Seasonal variability of heavy metal content and its chemical speciation in excess sludge by vermi-stabilization

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Abstract. Vermifiltration system has been reported to enhance sludge degradation and relieve the potential risks of heavy metals, whereas few studies focus on the seasonal variability of heavy metal content and its chemical speciation in excess sludge by vermi-stabilization. The results of this study showed that total contents of Zn, Pb, Cr and the treatment efficiency of vermifilter were higher in summer and lower in winter due to the temperature effect. While Cu was observed to not be dependent on season. Further investigation using a five-step fractionation procedure to evaluate the main chemical speciations of metals in the sludge showed that there were similar variations in the five fractions (F1-F5) between each season, which indicated that the chemical speciation of heavy metals were most affected by vermifiltration technique than seasonal factor. The transformations among the five fractions suggested that the vermifiltration significantly reduced the mobility and bioavailability of Zn and Pb due to their increased stable fractions and decreased unstable fractions. The higher stable fraction of Cu led to the stable morphology in sludge, which favoured the insignificant variations with seasons.

1. Introduction

Larger amount of excess sludge has been produced in municipal wastewater treatment plants (WWTPs) in China due to the development of urbanization during the last few decades [1]. Accordingly, a mixture of heavy metals, organic compounds, non-essential trace elements, and microorganisms included in excess sludge would have negative effects on water, soil and floral, thus the sludge needed to be treated before being discharged to the environment [2, 3]. However, the high heavy metal content in excess sludge likely caused a serious risk to soil when the treated sludge was used preferentially as the fertilizer. This will become a more serious problem if the toxic metals are taken up by plants or transported through water after heavy metal migration and transformation.

Vermifiltration system, a low-cost and eco-friendly treatment technique for sludge by the interaction of earthworms and microorganisms in conventional filtration systems, has been reported to enhance sludge degradation and relieve the potential risks of heavy metals [4-8]. Nevertheless, some uncertainties like a sudden leakage accident of metals, rainstorm and seasonal temperature change often affect the properties of sludge. Especially for the seasonal factor, the different sludge properties would lead to the changes of heavy metal contents and chemical speciations in sludge after vermi-stabilization. The purpose of this study is to investigate the variations of heavy metal content and its chemical speciation in excess sludge after vermi-stabilization at different seasons.

As we known, only using the measure of total heavy metal contents in sludge as the evaluation index of pollution does not give a comprehensive overview. Because it cannot evaluate the potential...
migration and transformation of the metals under field conditions. Therefore, the mobility and bioavailability of heavy metals are also major limitations of land application of excess sludge. The bioavailability of heavy metals in sludge is strongly dependent on its specific chemical speciation or ways of binding [9,10]. Its specific chemical speciation is fractionized into exchangeable fraction (F1), carbonate precipitated fraction (F2), Fe–Mn oxide binding fraction (F3), organic and sulfide binding fraction (F4), and the residual fraction (F5) [11]. F5 is often immobilized by insoluble salts such as phosphate and humic acid-like organic matters, and is regarded as the mostly stable fraction and no toxic to environment [12,13]. Many studies have reported the changes of heavy metal bioavailability due to the earthworm activity [14-16]. For example, Devliegher et al. thought that the decreasing bioavailability of heavy metals by earthworms was due to their redistributing of metal speciations [17]. Other researchers reported that the vermicomposting technology also played a positive role in stabilizing heavy metals in the treatment of waste [18-20].

Under field conditions, the dynamic variations of total content and chemical speciations of heavy metals were found to be dependent on the individual metal and sludge characteristic and the treatment processes involved. So far, few studies focus on the seasonal variability of heavy metal content and its chemical speciation in excess sludge by vermi-stabilization. The aim of this study is to investigate the impact of vermi-stabilization on the total content of selected heavy metals (Zn, Pb, Cr and Cu) at different seasons. And the variations of their corresponding chemical speciation (F1-F5) transformations with seasonal variability were further investigated.

