従前の都市ごみ埋め立て処分場周辺の水銀の分画、空間分布、ミミズへの生物濃縮および浸出水汚染土壌への除去

Mercuric fractionation, spatial distribution, earthworm bioaccumulation and removal of the leachate-contaminated soils around the traditional municipal solid waste (MSW) landfill

A dissertation submitted
By

HE Changhua
何 昌華

to
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Graduate School of Environmental & Symbiotic Sciences
Prefectural University of Kumamoto, Japan

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Thesis Reading Committee

Chairman: Prof. ISHIBASHI Yasuhiro
Committee member: Prof. ARIZONO Koji
Prof. ZHANG Daizhou
Prof. KOBAYASHI Jun

This thesis is dedicated to my family.

For their endless love, understanding, encouragement and constant support. My parents have been a source of motivation and strength during moments of despair and discouragement. They did not only raise and nurture me but also burdened themselves dearly over the years for my education and intellectual development. From mother's care and support is great for my kids have been shown always in incredible ways. Especially, I would like to thank my wife for giving me understanding and supports on my professional and academic commitments. Only she knows how much work has gone into this.

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Changhua HE
Prefectural University of Kumamoto, Japan
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Abstract

Since the mercury (Hg) contamination was found to threaten human health, direct discharge of Hg-containing municipal solid waste (MSW) into the environment has been declined substantially. Nevertheless, already happened high Hg concentration are still existing in water, soil, air and food of our living environment. For a long time, many questions remain unclear integrally on Hg potential risks of toxicity during the long-term variation trends. Remediation of Hg contaminated substances is always difficult to achieve in the Hg threat elimination for improving the public health. Even so, historical Hg contaminated sites also provide a good opportunity to study the characteristics of Hg environmental chemistry and get more information for understanding Hg polluted problem.

Hg is inevitably deposited in traditional landfills via a variety of Hg-containing Municipal solid waste (MSW) growth worldwide, such as household batteries, devices, fluorescent lamps, mirror coatings, medical waste, Hg amalgams, sewage sludge, and power plants waste. Hg can be accumulated and amplified in predatory animals and humans through the food chain, and eventually influence human health through several causal linkages directly and indirectly, such as neurotoxicity, mortality, and reproductive toxicity. However, it has been reported that approximately 70% MSW globally remains to be managed by traditional landfill owing to technological simplicity, ease of use and cost-effectiveness, and that most traditional landfills were built into mountainsides using the natural space between ridges to limit land usage, and there is no perfect liner system for preventing leachate leaks in the past and now, especially landfill built in last century. Hg of MSW will be deposited as a long-term pollutant, and can be released into the surrounding air, water, and soil through gas emissions and leachate from landfill during the MSW decomposition and excess rainwater infiltrating from landfill. It is confirmed that Hg is one of the most challenging among all MSW contaminations, because of its properties of persistence, immutability, not being degradable, and no beneficial biological function of Hg has been reported to date.

Earthworms play important roles in maintaining soil fertility and recycling organic matters, they spend most of their life in soil and play an important role in soil structure maintenance, and also function as a key component of food chains for providing animal proteins to the predators in local ecosystems, which are considered useful bio-indicators for assessing soil health and quality in numerous studies of contaminated sites and in laboratory experiments. Although numerous studies focused on the adverse influence of Hg contaminants in soil. Nevertheless, soil-Hg exists in different chemical forms that have different mobility, bioavailability, and toxicity to organisms. Soil T-Hg determination could not completely delineate the biological behavior and risk to environmental organisms. Particularly, the long-term transport tendency, spatial distribution, and biological transformation of Hg in landfill-leachate-contaminated soils (LLCS). This study is motivated that knowledge on the mercuric biogeochemical fractions, spatial distribution, earthworm bioaccumulation, immobilization and washing removal of the LLCS are limited, especially the field investigation on spatial distribution characteristics and relationships of the mercury biogeochemical fractions between the LLCS and native earthworms under considering other soil's physicochemical properties used field-collected samples are largely lacking. To our knowledge, there are have no studies on the changes of Hg fraction composition before and after soil washing treated by saturated Na_2S solution (ss-Na_2S) for Hg containing landfill-leachate-contaminated soils (LLCS). Therefore, it is essential to investigate the changes of different Hg fractions in LLCS by washing removal with ss-Na_2S treatment in order to accurately demonstrate the removal efficiency of the different Hg fraction by ss-Na_2S. The subsequent stable effect of treated LLCS under the controlled condition of the different pH values and equilibrium times in Hg-static leaching test, and then judge the removal efficiency of exchange leaching Hg in Hg-containing LLCS. Furthermore, exploring and improving the capabilities of management and governance for controlling the environmental risks of leaching Hg from Hg-containing LLCS around the traditional landfill. In this study, soil and earthworm samples were collected at the traditional landfill of "Sanpou" mountain (32.876°N, 129.743°E) in Nagasaki, southwestern Japan and built in 1975. In the present study included three parts.

