard dilution method. Total organic carbon (TOC) was analyzed by TOC analyzer (Elementar liqui TOC II, Germany). Total nitrogen (TN) was analyzed by alkaline potassium persulfate digestion-UV spectro photometric method (Lambda25/35/45, Perkin Elmer, USA). The ammonium (NH_4-N) and nitrate nitrogen (NO_3-N) were analyzed by the continuous flow analyzer (FIAstar™5000 Systems, FOSS, USA). Total (TP) and dissolved phosphorus (DP) were measured by VIS Spectrophotometer (Spectrumlab 725S, Lingguang Ltd., China). Total potassium (TK) was measured by flame photometer (FP640, Xingyi Ltd., China).

The total and dissolved concentrations of Zn, Cu and As were measured using flame atomic absorption spectrophotometer (PE A300, USA) and atomic fluorescence spectrophotometer (AFS-210, China), respectively. The liquid samples (RMS and DS) and solid samples (SFD) were digested using guaranteed reagent HNO_3 and $HClO_4$.

Student's *t*-test was used for assessing the variations of heavy metal concentrations between two kinds of feedstock (e.g., pig manures and dairy manures), and sampling sites (e.g., the southern part and northern part of Jiangsu Province). Linear regression analyses were used to determine the relationships of total and dissolved concentrations of Cu, Zn and As in RMS and DS. Stepwise regression was used to find out the critical factors impacting the contents and chemical fraction distributions of Zn, Cu and As in DS_{AD} and DS_{OP}. These statistical analyses were performed using procedures of SPSS (v.13.0).

Results and Discussion

Macronutrients in Digested and Raw Slurries

With the decomposition of organic matter in feedstock, COD was significantly ($p<0.05$) reduced during the AD treatment. The pH and ORP in DS were slightly declined (Table 1). Dry matter content (i.e., TS in Table 1) in the RMS was low in comparison with data in the literature [16]. Because farms in this investigation usually used dry manures to compost, causing the RMS to be quite dilute. Although TS was low, N and K contents in DS_{AD} were much higher than those mentioned in other literatures in China [29]. However, N and P contents in DS_{AD} were lower than the data presented in the study of Marcato et al. [10], which had similar content of dry matter in the RMS. In general, DS from large-scale AD plants was spread 50–60 m^3/ha per year to vegetable fields for additive fertilizer in China [30], leading to annual macronutrient inputs (such as N, P and K) lower than the requirements of most vegetables. Thus, the current application rate of DS could meet the general needs of the plants for nutrition without over-fertilizing N, P and K or causing any unwanted accumulation in arable lands.

Table 1 Characteristics of untreated slurry (RMS) and digested slurry (DS) from twenty-one large-scale anaerobic digestion plants together with intensive pig and dairy farms in Jiangsu Province, China

	Unit	RMS		DS	
		Pig farm (*n*=16)	Dairy farm (*n*=5)	Pig farm (*n*=16)	Dairy farm (*n*=5)
pH		7.34 to 8.30[a]	7.45 to 8.23	6.88 to 8.22	7.34 to 8.29
		7.91±0.06[b]	7.85±0.13	7.49±0.05	7.66±0.12
ORP	mV	−260 to −79	−266 to −87	−318 to −41	−302 to −20
		−168±14	−156±32	−140±14	−136±38
TS	g/l	0.20 to 49.61	3.98 to 33.90	0.14 to 42.70	0.26 to 34.64
		15.45±3.78	17.93±6.88	10.09±3.91	10.35±8.17
VS		0.07 to 33.52	1.68 to 24.24	–	–
		10.48±2.65	11.70±5.59	–	–
COD	mg/l	3,472 to 57,600	16,000 to 90,400	1,138 to 68,800	1,920 to 110,400
		20,743±3,799	52,932±20,393	13,034±4,809	34,864±19,872
SCOD		–	–	480 to 11,360	655 to 4,960
		–	–	2,928±855	2,739±888
BOD_5		–	–	218 to 7,090	445 to 4,885
		–	–	839±573	1,684±812
TOC		–	–	318.9 to 2,132.3	586.1 to 2,212.3
		–	–	901.2±91.7	1,292.5±183.1
TN		–	–	209.8 to 1,732.3	255.5 to 1,354.9
		–	–	815.2±80.7	674.4±129.5
NH_4–N		–	–	203.8 to 1,322.9	226.0 to 920.0
		–	–	704.6±64.8	554.2±94.3
NO_3-N		–	–	1.5 to 34.0	1.8 to 36.0
		–	–	8.7±1.4	14.4±3.9
TP		–	–	22.7 to 404.7	12.5 to 415.1
		–	–	112.0±18.6	187.4±55.9
DP		–	–	4.8 to 77.0	10.4 to 149.8
		–	–	26.1±3.3	59.6±18.8
TK		–	–	101.1 to 1,434.9	103.8 to 2,459.0
		–	–	547.3±70.2	880.4±262.6

pH hydrogen potential, *ORP* oxidation-reduction potential, *TS* total solid, *VS* volatile solid, *COD* chemical oxygen demand, *SCOD* soluble COD, *BOD_5* 5-day biological oxygen demand, *TOC* total organic carbon, *TN* total nitrogen, *NH_4-N* ammonium nitrogen, *NO_3-N* nitrate nitrogen, *TP* total phosphorus, *DP* dissolved phosphorus, *TK* total potassium

