Pollution and health risk assessment of heavy metals following repeated biogas slurry application in two vegetable fields

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Abstract. Repeated biogas slurry application in soil may cause accumulation of heavy metals. In the experiment, repeated biogas slurry application for 4 (N1) and 10 years (N2) in two vegetable fields, to detect heavy metal content (Zn, Cu, Cr, Pb) and assessment the risk of short- and long-term fertilizing on ecology and health. The result showed, long-term fertilization has increased the content of heavy metal. Zn and Cu in topsoil (0-20 cm) in field N2 exceed the standard, resulting in mild contamination. The value of hazard quotient (HQ) in the same land were as follows: Cr > Pb > Cu > Zn. In general, the noncarcinogenic risk and carcinogenic risk index in N2 were higher than those of N1. In the survey area, there was no noncarcinogenic risk and carcinogenic risk for adults and children. However, children are more sensitive to environmental changes than adults and should pay more attention to protect their health. Long-term monitoring and evaluation of heavy metal hazards is necessary to ensure the safety of biogas slurry used in farmland.

1. Introduction
With the development of animal husbandry, reasonable disposal of livestock excreta has become a stern challenge [19]. Irrigating biogas slurry to the field is considered feasible and highly recommended. Heavy metals are regarded as the main toxic elements threatening the agricultural ecological environment, which have attracted extensive attention [3]. Application of biogas slurry is one of the sources of heavy metals in soil [13]. Heavy metals are accumulated through the food chain and endanger human health. Excessive accumulation of heavy metals in the human body can affect the central nervous system, attack the immune system, cause damage to human body functions and even cancer [16].

Previous studies have focused on the impact of heavy metals on fertility or on the environment. It was found that the application of biogas slurry increased the content of trace elements and improved crop quality and yield [3]. But excessive biogas slurry has affected soil microorganisms, limited soil function, and increased the risk of soil and water pollution [5]. In the short term, microorganisms are negatively affected by heavy metals, but in the long term, the number of microorganisms increases due to adaptation to the environment. This study focuses on the distribution characteristics of heavy metals in agricultural soils at different depths, and conducts a comprehensive evaluation, including the harm of heavy metals in soils with different fertilization years to the environment and human body, in order to provide a reference for the evaluation of soil heavy metals.
2. Materials and methods

2.1. Site description
The experimental area is located in Mianyang City of Sichuan Province, China (30°42′-33°03′N, 103°45′-105°43′E). The climate of the region is characterized by subtropical monsoon climate with annual rainfall of 963.2 mm. Combination of planting and breeding is the local agricultural characteristics. In order to increase crop yields, farmers choose biogas slurry from nearby pig farms instead of chemical fertilizer to irrigate their fields.

2.2. Experimental method and sample treatment
The samples came from two fields, which mainly grew vegetables. One field was irrigated with biogas fertilizer for 4 years (N1) and the other for 10 years (N2). Soil samples were taken at random from three locations in each filed using a manual soil sampling drill to a depth of 40 cm at 10 cm spaces. To accurately determine the change of heavy metal concentration of field N2, the sampling depth was up to 60 cm.

After sampling, the samples bring to the laboratory and were first air-dried for a week. Then the samples were ground into fine particles and passed 0.15 mm sieve pore. The value of pH was tested by potentiometry. Soil samples were digestion by HNO3-HClO4-HF. Ultimately, all digested samples were diluted to 10 mL filtered. The concentration of Zn, Cu, Cr, Pb were determined by ICP-OES[14].

| Field | pH   | Zn (mg/kg) | Cu (mg/kg) | Cr (mg/kg) | Pb (mg/kg) |
|-------|------|------------|------------|------------|------------|
| N1    | 7.72 | 9.27       | 3.80       | 0.18       | 0.053      |
| N2    | 7.34 | 3.04       | 1.94       | 0.043      | 0.019      |