In the first part, the contents and spatial distribution of mercury (Hg), including soil-Hg fractionation and Hg-containing native earthworm *Bimastos parvus* (*B. parvus*) species, were investigated in the leachate-contaminated zone of a large traditional landfill, Japan. Soil-Hg was fractionated into 5 categories: F1/water soluble Hg (Hg-w), F2/human stomach acid soluble Hg (Hg-h), F3/organic-chelated (Hg-o), F4/elemental Hg (Hg-e), and F5/mercuric sulfide (Hg-s). The total mercury (THg) and methylmercury (MeHg) of native *B. parvus*, and the geochemical properties of soils were examined in this study. Soil T-Hg concentration ranged between 0.227 and 2.919 mg kg^{-1} dry weight (dw). The T-Hg and MeHg concentrations of *B. parvus* species ranged from 1.242 to 6.775 mg kg^{-1} dw and from 0.031 to 0.218 mg kg^{-1} dw, respectively. Percentages of soil-Hg fractions were in the order of F3/Hg-o > F4/ Hg-e > F5/ Hg-s > F1/Hg-w > F2 / Hg-h, and the fractions of Hg-o and Hg-e were 55.50% and 35.31%, respectively. Similar distributions and close correlations between the levels of *B. parvus* Hg and soil Hg-o, Hg-e, and Hg-s were observed in this study. The distribution of Hg in *B. parvus* was associated with soil organic matter (SOM) content and particle size (sand, clay); however, it was not correlated with Hg-w or Hg-h. The results indicated that easily bioavailable and soluble Hg fractions (Hg-w, Hg-h) of the soil were not appropriate to illustrate the distribution of Hg in native *B. parvus*. Instead, the stable soil-Hg fractions (Hg-o, Hg-e, and Hg-s) demonstrated good relationships of spatial distribution with *B. parvus* Hg in leachate-contaminated soil. It is advisable to preclude the evaluation of Hg biological distribution using soluble Hg fractions only. Stable Hg fractions in leachate-contaminated soil should also be included for assessing the biological distribution of Hg in leachate-contaminated soils.

In the second part, THg and methylmercury (MeHg) bioaccumulation was explored in the *Bimastus parvus* species of earthworm (*B. parvus*) native to the leachate-contaminated forest soils around a Hg-polluted traditional landfill in Japan. General soil properties and concentrations of THg and MeHg in forest soils and in *B. parvus* were determined. The results indicated that the average THg concentrations in *B. parvus* and in forest soils in the leachate-contaminated sites were 10.21 and 14.90 times higher than those in the reference sites, respectively, whereas similar average MeHg concentrations were observed in forest soils (< 0.01 mg kg^{-1}) and in *B. parvus* (0.100–0.114 mg kg^{-1}) across all sampled sites. The average bioaccumulation factors of THg in *B. parvus* (BAF_{THg}) in forest soil were similar between the leachate-contaminated sites and the reference sites. Cluster and regression analyses demonstrated that the *B. parvus* Hg (THg and MeHg) and soil THg were positively correlated with each other and with soil organic matter (SOM) and clay but were negatively correlated with sand and hardly correlated with silts and pH in leachate-contaminated forest soils. From these results, it was proposed that Hg exposure to food chains is possible through *B. parvus*. However, the role of *B. parvus* in MeHg production is not clear, and it is possible that the MeHg in *B. parvus* was firstly formed within forest soils and then accumulated in their tissues.

In the third part, the objective of this laboratory study is to investigate the effect of washing removal using saturated sodium sulfide solution (ss-Na_2S) for Hg-containing LLCS through considering the changes of soil THg concentration, 5-extracted soil Hg-fractions, soil Hg-static leaching test as a function of pH (1-12) and equilibrium time (0-4weeks), and the varied trends of leaching Hg concentration were simulated by Visual MINTEQ modeling according to the mineralogical composition analysis. Before and after ss-Na_2S washing treatment, the averaged THg concentration of LLCS subsamples were reduced by 22.02% only (from 1.8266 to 1.4244 mg kg^{-1}). However, the leaching Hg concentration of treated LLCS was reduced significantly and kept low level under a wide range of equilibrium time (6h - 4w) and pH value (1 - 12) static leaching conditions. Especially, the most of exchangeable Hg (F1/Hg-w, F2/Hg-h) and stable F5/Hg-s were to be washing removed from Hg-containing LLCS by ss-Na_2S significantly, the average concentration of F1/Hg-w, F2/Hg-h, F3/Hg-o, F4/Hg-e and F5/Hg-s were reduced by 96.15%, 95.58%, 23.85%, 5.92%, and 99.22%, respectively. Our study shows that ss-Na_2S washing treatment can significantly reduce the Hg leaching from LLCS. Considering the exchangeable Hg fraction (Hg-w, Hg-h) were the high mobility and potential bioavailability than other Hg fraction in soil, the ss-Na_2S ex situ treatment may be developed a cost-effective Hg immobilization and washing process for removing or reducing exchangeable Hg fraction (Hg-w, Hg-h) and non-mobile Hg fraction (Hg-s) from Hg-containing LLCS simultaneously for reducing or eliminating the Hg potential environmental risks of leaching Hg from LLCS under referencing the Hg safety guide of Japanese for soil. Our hypothesis is that soil-Hg fraction contents are diffused in small leachate ditches and scattered pools on the surface soil of the contaminated zone around the traditional MSW landfill, and another hypothesis is that Hg in earthworm tissues was accumulated via the gut uptake from the surface soil in the landfill-leachate-contaminated zone.