[a] Range

[b] Mean ± standard error of mean

Total Concentrations of Heavy Metals in Digested Slurries

Heavy metal contents in liquid and solid samples varied, to a large extent, among twenty-one farm-based AD plants (Table 2). Zn, Cu and As in digested pig and dairy slurries were primarily associated with those in RMS. The linear regression equations were $Zn_{DSAD}=2.032\times Zn_{RMS}-1.775$ (p=0.101), $Cu_{DSAD}=4.348\times Cu_{RMS}-6.371$ (p=0.049), and As_{DSAD}

Table 2 Total concentrations of Zn, Cu and As in untreated slurry (RMS), digested slurry (DS) and solid fraction of digestate (SFD) from twenty-one large-scale anaerobic digestion plants together with intensive pig and dairy farms in Jiangsu Province, China

	RMS (mg/l)		DS (mg/l)		SFD (mg/kg)	
	Pig farm[a]	Dairy farm[b]	Pig farm	Dairy farm	Pig farm	Dairy farm
Zn						
Range	3.20–17.00	11.10–14.40	1.02–93.20	0.26–46.60	399.70–671.20	14.10–13.20
Mean ± SE	12.75±1.65	6.96±2.60	20.66±6.99	17.45±8.49	477.08±40.45	57.13±29.34
Significance	ns		ns		***	
Cu						
Range	1.12–17.80	3.65–6.61	0.21–73.40	0.06–12.80	113.60–312.90	10.50–25.30
Mean ± SE	7.54±2.96	5.13±1.48	16.34±4.10	3.29±2.43	204.02±30.04	16.63±4.46
Significance	ns		*		**	
As						
Range	0.004–0.53	0.04–0.06	0.01–2.08	0.02–0.17	0.17–5.94	0.11–0.37
Mean ± S.E.	0.13±0.10	0.05±0.008	0.26±0.14	0.06±0.03	2.19±0.88	0.22±0.08
Significance	ns		ns		ns	

Student's *t*-test was used for assessing the variations of total concentrations of Zn, Cu and As between two kinds of feedstock (i.e., pig slurries and dairy slurries); significance level: *ns* not significant; *$p<0.05$, **$p<0.01$, ***$p<0.001$

[a] $n=16$

[b] $n=5$

$=1.895\times As_{RMS}+0.009$ ($p<0.001$), respectively. This implied that the raw manure quality was the most important factor to produce DS with low heavy metal concentrations. Total concentrations of Zn, Cu and As in DS were much lower than those in SFD (Table 2). It was because that most of the heavy metals associated with the suspended solids would deposit in the bottom of the digester during a relatively long period [10], resulting in the larger accumulation amount of heavy metals in solid matter [31]. Thus, no significantly positive linear relationship was found between heavy metals in RMS and long-accumulated SFD.

In most investigated AD plants in pig farms, total concentrations of Zn, Cu and As in DS_{AD} were concentrated in <10, <5 and 0.02–0.1 mg/l, respectively; and <2 and 10–30, <1, and 0.02–0.1 mg/l, respectively, in DS_{AD} from dairy farms (Fig. 3). Total concentrations of Zn, Cu and As in digested pig slurries were higher than those in digested dairy slurries (Table 2), which contributed to the higher dietary supply of those elements to pigs [13]. In addition, less amount of Cu was excreted by dairies [32]. Thus, the total concentrations of Zn, Cu and As in raw dairy manure slurries were lower than those in raw pig manure slurries (Table 2), which corresponded with other studies [13, 14]. Given that the raw manure quality is the critical factor to produce DS with low heavy metal content, it is easy to understand why the metal concentrations, especially Cu ($p<0.05$; Table 2), were lower in digested dairy slurries.