2.3. Pollution assessment methods

2.3.1. Ecological risk
In this study the standard value (Sij) referenced Chinese soil environmental quality risk control standard (6.5 < pH<7.5: SZn=250, SCu=100, SCr=200, Spb=120 mg/kg; 5.5 < pH<6.5, SZn=200, SCu=50, SCr=150, Spb=90 mg/kg)[4]. The assessment methods and the ecological risk assessment standard are presented in Table 1 and Table 2, respectively.

| Number | Evaluation method                          | Equation                                      | Major parameters                                      |
|--------|-------------------------------------------|-----------------------------------------------|------------------------------------------------------|
| 1      | Single factor pollution index              | \[ P_i = \frac{C_i}{S_i} \]                   | \[ C_i \] and \[ S_i \]: the concentration of individual heavy metal in the soil sample and standard value (mg kg\(^{-1}\)); |
| 2      | Nemerow pollution index                    | \[ P = \frac{P_{\text{max}}^2 + P_{\text{ave}}^2}{2} \]\(^{1/2}\) | \[ P_{\text{max}} \]: maximum single pollution index; \[ P_{\text{ave}} \]: average of all single factor pollution indices; |
| 3      | Ecological pollution index                | \[ E_i = \frac{T_i \cdot C_i}{C_b} \]        | \[ T_i \]: the potential ecological risk of each heavy metal; \[ T_i \]: biological toxic factor of individual element; \[ C_b \]: the concentration of individual heavy metal in the background, Zn=95, Cu=45, Cr=0.3, Pb=20 mg kg\(^{-1}\); |
|        | RI = \[ \sum_{i=1}^{n} E_i \]            | \[ R_{i} \]: the potential ecological risk of various heavy metal |
### Table 2. Standard degree of indicators in the assessment

| Single factor pollution index | Nemerow pollution index | Ecological pollution index |
|-------------------------------|------------------------|--------------------------|
| Pollution level               | Index                  | Risk level               | $E_i$ | $RI$ |
| Acceptable $P_i \leq 1$       | Clean $P \leq 0.7$     | Low                      | $E_i \leq 40$ | $RI \leq 90$ |
| Slightly $1 < P_i \leq 2$     | Cleanliness (Cordon)   | Moderate                  | $40 \leq E_i \leq 80$ | $90 \leq RI \leq 180$ |
| Moderate $2 < P_i \leq 3$     | Slightly $1 \leq P \leq 2$ | Considerable             | $80 \leq E_i < 160$ | $180 \leq RI < 360$ |
| Heavy $P_i > 3$               | Moderate $2 \leq P \leq 3$ | High                     | $160 \leq E_i < 320$ | $360 \leq RI < 720$ |
| Heavy $P > 3$                 | Very high $E_i \geq 320$ | $RI \geq 720$            |

#### 2.3.2. Health risk assessment

According to a large number of studies, there are two main ways to assess the human health risk of heavy metal contaminated soil: direct oral intake of soil particles and skin contact with soil [11]. The calculation formulas and parameters of health risk value are presented in Table 3 and table 4 [10].

Ordinarily, if HI is less than 1, it shows that the potential health risks of exposed people will not be obvious, and vice versa. ACR values are divided into three levels: below $1 \times 10^{-6}$ (almost no effect on health), range $1 \times 10^{-6}$ to $1 \times 10^{-4}$ (acceptable), above $1 \times 10^{-4}$ (unacceptable).

Table 3. Calculating models of noncarcinogenic and carcinogenic risk in two exposure pathways [18]

| Number | Exposure routes | Instruction | Equation |
|--------|----------------|-------------|----------|
| 1      | Oral ingestion | The average daily intake of soil particle from oral ingestion | $ADI_{oi} = \frac{C_i \cdot SIR \cdot EF \cdot ED}{BW \cdot AT \cdot 10^6}$ |
|        | Hazard quotient | | $HQ_{oi} = \frac{ADI_{oi}}{RF_{D_{oi}}}$ |
| 2      | Skinf contact  | The average daily intake of dermal contact | $ADI_{di} = \frac{C_i \cdot SA \cdot EF \cdot ED \cdot SAF \cdot ABS}{BW \cdot AT \cdot 10^6}$ |
|        | Hazard quotient | | $HQ_{di} = \frac{ADI_{di}}{RF_{D_{di}}}$ |
| 3      | Oral ingestion and skin contact | Noncarcinogenic risk | $HI = HQ_{oi} + HQ_{di}$ |
| 4      | The aggregate carcinogenic risk | | $ACR = ADI_{oi} \cdot SF_{oi} + ADI_{di} \cdot SF_{di}$ |
| 5      | The contribution rate | $R_i = \frac{100 \cdot (HQ_{oi} + HQ_{di})}{HI}$ |