In summary, Hg was fractionated into 5 fractions in the LLCS, main components of the LLCS Hg fractions were Hg-o (55.50%) and Hg-e (35.31%). Hg distribution in *B. parvus* and LLCS showed a dependence on SOM and clays, significantly. Distributions of *B. parvus* Hg and soil stable Hg fractions were similar. Soluble Hg fractions cannot associate with Hg distribution in native *B. parvus*. *B. parvus* showed a high ability to accumulate THg and MeHg in both leachate-contaminated and reference forest soils. Comprehensive findings indicated that the role of *B. parvus* in MeHg production is not clear, and it is possible that the MeHg in *B. parvus* was firstly formed within forest soils and then accumulated in their tissues. Considering the exchangeable Hg fraction (Hg-w, Hg-h) were the high mobility and potential bioavailability than other Hg fraction in soil, the ss-Na_2S ex situ treatment may be developed a cost-effective Hg immobilization and washing process for removing or reducing exchangeable Hg fraction (Hg-w, Hg-h) and non-mobile Hg fraction (Hg-s) from Hg-containing LLCS for reducing or eliminating the Hg potential environmental risks from leaching Hg, simultaneously.

Keywords:

Landfill-Leachate-Contaminated Soils (LLCS); Mercury (Hg) Fractionations, Spatial Distribution; Earthworms; Bioaccumulation; pH-dependent Static leaching; Sodium Sulfide Solution (ss-Na_2S); Washing Removal.

Contents

CHAPTER 1

Spatial distribution characteristics of mercury in the soils and native earthworms (*Bimastos parvus*) of the leachate-contaminated zone around a traditional landfill

Citation:

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★ Chapter Profile - 1

The contents and spatial distribution of mercury (Hg), including soil-Hg fractionation and Hg-containing native earthworm *Bimastos parvus* (*B. parvus*) species, were investigated in the leachate-contaminated zone of a large traditional landfill, Japan.Soil-Hg was fractionated into 5 categories: F1/water soluble Hg (Hg-w), F2/human stomach acid soluble Hg (Hg-h), F3/organic-chelated (Hg-o), F4/elemental Hg (Hg-e), and F5/mercuric sulfide (Hg-s). The total mercury (T-Hg) and methylmercury (MeHg) of native *B. parvus*, and the geochemical properties of soils were examined in this study.

Soil T-Hg concentration ranged between 0.227 and 2.919 mg kg^{-1} dry weight (dw). The T-Hg and MeHg concentrations of *B. parvus* species ranged from 1.242 to 6.775 mg kg^{-1} dw and from 0.031 to 0.218 mg kg^{-1} dw, respectively. Percentages of soil-Hg fractions were in the order of F3/Hg-o > F4/ Hg-e > F5/ Hg-s > F1/Hg-w > F2 / Hg-h, and the fractions of Hg-o and Hg-e were 55.50% and 35.31%, respectively. Similar distributions and close correlations between the levels of *B. parvus* Hg and soil Hg-o, Hg-e, and Hg-s were observed in this study. The distribution of Hg in *B. parvus* was associated with soil organic matter (SOM) content and particle size (sand, clay); however, it was not correlated with Hg-w or Hg-h.

The results indicated that easily bioavailable and soluble Hg fractions (Hg-w, Hg-h) of the soil were not appropriate to illustrate the distribution of Hg in native *B. parvus*. Instead, the stable soil-Hg fractions (Hg-o, Hg-e, and Hg-s) demonstrated good relationships of spatial distribution with *B. parvus* Hg in leachate-contaminated soil. It is advisable to preclude the evaluation of Hg biological distribution using soluble Hg fractions only. Stable Hg fractions in leachate-contaminated soil should also be included for assessing the biological distribution of Hg in leachate-contaminated soils.