In general, manures were applied at the rate of 250 kg total N/ha per year in accordance with the maximum recommended application rate in the Code of Good Agricultural Practice for the Protection of Water [33]. Based on total N concentration in DS_{AD} (Table 1),

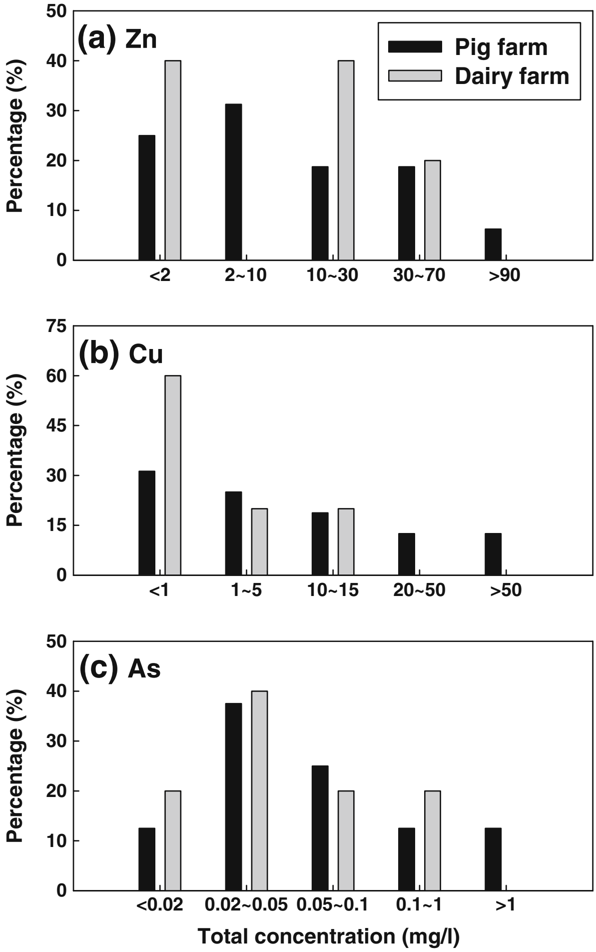

Fig. 3 Distribution of total concentrations of heavy metals in digested slurries (DS) from 21 investigated AD plants together with intensive pig and dairy farms in Jiangsu Province, China. *Y* axis represents the percentage of DS samples at different concentrations in total investigated samples

digested slurry application at the rate of 250 kg total N/ha per year was estimated to raise the metals by 5.88 kg/ha Zn, 4.97 kg/ha Cu and 0.079 kg/ha As (in digested pig slurries), respectively, and 6.81 kg/ha Zn, 0.76 kg/ha Cu and 0.025 kg/ha As (in digested dairy slurries), respectively, on average. Compared with heavy metal loadings from undigested animal manures [12, 15], the land application of digested slurries should raise enough concerns on the heavy metal pollution, despite its fertilizer value.

Regional Variations of Total Concentrations of Heavy Metals in Digested Slurries

Among twenty-one investigated AD plants, eleven pig farms and three dairy farms were located in the southern part of Jiangsu Province (Fig. 1). There were mainly two reasons for the higher number of AD plants in the southern areas. Firstly, there was a large number of