2. Materials and methods

2.1. Raw excess sludge and vermi-filtration treatment

Raw excess sludge (RES) used in this study was taken from the secondary sedimentation tank of Guang Da Municipal WWTP in Jinan, China monthly from 2015 to 2016. The earthworms, purchased from a farm in Jinan, were cultured in the vermi-filter (diameter of 30 cm and height of 90 cm, made of Teflon, showed in Figure 1) at an initial earthworm density of approximating 32 g/L (fresh weigh basis). A filter material (ceramsite) height of 80 cm was placed in the vermi-filter to entrap the sludge and support the earthworms. The hydraulic load of the vermi-filter was 3 m$^3$/m$^2$.d, and the volatile solid (VS) concentration of diluted RES was 292-320 mg/L.

During a 12-month steady operation (earthworms were healthy), the vermi-filter treated sludge (VTS) was collected three times a month to determine the total contents and speciation of heavy metals (Zn, Pb, Cr and Cu). The concentrated VTS was firstly centrifuged at 9000 rpm for 15 min after natural sedimentation for 2 h; and then the residua were freeze-dried for 48 h. The samples were passed through 0.15 mm mesh prior to further analysis. The RES was sampled and treated using the same process described above on the same days.

Figure 1. Schematic diagram of the vermi-filter (with earthworms in the filter bed).
The important properties of sludge pertaining to the study, such as pH, the ratio of VS to total suspended solid (TS) (VS/TS), total N and C were determined following the reference [21]. The C/N ratio and total organic C were measured using an elemental analyzer Vario EL III (German).

2.2. Determination of heavy metals
The sludge samples needed to be digested prior to heavy metal contents being determined based on the reference [22]. These samples were digested in a microwave (Berghof, Germany) after being acidified with a mixture of 6 ml HNO₃, 2 ml HCl and 2 ml HF. The procedure went as follows: a 10 min gradual temperature increase to 200 °C (1000 W, 106 Pa), a 15 min step of 200 °C and a ventilated cooling stage. Then the digested samples were heated on a hot plate (120 °C) until they became near dry for inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 2100 DV, USA) analysis. The heavy metal chemical speciations of five fractions (F1, F2, F3, F4, and F5) were determined by a five-step sequential extraction procedure proposed by Tessier et al. [8,11].

2.3. Statistical analysis
Samples of RES and vermifilter treatment sludge (VTS) were respectively collected from wastewater treatment and vermifilter at the beginning, middle, and end of each month. All assays were conducted in triplicate, and the results were expressed as mean ± standard deviation. An analysis of variance (ANOVA) was used to test the significance of results, and p < 0.05 was considered to be statistically significant.

3. Results and discussion
3.1. Performance of sludge stabilization by vermifilter
The treatment performance of vermifilter was firstly investigated, and the degree of the sludge stabilization at each month was assessed by the VS/TS (Figure 2). In this study, seasons were divided as follows: spring was March 2016 to May 2016; summer was June 2016 to August 2016; autumn was September 2015 to November 2015; winter was December 2015 to February 2016. Figure 2 showed that the variation trend of VS/TS in the RES was similar to that in the VTS during the whole year, and the VS/TS value was higher in winter and spring, and lower in summer and autumn, suggesting a relative strong relationship with temperature. The VS/TS value of the RES and VTS were 70.2-79.1% and 57.9%-68.6% during the whole year, and their comparisons indicated that the vermifiltration greatly improved the degradation of the organic matter in the RES, which was consistent with the report of Zhao et al. [1], who thought that the sludge stabilization in vermifilter was achieved by earthworms digestion and earthworm-microorganism interactions.