Keywords: Mercury, Soils, Distribution, Earthworms, Landfill

1. Introduction

Mercury (Hg) is a widely-distributed contaminant in the biosphere (air, water, and soil) and is considered as one of the most toxic species because of its adverse effects on the ecosystems and human health through subsequent biological magnification of methyl (Me)Hg compounds in the food chain (Barbosa et al., 2003; Feng and Qiu, 2008; Guédron et al., 2013). Hg is also considered as one of the top ten toxic elements for public health concern by the World Health Organization (WHO) (Da Silva et al., 2016). No beneficial biological function of Hg has been reported to date (Ernst et al., 2008). The major sources of environmental Hg contamination include natural deposit (e.g., geologic erosion, volcanoes, etc.) and extensive emissions owing to anthropogenic activities. Hg persists widely in the soil, water, air, and wildlife (Gworek et al., 2015).

Municipal solid waste (MSW) growth is a serious challenge to many developing urban areas worldwide. In 2002, it was estimated that on a global average, 2.9 billion urban residents generated about 0.64 kg MSW per person day^{-1}, which will increase to 1.2 kg MSW per person day^{-1} by 3 billion urban residents by 2012, and then to 1.45 kg MSW per person day^{-1} by 4.3 billion urban residents by 2025 (Hoornweg D, 2012). It is estimated that about 51.6 million tons of MSW were generated in Japan in 1998, of which 78% was incinerated that contained about 16 tons of Hg (Takaoka et al., 2002).

There are many disposal methods for MSW based on physical, chemical, thermal or biological processes, such as landfills, incineration, composting, and recycling/recovery (Mukherjee et al., 2004). Traditional landfill remains the primary method to manage the disposal of MSW globally owing to technological simplicity, ease of use and cost-effectiveness. Landfills have been used for treating 70% of MSW disposal (Li et al., 2010; Zhu et al., 2013). Hg-containing items present in MSW landfill are difficult to avoid, which include used batteries, fluorescent lamps, ignition switches, medical waste, Hg amalgams, sewage sludge, and power plants waste (Mukherjee et al., 2004). In fact, Hg-containing MSW need special purification treatment before landfilling.

Once Hg MSW is buried in the landfill, it will be deposited as a long-term pollutant, and can be released into the surrounding air, water, and soil through gas emissions and leachate from landfill, which will eventually influence human health through several causal linkages directly and indirectly (Cheng and Hu, 2012; Ding et al., 2007; Franchini et al., 2004; Mukherjee et al., 2004; Porta et al., 2009). Hg concentration in large MSW landfills ranged between 0.17 and 46.22 mg kg^{-1} in Guiyang and 0.24 and 1.271 mg kg^{-1} in Wuhan City, China (Li et al., 2010), and 0.03 to 16.8 mg kg-1 in Florida, USA (Wang et al., 2012). Hg^0 concentration varied between 3.45 and 2952 ng m^{-3} in surface air, which was observed at the Nan-Ji-Do landfill in Korea (Kim and Kim, 2002) (Table S1-4).

Leachate-contaminated zone around the traditional landfill is gradually formed by the released leachate during the MSW decomposition and excess rainwater infiltrating from landfill. It has been reported that many environmental hazardous pollutants can be released into the soil through leachate from landfill, including heavy metals (e.g., Hg, Pb, Fe, Cu, Cd), dissolved macro organic matter, inorganic, and xenobiotic organic compounds (Peter et al., 2002). For Hg, the released content from landfill leachates had a wide alteration, whose T-Hg concentration ranged between 0.05 and 160 μg L^{-1} (Lee et al., 2016). A series of physicochemical conversions occurred when Hg was released into the soil by leachate. These reactions were influenced by environmental conditions such as bacteria, temperature, pH, and humic acid content.

Hg ecological effects are influenced by the formation of organic and inorganic Hg compounds, and with different mobility and bioavailability in the soil (Gavilán-García et al., 2008; Lindberg et al., 2001; Lovley, 2000; Nriagu, 1979). In particular, some Hg can be methylated into methyl (Me)Hg under anaerobic conditions of landfill, which will enhance bio-magnification in the terrestrial and aquatic food web and might cause adverse human health effects (Cristol et al., 2008; Rieder et al., 2011).

Earthworms play important roles in maintaining soil fertility and recycling organic matters (Tang et al., 2016), and also function as a key component of food chains for providing animal proteins to the predators in local ecosystems (e.g., many mammals, birds, fish, insects, and reptiles) (Han et al., 2012). It was reported that soil-Hg is the main source of bioaccumulation of Hg in earthworm, and the bioaccumulation of earthworm to Hg was affected by different soil-Hg levels (Burton et al., 2006; Han et al., 2012). Accumulated Hg in earthworms could be biological distributed in the trophic levels along the related food chain (Zhang et al., 2009). Currently, little is known about the relationships between the spatial distributions of soil-Hg fractions and Hg bioaccumulation in earthworms, despite the significant roles of earthworms to accumulate soil-Hg (Chen, 2010; Kamitani and Kaneko, 2007; Nahmani et al., 2007). To our knowledge, a few studies have been performed focusing on Hg fractions in leachate-contaminated soils. In addition, these studies mainly focused on the contamination of gas phase Hg emitted from landfill (Lee et al., 2016).