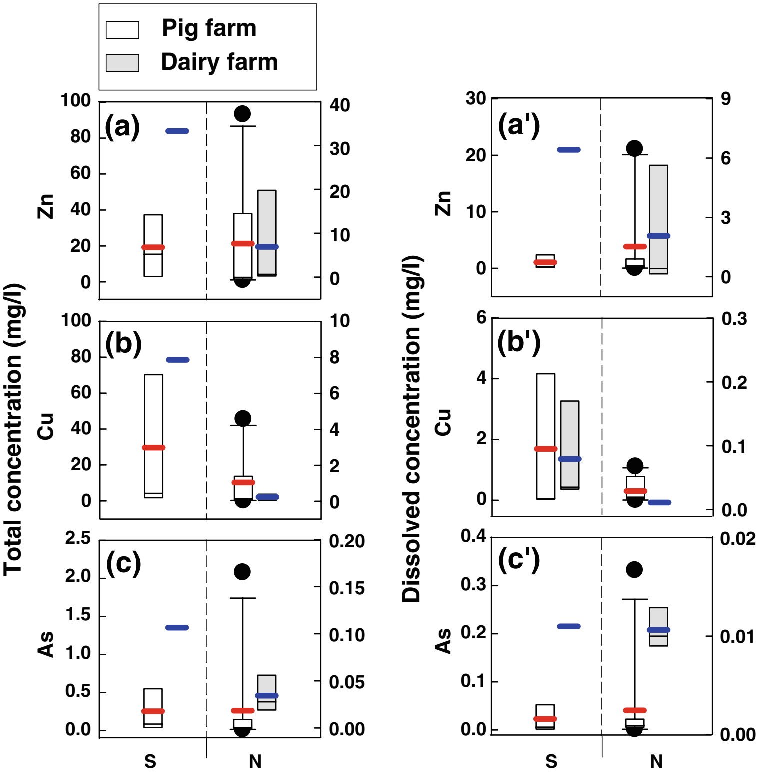

Fig. 4 Total and dissolved concentrations of Zn (**a**, **a′**), Cu (**b**, **b′**) and As (**c**, **c′**) in digested manure slurries from anaerobic digestion plants in the southern part (S) and northern part (N; including the middle part) of Jiangsu Province. Eleven pig farms and three dairy farms were located in the southern part; the rest were in the middle and northern parts. Dissolved fraction was the liquid part of digested slurries filtered through 0.45-μm millipore filter paper. The legend applies for all panels. The *upper* and *lower edges* of the box indicate the 75th and the 25th percentiles of the data set, respectively. The ***red*** line and *black* line indicate the mean and medium values, respectively. The *vertical line* indicates the minimum and maximum data values. The *black dots outside the ends of the vertical lines* represent the outlier or suspected outlier. The *left y*-axis represents digested pig slurries, and the *right y*-axis denotes digested dairy slurries. Note that the graphic scales for the two types of digested slurries are different

intensive farms in the southern part, contained in the most active and developed Yangtze River Delta region. Development of clean energy plant has drawn much more attention from scientists, the public as well as the central government in this region, to control and prevent non-point source pollution [34]. Secondly, the southern part consists of the most developed economic zones of Jiangsu Province. Rapid development of farm-based AD plant systems received strong government support and subsidies from local government [2].

In digested pig slurries, the effects of sampling site on total concentrations of Zn and As were marginal to absent (Fig. 4a, c); while total concentrations of Cu was significantly (p=0.049) higher in the southern part of Jiangsu Province (Fig. 4b). In digested dairy slurries, total concentrations of Zn (p=0.013), Cu (p=0.031) and As (p=0.003) from the southern part were significantly higher than those from the northern part (Fig. 4a-c). Zinc, Cu and As contents in feedstock from the southern part were 12.1%, 13.7% and 3.2% (raw pig manure), and 10.7%, 20.3% and 2.1% (raw dairy manure) higher than those from the middle and northern parts, respectively, according to our investigation data. One reason was the variations in veterinary supplement and drug usage in different regions [13–15]. Another reason was the regional differences of human activities. In recent decades, with the rapid development of industry, agriculture, traffic and transportation, heavy metal contamination becomes a serious issue in some areas, like Tai Lake region in southern Jiangsu Province [35]. Many studies found that heavy metal contents in soils and plants were higher in rapidly developing regions of southern Jiangsu Province [26, 36]. Wan et al. [36] showed that the concentrations of Zn, Cu and As in soils from this region were higher than their background values set by State Environmental Protection Administration of China (1995). The heavy metal polluted soils, water, air, and plants would in turn affect the heavy metal level in animals and manures through food chain.

Chemical Fractions of Heavy Metal in Digested Slurries

Dissolved fractions (through 0.45-μm millipore filter paper) of Zn, Cu and As accounted for 1–74%, 1–33% and 2–53% of the total concentrations, respectively, in digested pig slurries, and 18–65%, 12–58% and 3–68%, respectively, in digested dairy slurries. The proportion of dissolved fractions to total concentrations was higher than that reported by Marcato et al. [10]. Dissolved fractions of heavy metals did not necessarily increase according to the increase of total concentrations in digested pig slurries (F=6.227, p=0.026 for Cu; F=188.87, p<0.001 for As) and digested dairy slurries (F=41.825, p=0.008 for Zn; F=42.788, p=0.007 for Cu). This indicated that the chemical forms of heavy metals in DS_{AD} were not only dependent on heavy metal contents in RMS but also on the conditions of operation and storage. Perhaps for this reason, the trends of dissolved concentrations of heavy metals in DS_{AD} were not evident with sampling site (Fig. 4a′–c′), compared with total concentrations (Fig. 4a–c).

During the AD treatment, different speciations of heavy metals would transform between liquid and solid phases [37]. In our study, ORP, pH and macroelements (e.g., N, P and C) were the major contributors to the chemical fractions of heavy metals in DS (Table 3). In raw manure, Zn and Cu were mostly present in the organically bound fractions [13, 21], and As in organic-arsenicals form like roxarsone and *p*-arsanilic acid in fresh dried manure [12]. During AD treatment, an aqueous phase in contact with a solid phase in RMS was enlarged by adding water, i.e., TS decreased from 18% and 17% in fresh pig and dairy manure to below 2% in RMS (Table 1). Meanwhile, organic compounds (especially the small particles) in RMS degraded, which would influence forms of heavy metals binding to these substances [10]. In alkalescent and anaerobic condition (pH = 7 to 8, ORP = −318 to −20; Table 1), Zn (II) was easily reduced to Zn(0) and Cu(I) to Cu(0), based on the Standard Electrode Potential, whereas the degradation of arsenate and arsenite [As(III) as H_3AsO_3 and $H_2AsO_3^-$] from the organic arsenicals were more prevalent [38]. The microbiological oxidation/reduction of heavy metals also impacted the speciation and adsorption in solid fractions of DS, due to the gain/loss of electrons [38]. It was supposed that the dissolved fraction of heavy metals would increase during the AD treatment.