Table 4. Major parameters in formula [9]

| Parameter | Meaning | Value | Unit |
|-----------|---------|-------|------|
| $SIR$     | Soil ingestion rate | Adult 100, children 200 | mg day$^{-1}$ |
| $EF$      | Exposure frequency | 350 | day$^{-1}$ |
| $ED$      | Exposure duration | Adult 24, children 6 | year |
| $BW$      | The average body weigh | Adult 63.3, children 15.9 | kg |
| $AT$      | The average contact time | carcinogenic risk 26280 | day |
| $RfD_{oi}$ | Reference dose of individual heavy metal taken orally | Zn=0.3, Cu=0.04, Cr=0.003, Pb=0.0035 | mg kg$^{-1}$ day$^{-1}$ |
| $SA$      | The exposed skin surface area | Adult 0.153, children 0.086 | m$^2$ |
| $SAF$     | The skin adherence factor | Adult 0.49, children 0.65 | mg cm$^{-1}$ day$^{-1}$ |
ABS  The dermal absorption factor  

$Zn=0.02, Cu=0.1, Cr=0.04, Pb=0.006$  

$RfD_{di}$  Reference dose of a single heavy metal through the dermal contacts  

$Zn=0.06, Cu=0.012, Cr=0.0006, Pb=0.000525$  

$SF_{oi}$  The cancer slope factor of heavy metal $i$ via oral ingestion  

$Cr=0.5, Pb=0.0085$  

$SF_{di}$  The cancer slope factor of heavy metal $i$ via oral dermal contact  

$Cr=42, Pb=0.042$  

3. Results and discussion

3.1. Pollution risk assessment of soil heavy metal

The pH of N1 and N2 were measured to be 6.31 and 6.90, respectively, demonstrating the soil in the sample plots were weakly acid. The trend of single factor pollution index of soil heavy metals was consistent with that of corresponding soil heavy metal concentrations. The calculation results are presented in Table 5. In N2, the pollution risk showed decreasing tendency with the increase of depth, but the lowest pollution risk was at the depth of 30-40 cm (Figure 1). Because other migration and adsorption of heavy metals may exist at the depth of 30-40 cm, leading to the lowest risk. In field N1, the pollution risk did not change significantly with the depth. The contamination level caused by heavy metal among N1 generally lower than N2, which may be related to the years of fertilization. Continuous fertilization has promoted heavy metal accumulation [17]. For topsoil, $P_{Zn} > P_{Cu} > P_{Cr} > P_{Pb}$., there were no excessive heavy metals in cultivated land N1 displayed by $P_{i}$, but the surface soil (0-20 cm) in N2 was slightly polluted by Zn and Cu. In livestock feed, Zn and Cu are important additives that promote growth [7].

In the study, the advantages of $P$, $E_i$ and $RI$ were used to evaluate the metal hazards in soil samples for more comprehensive analysis. While field N1 was at a clean level, Nemerow pollution index indicated that the soil in N2 (0-10 cm) was slightly polluted. $E_i$ indicates the sensibility of living organisms to harmful substances and explains the possible ecological risks of different heavy metals [8]. $RI$ is used to assess the sensitivity of diverse biological populations and integrated environmental impacts. $P$ exceeded the standard value, but $E_i$ were far less than 40 and $RI$ were far less than 90. Hence, low risk existed in the two study areas. It is similar to that of Baran et al., which explains that Zn has no ecological risk to biological group [2].