Although numerous studies focused on the adverse influence of Hg contaminants in soil, knowledge on the long-term transport tendency, spatial distribution, and biological transformation of Hg in landfills is limited (Bravo et al., 2014; Zhu et al., 2013). Furthermore, soil-Hg exists in different chemical forms that have different mobility, bioavailability, and toxicity to organisms (Issaro et al., 2009). Soil T-Hg determination could not completely delineate the biological behavior and risk to environmental organisms (Issaro et al., 2009; Pinedo-Hernández et al., 2015). Therefore, the status of Hg chemical forms and their accumulation in organisms needs to be further quantified. However, to understand and quantify Hg chemical forms in soils is an elusive goal, because the Hg specific compounds in conjunction with their interaction with the soil native matrix will determine the Hg mobility and bioavailability together under different environmental conditions (Bloom et al., 2003). Particularly, the alterations of Hg in complex environmental conditions of MSW landfill was hard to predict (Lee et al., 2016). Soil-Hg fractions were performed by the sequential extractions to better understand the different characteristics of Hg geological solid phase (e.g., possible mobility, eco-toxicological) in soils. For separating the “make sense” biogeochemical fraction, rather than getting the Hg specific compounds (Bloom et al., 2003).

To characterize Hg fractions, mobility, and bioavailability in leachate-contaminated soils, this study is a field investigation on the relationships between Hg fractions, and spatial distributions of native earthworms in a leachate-contaminated at a mercury polluted traditional MSW landfill. The main objective was to improve the understanding and prediction on the relationships between soil-Hg fractions, long-term variation trends, and potential toxicity risks in the leachate-contaminated soils. Our hypothesis is that soil-Hg fraction contents are diffused in small leachate ditches and scattered pools on the surface soil of the contaminated zone around the traditional MSW landfill, and another hypothesis is that Hg in earthworm tissues was accumulated via the gut uptake from the surface soil in the landfill-leachate-contaminated zone.

2. Materials and methods

2.1 Site description

The MSW landfill was built at the “Sanpou” mountain of Nagasaki, North-West of Kyushu island (32.876°N, 129.743°E), Japan in 1975 (Fig. 1-1). MSW were mainly incinerated ashes, industrial wastes, and building debris. During the early period, because of the lack of experience for monitoring environmental influences, nonstandard MSW were directly dumped into this landfill, which were also mixed with Hg-containing garbage (e.g., damaged household products, switch devices, fluorescent light, consumer electronics) after the local big flood of Nagasaki in 1982. In addition, proper sanitary treatment for preventing leachate leak was not considered at the beginning of the landfill construction. For these reasons, a leachate-contaminated zone was gradually formed around the MSW landfill, about 500 m below the landfill level. According to the environmental reports in 1998, the T-Hg concentrations in the leachate-contaminated soils ranged between 0.81 and 8.48 mg kg^{-1} (SanPouZan Commission of Water Environment Protection). These mercury values were extremely higher than that of local background levels (0.06 mg kg^{-1}) during the same period (Arisawa et al., 2000; Hidenori ITO, 2012).