Although Zn, Cu and As in digested slurries were conserved without any loss during AD process, their chemical fractions had been changed. So far, there is no regulation for digestate application to arable lands in the world. In China, the application amount of solid fraction always referred to the standard of sewage sludge [30]. As mentioned above, the application of liquid fraction (i.e., digested slurry) should bring sufficient attention to its mobility in soils and toxicity to plants caused by the alternative chemical forms of heavy metals.

Table 3 Stepwise regression analysis between total and dissolved concentrations of Zn, Cu and As, and physical and chemical properties of digested slurry in anaerobic digesters (DS_{AD}) and oxidation ponds (DS_{OP}) from twenty-one large-scale anaerobic digestion plants together with intensive pig and dairy farms in Jiangsu Province, China

		DS_{AD}				DS_{OP}	
		Pig farm		Dairy farm			
Zn	Total concentration	SCOD[a]	0.592[b]	COD	0.787	ORP	0.775
		TP	0.962				
	Dissolved concentration	NH_4-N	0.602	–	–	ORP	0.818
Cu	Total concentration	ORP	0.341	COD	0.992	ORP	0.825
						NO_3-N	0.929
	Dissolved concentration	TP	0.529	COD	0.859	ORP	0.818
		NH_4-N	0.704	TP	0.997	NO_3-N	0.885
As	Total concentration	TP	0.365	COD	0.931	–	–
	Dissolved concentration	–	–	–	–	pH	0.458
						COD	0.858

For abbreviations, see Table 1

[a] Variables entered

[b] Adjusted R^2

Effects of Oxidation Ponds

Two-pond system was considered very effective in the removal of suspended solid, COD and biological oxygen demand (BOD), but were ineffective in removing heavy metals, such as Cu, Zn and As in slurries from pig and dairy farms [39]. In our study, not only primary-oxidation-pond systems but also multi-oxidation-pond systems could cripple Zn, Cu and As (Table 4; Fig. 5a, d, g), regardless of the substantial difference in total concentrations in DS_{AD} among different sampling sites. The removal efficiencies of primary oxidation pond for total Zn, Cu and As in DS were 37–98%, 52–95% and 78–95%, respectively (Table 4); while in tertiary oxidation pond, all the removal efficiencies were more than 95% (Fig. 5a, d, g). It was clear that the removal effect of the total contents of heavy metals was better in multi-oxidation-pond systems than that in primary-oxidation-pond systems, and removal effect of Cu was better than Zn and As. Oxidation pond system, as a solid–liquid separation device, was designed to remove both coarse materials and particles in order to significantly reduce the nutrient contents in the slurries [39]. Heavy metals binding to these solids would deposit, consequently causing total contents of heavy metals in DS_{OP} to drop.

The dissolved concentrations of Zn and Cu in DS_{OP} from certain sampling sites were higher than those in DS_{AD} (negative values in Table 4; Fig. 5b, e). Oxidation pond might have changed the physical and chemical characteristics of DS_{AD}, e.g., pH, ORP, contents and chemical forms of N and P (Table 3), as well as microbial activities in it. These changes added complexity to the absorption-disassociation process of heavy metals, and consequently increased the dissolved fraction of Zn and Cu in DS_{OP}. However, all the percentages of dissolved fractions in total concentrations of Zn and Cu were higher than those in DS_{AD} (Fig. 5c, f). As mentioned above, decrease in total concentrations and increase in dissolved concentrations were the main contributors to the increased proportions.

Table 4 Removal efficiency of primary-oxidation-pond systems to total and dissolved concentrations of Zn, Cu and As in digested slurry

		Pig farm (n=3)[a]	Dairy farm (n=1)
Total concentration (mg/l)	Zn	79.1 to 97.9[b]	36.5
	Cu	52.4 to 95.4	71.8
	As	92.2 to 94.6	78.0
Dissolved concentration[c] (mg/l)	Zn	−154.4 to 29.3	82.9
	Cu	−63.6 to 83.5	62.9
	As	14.3 to 93.3	67.7

[a] Four well-designed and operating anaerobic digestion plants with primary-oxidation-pond device were collected in our investigation, including three together with pig farms in Dafeng (DF), Nantong (NT) and Suqian (SQ) located in the northern part, and one together with dairy farm in Nanjing (NJ) located in the southern part of Nanjing Province (see Fig. 1). All the samples were collected at ~1 m from the edge of oxidation ponds and ~0.5 m depth from the surface

[b] Removal efficiency (%) = $(DS_{AD}-DS_{OP})/DS_{AD} \times 100$; DS_{AD} and DS_{OP} denote the digested slurries from anaerobic digester and oxidation pond, respectively. Note that negative values indicate that the concentrations in DS_{AD} is smaller than those in DS_{OP}