![Figure 2. Variations of VS/TS in the RES and VTS during the whole year.](image)

By the vermifiltration of sludge, there were significant different in the physico-chemical properties between the RES and VTS. For example, average value of pH was decreased from 7.2 in the RES to 6.5 in the VTS, average values of total C and N were decreased from 310.6 and 37.7 mg/kg-VS in the RES to 211.3 and 25.3 mg/kg-VS in the VTS, respectively. For their specific seasonal variations, they were also observed to be interconnected with temperature. The values of pH, total organic C and N
were higher in winter and spring than those in summer and autumn. The different physic-chemical characteristics of sludge would result in the variations of the total content and the chemical speciation of heavy metals. Thus, the comparisons of heavy metal contents between the sludge before (RES) and after (VTS) vermifiltration during the whole year were then investigated in the following text.

3.2. Seasonal variations of heavy metal contents by vermifiltration

Figure 3. Comparisons of total contents of Zn, Pb, Cr, and Cu between the RES and VTS during the whole year.

Figure 3 shows the monthly variations of selected heavy metal (Zn, Pb, Cr, Cu) contents in the RES and VTS at different months from Sep. 2015 to Aug. 2016. It can be seen that total contents of these metals were increased in the VTS compared with the RES, especially for Zn, which seemed to heighten its potential risk to the environment; however, all heavy metal contents obtained in the VTS were well below the threshold value required in China for land application [23]. The increases of the total contents of Zn, Pb and Cr were mainly due to the fact that the organic matters in the sludge were biodegraded by the earthworms and the interactions of earthworm-microorganisms in the vermiclizer. A similar phenomenon was found that total contents of heavy metals might be increased after sludge stabilization by composting, liquefaction and phytostabilization [24-26], and all these were due to the degradation of organic matters. While some studies pointed that total contents of heavy metal were decreased during composting process, with the reason being that metals were released from the decomposed organic matter [26,27]. In this study, the detected soluble metal ions in the effluent were negligible. Therefore, the higher total contents of Zn, Pb and Cr in the VTS than those of RES mainly
resulted from the organic matter degradation by vermifiltration. The reason for the inconspicuous variation of Cu during the whole year was likely due to its stable characteristic.

Heavy metal composition of excess sludge can be extremely variable depending on the weather and human activities. Furthermore, the average value of heavy metal total contents in the RES and VTS at each season was included in Table 1. It can be observed that total contents of Zn, Pb and Cr in both RES and VTS were higher in summer and lower in winter, and the similar phenomenon was also found by Garcia [28]. This suggested that the temperature was a key factor affecting total contents of heavy metals in excess sludge. Taking Zn as an example, since of Zn having the highest percentage (> 70%) among the four metals, its total contents in the RES and VTS were 1950 and 1548 mg/kg-VS in summer. When the lower temperature (winter) came, total contents of Zn in the RES and VTS were all decreased, and its concentration was 1394 and 1052 mg/kg-VS, respectively. We suspected that the activity of the microorganisms was easily affected by temperature, which resulted in more organic matter was degraded under the higher microbial activity condition in summer. Since heavy metals are non-biodegradable, their concentrations in the VTS tended to be increased. All total contents of the selected heavy metals in the RES were below the values reported by other authors [24-26], which were probably related to the low heavy metal concentrations of area characterized domestic sewage in Jinan.

| Table 1. Total contents of heavy metals (Zn, Pb, Cr and Cu) in the RES and VTS at each season. |
|---------------------------------------------------------------|
| **Autumn** | **Winter** | **Spring** | **Summer** |
| RES | VTS | RES | VTS | RES | VTS | RES | VTS |
| Zn | 1337±183 | 1796±223 | 1052±86 | 1394±129 | 1312±183 | 1701±237 | 1548±6 | 1950±37 |
| Pb | 122±3.5 | 147±2.5 | 112±8.3 | 135±7.8 | 130±2.9 | 157±1.8 | 135±1.8 | 145±1.0 |
| Cr | 113±6.5 | 132±4.6 | 93±7.4 | 108±8.9 | 98.6±8.3 | 108±9.1 | 124±4.9 | 131±5.5 |
| Cu | 310±4.4 | 361±6.5 | 324±2.1 | 369±2.6 | 314±3.8 | 358±4.5 | 311±1.9 | 388±5.1 |

3.3. Variations of heavy metal chemical speciations by vermifiltration

The specific chemical speciations of Zn, Pb, Cr and Cu in the VES and VTS were further investigated (Figure 4) to comprehensively evaluate their potential risk. The data from Figure 4 were determined based on the sludge samples collected in the beginning, middle and end of April in 2016. In this study, the proportions of heavy metal fractions at other months were also analyzed (data not shown), and their variation trends were found to be in correspondence with the results showed in Figure 4. The reason being that the variations of heavy metal speciation caused by vermifiltration technique was much higher than the effect caused by the seasonal variation. Thus, the transformations among the five fractions between the RES and VTS were the key discussion in this study.