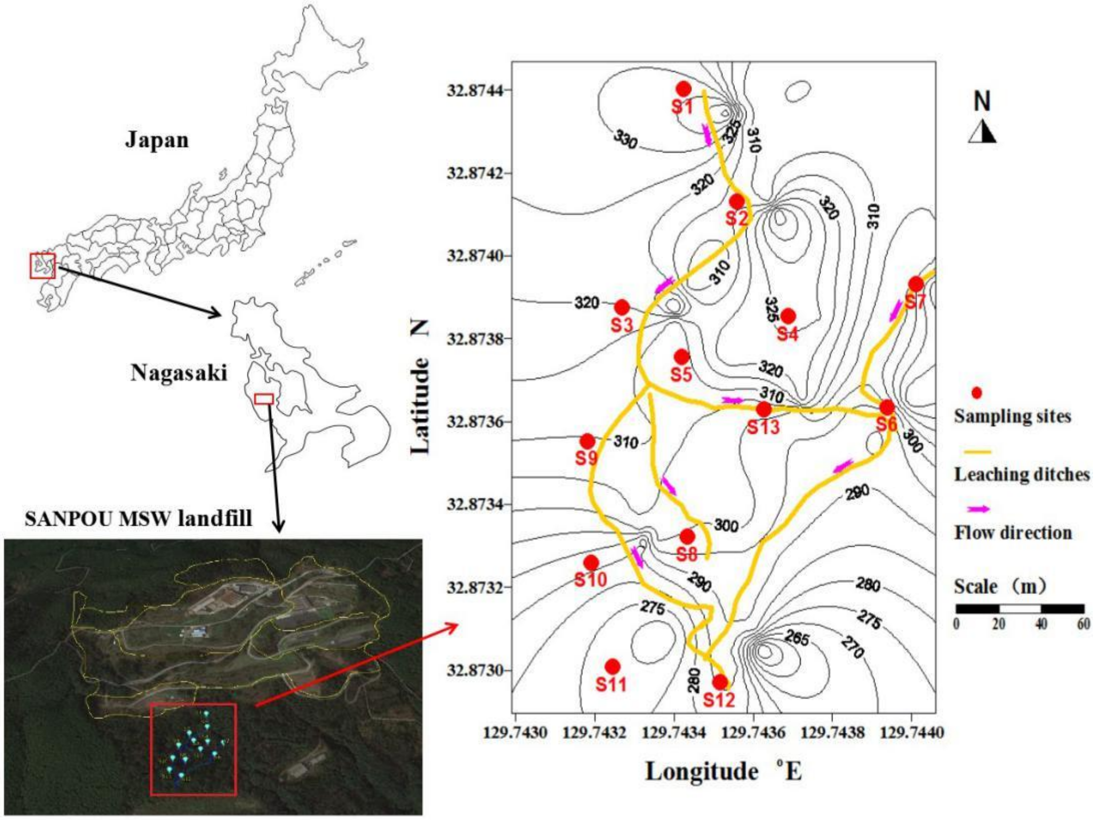


Fig. 1-1 Location of sampling sites.

Three small ditches and a few scattered pools were formed by the leachate and rainwater on the surface of the leachate-contaminated zone. The leachate flowed very slowly, about 1-10 cm depth in these ditches and pool zones, and the leachate wetlands were found at some places. This zone was also chosen for the soil-Hg survey in 1998. The control sites (C1-C3) for naturally occurring Hg background levels in the soil were selected in another mountain, about 1.6 km away from the study sites. Currently, the whole leachate-contaminated zone of MSW landfill and the control sites were covered with densely-grown trees of the local national forest.

2.2 Field sampling of soils and earthworms

The leachate-contaminated zone was approximately 250 × 180 m^2. The sampling sites were along the edge of the surface ditches and around the scattered pools formed by the leachate. A relatively soft and wet area devoid of rocks was selected and dug with small spades to search for earthworms. Earthworms and soils were sampled manually at an average depth of 0-15 cm and 0.25 m^2 at sampling sites (S1-S13) and control sites (C1-C3) using disposable gloves. The earthworms were kept in the soil samples collected from the same sites in polyethylene terephthalate (PET) bottle and brought to the laboratory under dark conditions (Yu and Yan, 2003). The sampling period was on November 2016. The weather was sunny, and the temperature was about 22 ± 4 °C (Fig. 1-1 and Fig. S1-1).

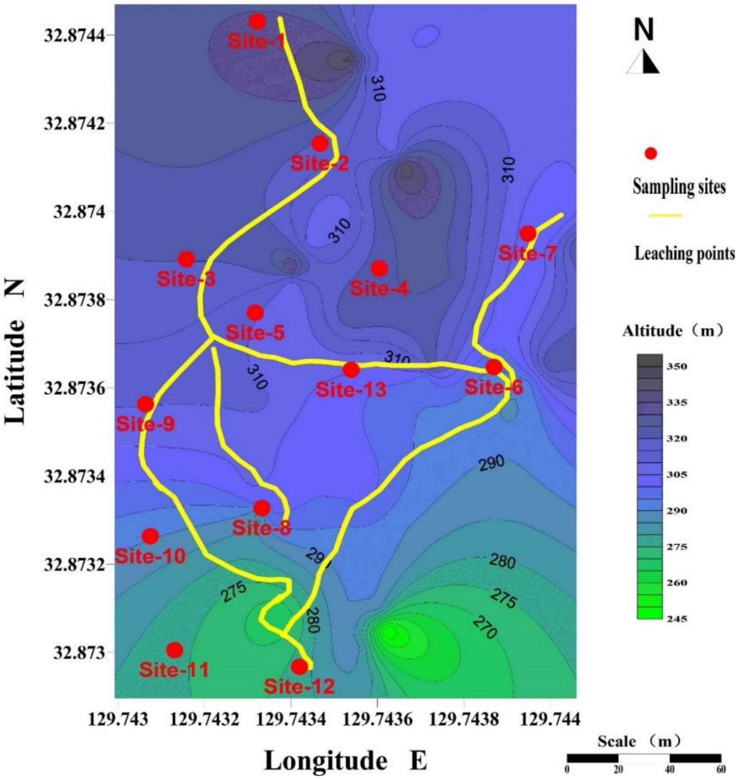


Fig. S1-1 Geomorphic feature of the sampling sites

2.3 Sample pre-treatment of earthworms and soils

The earthworms were picked up with clean plastic forceps and immobilized with deionized water to remove the adhering soil (Ernst et al., 2008; Hojdová et al., 2015; Zhang et al., 2009). After the enumeration of earthworms in the samples, mature earthworms (mature body with obvious clitella) were selected and placed on a glass plate with moistened filter papers at the bottom and used for identification (Fig. S1-2). Earthworms were kept at room temperature (22 ± 2 °C) in the dark for 3 days to empty their gut contents (Buch et al., 2017). These earthworms were identified, weighed, counted, and measured (Minamiya, 2014; Sims and Gerard, 1985).