[c] Dissolved fraction was the liquid part of digested slurry filtered through a 0.45-μm millipore filter

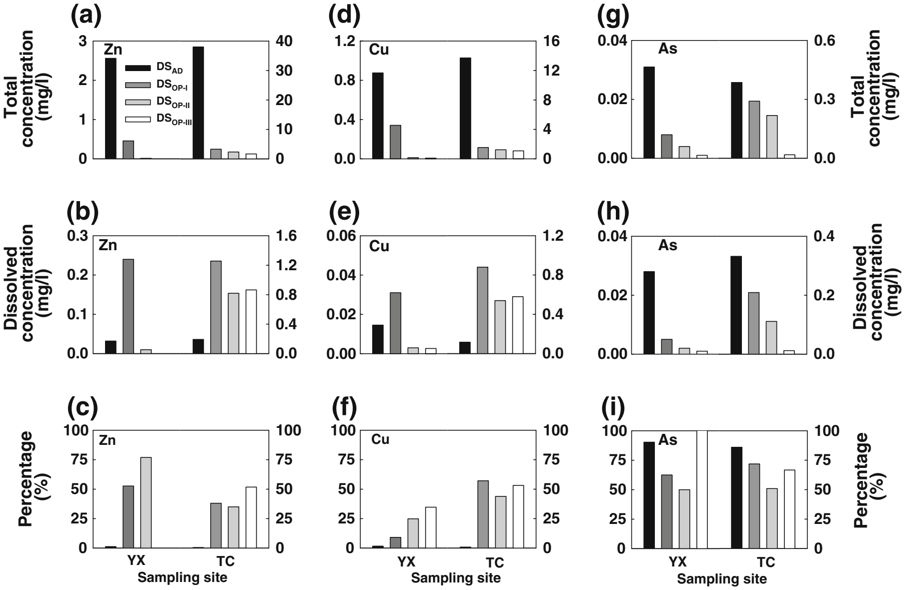

Fig. 5 Total and dissolved concentrations of Zn (**a**–**c**), Cu (**d**–**f**) and As (**g**–**i**) and the percentage of dissolved fractions in total concentrations in digested slurries from anaerobic digester (DS_{AD}) and multi-oxidation-pond systems together with pig farms in Yixing (*YX*) and Taicang (*TC*), the southern part of Jiangsu Province. Dissolved fraction denotes the liquid part of digested slurry filtered through 0.45-μm millipore filter paper. $DS_{OP\text{-}I}$, $DS_{OP\text{-}II}$ and $DS_{OP\text{-}III}$ represent the digested slurries in primary, secondary and tertiary oxidation ponds, respectively. Note that the graphic scales for the two sampling sites are different

As for variable valence metalloid As, arsenate and arsenite forms have different adsorptive affinities to various compounds which strongly affect their concentrations in the aqueous phase [40]. In our study, dissolved concentration of As significantly decreased in both primary-stage (Table 4) and multi-stage oxidation pond systems (Fig. 5h). This might be because As(III) in DS was oxidated to As(V) in oxidation ponds, which had more electric charges and adsorbed more strongly to solids [41]. The percentage of dissolved fraction to total contents did not show similar trends in DS_{OP} between different sampling sites (Table 4; Fig. 5i). The chemical forms of As in DS_{OP} might be more complicated in natural sediment process. Given its biological toxicity, the changed mechanisms of chemical forms of As in animal manures during AD treatments and management processes should be paid more attentions in further studies.

Conclusions

In this investigation, total concentrations of Zn, Cu and As were high in digested slurries from twenty-one large-scale AD plants together with intensive pig and dairy farms in Jiangsu Province, China. So, the land application of digested slurries should be paid sufficient attention due to the heavy metal pollution it brings. During AD treatment of livestock manures, various conditions of operation and management impacted the decomposition of organic matter, water content, pH, ORP as well as microbial activities, and consequently affected the chemical fractions of heavy metals. Lowering the dietary supply of heavy metals to animals would be the most effective way to reduce total contents of heavy metals in digested slurries. Meanwhile, with the increased human activities (such as industry, agriculture and urbanization), the interregional difference in environmental heavy metals should be brought into consideration, because the level of heavy metals in animals and manures would be influenced by the heavy metal-polluted soils, water, air, and plants through food chain. Oxidation pond system was effective in the removal of heavy metals in DS through crippling suspended solids, BOD, and nutrients (e.g., N, P). Furthermore, oxidation pond would impact heavy metal speciation with changes of pH and ORP. Thus, it is suggested that digested slurries from AD plants together with pig and dairy farms should be treated biologically using multi-stage pond systems before spreading on fields. Note that the chemical forms of As (variable valence metalloid) were more complicated during the processes of AD and natural sediment. Given its biological toxicity, the change mechanism of chemical forms of As during AD treatment and management processes of manure slurries should be given more attention in further studies.