![Figure 4](image.png)

*Figure 4. Proportions of Zn, Pb, Cr, Cu fractions (F1, F2, F3, F4, and F5) in the RES and VTS at April 2016.*
According to the reference [29], F1 represents the readily bioavailable proportion of metal and it can be defined as toxic. As seen from Figure 4, F1 percentage of Zn, Pb, Cr and Cu in the RES were low to 3%, suggesting its faint and direct toxicity to the environment [25,30]. After vermifiltration, F1 percentages of Zn and Pb were observed to be increased \((p<0.05)\), while its variations in Cr and Cu were negligible. F2 and F3 are sensitive to pH and can be easily transformed into F1 under weak acid condition [31,32]. F2 proportion of Zn and Pb were found to be significantly different \((p<0.05)\) before and after vermifiltration, and their portions decreased from 5.81\% and 7.54\% to 3.32\% and 3.33\%, respectively. F3 was the largest percentage among the five fractions of Zn in both the RES and VTS, which were 66.71\% and 32.37\%, respectively. This phenomenon was consistent with the report of Peruzzi [24]. The similar decreasing F3 in Pb and Cu were also observed after vermifiltration, while its variation for Cr was inconspicuous. F4 of Zn, Pb and Cu in the VTS were clearly higher than those in the RES, which indicated that the vermifiltration technique could promote Zn, Pb and Cu bound to organics and sulfide and decrease their bioavailability. F5 is immobilized by insoluble salts such as phosphate and humic acid-like organic matters, and is regarded as the mostly stable fraction and no toxic to environment [12,13]. F5 was the main chemical speciation of Pb, Cr and Cu, of the proportion were 43.16\%, 33.43\% and 43.62\% in the RES, and 48.95\%, 43.72\% and 45.80\% in the VTS, respectively, indicating an insignificant variation \((p>0.05)\). As for Zn, its F5 fraction was increased from 16.14\% to 37.34\% after vermifiltration \((p<0.05)\). Due to the multivalent characteristic of Cr, it can be existed stably in the anionic speciations of \(\text{Cr}_2\text{O}_3\), chromate \((\text{CrO}_4^{2-})\) or dichromate \((\text{Cr}_2\text{O}_7^{2-})\) in the vermifiltration system, which accordingly made its faint complexation with the negative charged sludge and resulted in the weaker transformations among the five fractions. The sum of F4 and F5 fractions of Cu was greater than 83.54\%, which was the highest among the four heavy metals and the most stable. The higher stable fraction of Cu led to the stable morphology in sludge, which favoured the insignificant variations with seasons.

4. Conclusion
The marked organic degradation in excess sludge by vermifiltration technique indeed enhanced total contents of selected heavy metals (Zn, Pb, Cr and Cu). Total contents of Zn, Pb and Cr in excess sludge before and after vermifiltration were found to change with the seasons due to the temperature effect; while this phenomenon does not apply to Cu. Further investigation of the chemical speciation transformations showed that there were similar variations in the five fractions (F1-F5) between each season, which indicated that the chemical speciations of heavy metals were most affected by vermifiltration technique than seasonal factor. Further analyses of transformations among the five fractions suggested that the vermifiltration significantly reduced the mobility and bioavailability of Zn and Pb due to their increased stable fractions and decreased unstable fractions. The higher stable fraction of Cu led to the stable morphology in sludge, which favoured the insignificant variations with seasons.

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