Among the 13 sampling sites in the leachate-contaminated soil, S1 earthworms died during the period of pre-treatment for depurating the earthworm gut contents, because of our negligence that the moisture of the filter papers was not reduced for small quantity earthworms from S1 during pre-treatment. It is most likely that the skin of earthworms could not breathe well which leading to lead the death and decay during the pretreatment period. Hence, only earthworms from 12 sites were analyzed in this study. Mature earthworms with obvious developed clitella and lengths of 4.5 – 5.5 cm were selected for further analysis. These earthworms were frozen, freeze-dried (-20 °C), weighed and ground to a fine powder with agate mortar-pestle. Then, they were stored in PET tubes for subsequent analyses (Buch et al., 2017; Hojdová et al., 2015). Approximately, 1 kg of fresh soil collected by quartering techniques from each site was homogeneously mixed and weighed. Then, the sample was completely air dried under dark condition to a constant weight under room conditions (25 ± 3 °C temperature, ~40% humidity). The sample was weighed again after drying to determine the soil moisture. All dried soil samples were crushed by mortar-pestle and sieved with a 2-mm Nylon sieve prior to the analysis (Fig. S1-2). (Buch et al., 2017; Da Silva et al., 2016; Fernández-Martínez and Rucandio, 2013).

Fig. S1-2 Sample pre-treatment of earthworms and soils

2.4 Reagents and materials

All reagents used for analyzing Hg in soil and earthworm were analytical grade in this study. The $HgCl_2$ standard solution ($HgCl_2$ 1,000mg/L) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Glacial acetic acid, hydrochloric acid (HCl), 5M sodium hydroxide (NaOH), nitric acid (HNO_3), hydrogen bromide, methyl-isobutyl ketone (MIBK, atomic absorption spectrometry grade), copper chloride dehydrates ($CuCl_2 \cdot 2H_2O$), hexane and toluene (HPLC, analysis grade), L-Cysteine (L-Cys) were purchased from Kanto Chemical Co. (Tokyo, Japan). Sodium acetate trihydrate (NaOAc: $CH_3COONa \cdot 3H_2O$) were purchased from Merck (Darmstadt, Germany). Milli-Q water was prepared using an Ultra-pure water system (Merck Millipore, Tokyo, Japan). Fresh

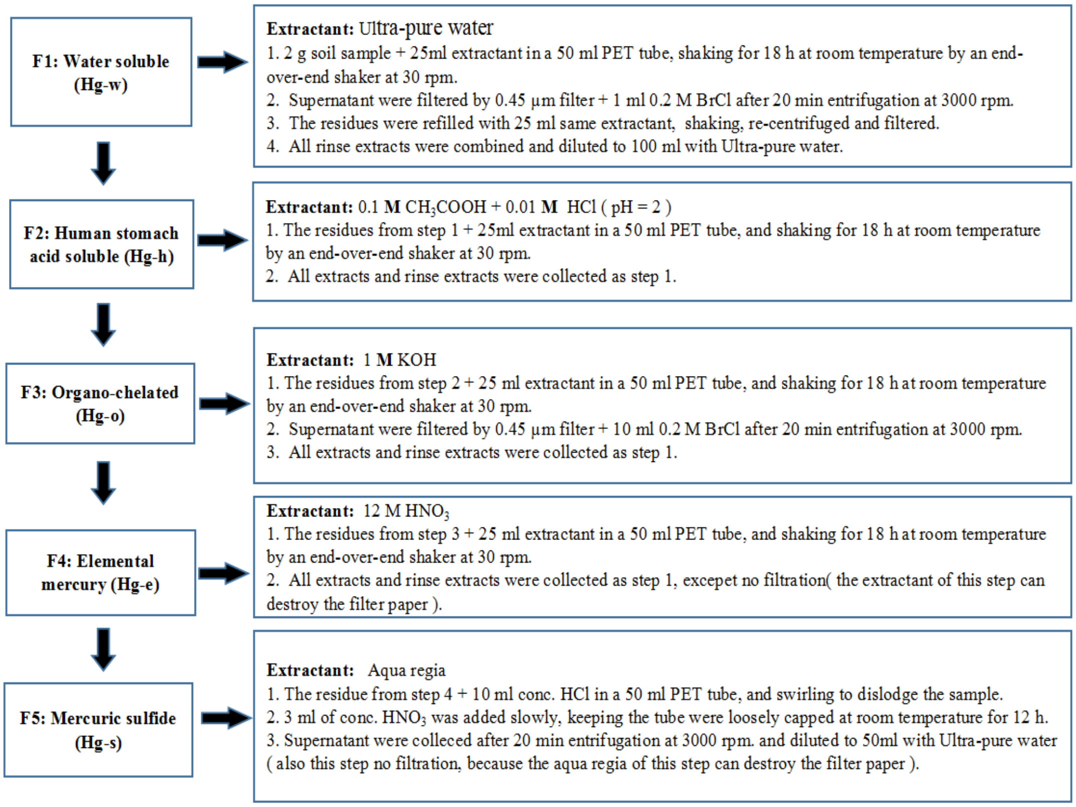
0.1% L-Cysteine solution and 0.2% L-Cysteine +2%NaOAc solution were prepared for each experiment. All analytical procedure and extraction materials (beaker, vessels, centrifuge tubes, etc.) were conducted ultra-clean handling to avoid cross-contamination and then soaking and washing with a detergent (Extran MA02, Merck, Ltd.) and Milli-Q water rinsing 3 times. And then following with soaking into 20% HNO_3 24h and Milli-Q water rinsing 3 times. Finally, all materials were air-dried in clean environment.