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References

1. Yan, L., Li, J. M., & Ren, Y. X. (2006). Symbiosis effect of rural biogas project. *Transactions CSAE, 22* (S1), 89–92.
2. Lu, J. B., Zhu, L., Hu, G. L., & Wu, J. G. (2010). Integrating animal manure-based bioenergy production with invasive species control: a case study at Tongren Pig Farm in China. *Biomass and Bioenergy, 34*(6), 821–827.

3. Gebrezgabher, S. A., Meuwissen, M. P. M., Prins, B. A. M., & Oude Lansink, A. G. J. M. (2010). Economic analysis of anaerobic digestion—a case of green power biogas plant in the Netherlands. *NJAS–Wageningen Journal of Life Sciences, 57*(2), 109–115.
4. Börjesson, P., & Berglund, M. (2006). Environmental systems analysis of biogas systems: part I. Fuel-cycle emissions. *Biomass and Bioenergy, 30*(5), 469–485.
5. Murphy, J. D., & Power, N. (2009). Technical and economic analysis of biogas production in Ireland utilizing three different crop rotations. *Applied Energy, 86*(1), 25–36.
6. Amon, T., Amon, B., Kryvoruchko, V., Machmüller, A., Hopfner-Sixt, K., Bodiroza, V., et al. (2007). Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. *Bioresource Technology, 98*(17), 3204–3212.
7. Weiland, P. (2006). Biomass digestion in agriculture: a successful pathway for the energy production and waste treatment in Germany. *Engineering in Life Science, 6*(3), 302–309.
8. Chen, Y., Yang, G. H., Sweeney, S., & Feng, Y. Z. (2010). Household biogas use in rural China: a study of opportunities and constraints. *Renewable and Sustainable Energy Reviews, 14*(1), 545–549.
9. Gómez, X., Cuetos, M. J., García, A. I., & Morán, A. (2005). Evaluation of digestates stability from anaerobic process by thermogravimetric analysis. *Thermochimica Acta, 426*(1–2), 179–184.
10. Marcato, C. E., Pinelli, E., Pouech, P., Winterton, P., & Guiresse, M. (2008). Particle size and metal distributions in anaerobically digested pig slurry. *Bioresource Technology, 99*(7), 2340–2348.
11. Jondreville, C., Revy, P. S., & Dourmad, J. Y. (2003). Dietary means to better control the environmental impact of copper and zinc by pigs from weaning to slaughter. *Livestock Production Science, 84*(2), 147–156.
12. Silbergeld, E. K., & Nachman, K. (2008). The environmental and public health risks associated with arsenical use in animal feeds. *Annals of the New York Academy of Sciences, 1140*, 346–357.
13. Cang, L., Wang, Y. J., Zhou, D. M., & Dong, Y. H. (2004). Heavy metals pollution in poultry and livestock feeds and manures under intensive farming in Jiangsu Province, China. *Journal of Environmental Sciences, 16*(3), 371–374.
14. Dong, Z. R., Chen, Y. D., Lin, X. Y., Zhang, Y. S., & Ni, D. H. (2008). Investigation on the contents and fraction of heavy metals in swine manures from intensive livestock farms in the suburb of Hangzhou. *Acta Agriculturae Zhejiangensis, 20*(1), 35–39.
15. Suo, C., Li, Y. X., Zhang, Z. Q., Han, W., Xiong, X., Li, W., et al. (2009). Residual character of Zn in feeds and their feces from intensive livestock and poultry farms in Beijing. *Journal of Agro-Environment Science, 28*(10), 2173–2179.
16. Nicholson, F. A., Chambers, B. J., Williams, J. R., & Unwin, R. J. (1999). Heavy metal contents of livestock feeds and animal manures in England and Wales. *Bioresource Technology, 70*(1), 23–31.
17. Garbarino, J. R., Bednar, A. J., Rutherford, D. W., Beyer, R. S., & Wershaw, R. L. (2003). Environmental fate of roxarsone in poultry litter: I. Degradation of roxarsone during composting. *Environmental Science & Technology, 37*(8), 1509–1514.
18. Su, D. C., & Wong, J. W. C. (2004). Chemical speciation and phytoavailability of Zn, Cu, Ni and Cd in soil amended with fly ash-stabilized sewage sludge. *Environment International, 29*(7), 895–900.
19. Ko, H. J., Kim, K. Y., Kim, H. T., Kim, C. N., & Umeda, M. (2008). Evaluation of maturity parameters and heavy metal contents in composts made from animal manure. *Waste Management, 28*(5), 813–820.
20. He, M. M., Li, W. H., Liang, X. Q., Wu, D. L., & Tian, G. M. (2009). Effect of composting process on phytotoxicity and speciation of copper, zinc and lead in sewage sludge and swine manure. *Waste Management, 29*(2), 590–597.
21. Nachman, K. E., Graham, J. P., Price, L. B., & Silbergeld, E. K. (2005). Arsenic: a roadblock to potential animal waste management solutions. *Environmental Health Perspectives, 113*(9), 1123–1124.
22. Zhang, X. Z., Zhang, F. S., Li, Y. X., Han, W., & Yang, M. (2009). The effects of manure composing on distribution of Cu and Zn speciations in soils. *Journal of Agro-Environment Science, 28*(9), 1975–1979.
23. Wang, W. S., Shen, X. Q., Wen, B., & Zhang, S. Z. (2003). Relationship between the extractable metals from soils and metals taken up by Maize roots and shoots. *Chemosphere, 53*(5), 523–530.
24. Tripathi, R. D., Srivastava, S., Mishra, S., Singh, N., Tuli, R., Gupta, D. K., et al. (2007). Arsenic hazards: strategies for tolerance and remediation by plant. *Trends in Biotechnology, 25*(4), 158–165.
25. Khan, S., Cao, Q., Zheng, Y. M., Huang, Y. Z., & Zhu, Y. G. (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental Pollution, 152*(3), 686–692.
26. Huang, M. L., Zhou, S. L., Sun, B., & Zhao, Q. G. (2008). Heavy metals in wheat grain: assessment of potential health risk for inhabitants in Kunshan, China. *The Science of the Total Environment, 405*(1–3), 54–61.
27. Gan, S. W., Xu, Z. B., & Huang, W. (2008). Key technology for ecological application of large-scale biogas project. *Chinese Journal of Eco-Agriculture, 16*(5), 1293–1297.