| Fields | Depth | $P_{Zn}$ | $P_{Cu}$ | $P_{Cr}$ | $P_{Pb}$ | $P$ | $E_{Zn}$ | $E_{Cu}$ | $E_{Cr}$ | $E_{Pb}$ | $RI$ |
|--------|-------|----------|----------|----------|----------|-----|----------|----------|----------|----------|------|
| N1     | 0-10  | 0.40     | 0.70     | 0.31     | 0.18     | 0.75| 3.90     | 1.02     | 4.15     | 9.91     |      |

Figure 1. The value of single factor pollution index ($P_i$)

Figure 2. Noncarcinogenic risk index (HI) for different population groups
Repeated biogas slurry application will increase the concentration of heavy metals. Generally, the content of heavy metals is higher in the topsoil layer. The concentration of Zn and Cu easily exceeds China's agricultural soil standards, causing the soil to be slightly polluted. Nevertheless, human exposure through oral intake and skin contact with heavy metals will not cause noncarcinogenic risk, and the carcinogenic risk is acceptable. Children are more sensitive than adults and need to be wary of them being harm. Continuous attention should be paid to changes in heavy metal content during the application of biogas slurry fertilizer.

### 3.2. Health risk analysis

The human body is easily in contact with surface soil (0-10 cm), so this study mainly focuses on the impact of heavy metal of surface soil on the human body. As shown in Table 6, whether children or adults, field N1 or N2, the value of HQ increased in turn: Zn < Cu < Pb < Cr, which Eziz's study also confirmed this conclusion [6]. The total HQCr and HQPb accounted for nearly 85% of the gross HQ value, especially that of N1 was exceeded 92%. Mirzaei also discovered that noncarcinogenic risk of Cr and Pb exceeded other tested heavy metals [15]. In the study, oral intake accounts for above 99% of all exposure pathways. Oral inhalation was the most important exposure ways, owing to the high daily intake of oral intake.

| Depth (cm) | N1 | N2 |
|-----------|----|----|
| 0-10      | 0.16 | 1.54 |
| 10-20     | 0.78 | 1.48 |
| 20-30     | 0.58 | 1.13 |
| 30-40     | 0.53 | 0.39 |
| 40-50     | 0.54 | 0.77 |
| 50-60     | 0.54 | 0.61 |

Table 6. The values of hazard quotient and carcinogenic risk index

| Depth (cm) | N1 | N2 |
|-----------|----|----|
| 0-10      | 0.12 | 1.54 |
| 10-20     | 0.78 | 1.48 |
| 20-30     | 0.58 | 1.13 |
| 30-40     | 0.53 | 0.39 |
| 40-50     | 0.54 | 0.77 |
| 50-60     | 0.54 | 0.61 |

All HI were below 1, and HImax was 0.32. So, there were no noncarcinogenic risk for adults and kids (Figure 2). However, the noncarcinogenic risk for kids were higher than that for adults. Kids are most likely to take it orally by their mouths and hands [1]. On the whole, the HI value of field N2 decreased with the increase of sampling depth, while that of field N1 has no evident differences. The carcinogenic risk of Zn first decreased with the increase of sampling depth, while the highest ACR in N2 was in 10-20 cm. The migration and accumulation of heavy metals were affected by the length of biogas slurry application [17]. The sensitivity of children to carcinogenic risk was still higher than that of adults [12].

### 4. Conclusion

Repeated biogas slurry application will increase the concentration of heavy metals. Generally, the content of heavy metals is higher in the topsoil layer. The concentration of Zn and Cu easily exceeds China's agricultural soil standards, causing the soil to be slightly polluted. Nevertheless, human exposure through oral intake and skin contact with heavy metals will not cause noncarcinogenic risk, and the carcinogenic risk is acceptable. Children are more sensitive than adults and need to be wary of them being harm. Continuous attention should be paid to changes in heavy metal content during the application of biogas slurry fertilizer.
Acknowledgments
This work was supported by Mianyang Science and technology project (2018YFZJ019) and Key Laboratory of Development and Application of Rural Renewable Energy, Ministry of Agriculture and Rural Affairs, China (2020-002).

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