28. Fuentes, A., Lloréns, M., Sáez, J., Soler, A., Aguilar, M. I., Orthño, J. F., et al. (2004). Simple and sequential extractions of heavy metals from different sewage sludges. *Chemosphere, 54*(8), 1039–1047.
29. Houghton, J. I., Burgess, J. E., & Stephenson, T. (2002). Off-line particle size analysis of digested sludge. *Water Research, 36*(18), 4643–4647.
30. Zhong, P., Li, Z. B., Li, Q. R., & Wang, Z. Y. (2007). Contents of selected nutrients and heavy metals in biogas slurry. *Journal of Agro-Environment Science, 26*(S), 165–171.
31. Alonso, E., Villar, P., Santos, A., & Aparicio, I. (2006). Fraction of heavy metals in sludge from anaerobic wastewater stabilization ponds in southern Spain. *Waste Management, 26*(11), 1270–1276.
32. Li, X. L., He, W. L., & Dong, S. L. (2006). Contamination by heavy metals in feed and measures of prevention and control. *Feed Industry, 27*(17), 48–51.
33. MAFF (1991). Code of Good Agricultural Practice for the Protection of Water. MAFF publications, London (PB0587).
34. Ye, C., Xu, Q. J., Kong, H. N., Shen, Z. M., & Yan, C. Z. (2007). Eutrophication conditions and ecological status in typical bays of Lake Taihu in China. *Environmental Monitoring and Assessment, 135* (1–3), 217–225.
35. Zhao, Y. F., Shi, X. Z., Huang, B., Yu, D. S., Wang, H. J., Sun, W. X., et al. (2007). Spatial distribution of heavy metals in agricultural soils of an industry-based peri-urban area in Wuxi, China. *Pedosphere, 17* (1), 44–51.
36. Wan, H. Y., Zhou, S. L., & Zhao, Q. G. (2005). Spatial variation of content of soil heavy metals in region with high economy development of south Jiangsu province. *Scientia Geographica Sinica, 25*(3), 329–334.
37. Temminghoff, E. J. M., Van der Zee, S. E. A. T. M., & de Haan, F. A. M. (1997). Copper mobility in a copper-contaminated sandy soil as affected by pH and solid and dissolved organic matter. *Environmental Science & Technology, 31*(4), 1109–1115.
38. Oremland, R. S., & Stolz, J. F. (2003). The ecology of arsenic. *Science, 300*(5621), 939–944.
39. Bolan, N. S., Khan, M. A., Donaldson, J., Adriano, D. C., & Matthew, C. (2003). Distribution and bioavailability of copper in farm effluent. *The Science of the Total Environment, 309*(1–3), 225–236.
40. Zobrist, J., Dowdle, P. R., Davis, J. A., & Oremland, R. S. (2000). Mobilization of arsenite by dissimilatory reduction of adsorbed arsenate. *Environmental Science & Technology, 34*(22), 4747–4753.
41. Smedley, P. L., & Kinniburgh, D. G. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. *Applied Geochemistry, 17*(5), 517–568.