2.5 Sample analysis

Soil properties including the soil pH value was measured in 0.01 mol/L $CaCl_2$ with 1:2.5 (soil: extractant solution ratio) using a pH meter (Cyberscan pH 1100, Eutech instruments), and the electrical conductivity (EC) was determined in Milli-Q water with 1:2 (soil: Milli-Q water ratio) using a conductivity meter (B-771 Compact conductivity Meter, HORIBA Ltd., Kyoto, Japan) (Pannu et al., 2014). The classification of soil particle size distribution (PSD) followed the USDA Texture Classes, and the particles were divided into three classifications by using a 0.053-mm sieve. The particles were characterized according to their size as: sands (2.0-0.05 mm); silts (0.05-0.002 mm); and clays (<0.002 mm), following the simplified method for soil sample PSD analysis (Kettler and Doran, 2001).

The total organic carbon (TOC) and total nitrogen (TN) in the soils were measured using an elemental analyzer (Flash Elemental analyzer EA1112Series). The freeze-dried and sieved soils were treated by 2N HCl to remove inorganic carbonate, and dried again under vacuum condition. The TOC and TN were measured according to previously reported methods (Tsutsumi et al., 2015). Glycine expression was used as a standard to calculate the content of TOC and TN. The soil organic matter (SOM) content and the loss on ignition (LOI) was determined by igniting the dry soil for 2 h at 550 °C in a muffle furnace (ISUZU AT-S13) (Guédron et al., 2013; Haris et al., 2017; Heiri et al., 2001). The major chemical components of SiO_2, Al_2O_3, Fe_2O_3, CaO, and MnO in the powdered soil samples (S2, S4, S6, S8, S11) were analyzed in the lab of Asahi Environment Analysis Center in Kumamoto (Japan) for X-Ray Diffraction (XRD) (Fernández-Martínez et al., 2015).

The extraction procedures of soil-Hg fractionation followed a validated five-step sequential extraction scheme (Fig. S1-3) (Bloom et al., 2003). The soil-Hg was divided into 5 extract fractions (F1-F5) to independently determine the T-Hg content, including fraction of water soluble Hg (F1/Hg-w), fraction of human stomach acid soluble Hg (F2/Hg-h), fraction of organic-chelated (F3/ Hg-o), fraction of elemental Hg (F4/Hg-e), and fraction of mercuric sulfide (F5/Hg-s). Except F5 extraction, 1.25 mL of 0.2 M bromine monochloride (BrCl) solution was used as an oxidant to preserve T-Hg in F1, F2, and F4 extractions, and for F3 extraction, 10mL 0.2 M BrCl solution was used, because of its higher acid neutralizing capacity.



F1: Water soluble (Hg-w)

Extractant: Ultra-pure water

1. 2 g soil sample + 25ml extractant in a 50 ml PET tube, shaking for 18 h at room temperature by an end-over-end shaker at 30 rpm.
2. Supernatant were filtered by 0.45 μm filter + 1 ml 0.2 M BrCl after 20 min entrifugation at 3000 rpm.
3. The residues were refilled with 25 ml same extractant, shaking, re-centrifuged and filtered.
4. All rinse extracts were combined and diluted to 100 ml with Ultra-pure water.

F2: Human stomach acid soluble (Hg-h)

Extractant: 0.1 **M** CH_3COOH + 0.01 **M** HCl (pH = 2)

1. The residues from step 1 + 25ml extractant in a 50 ml PET tube, and shaking for 18 h at room temperature by an end-over-end shaker at 30 rpm.
2. All extracts and rinse extracts were collected as step 1.

F3: Organo-chelated (Hg-o)

Extractant: 1 **M** KOH

1. The residues from step 2 + 25 ml extractant in a 50 ml PET tube, and shaking for 18 h at room temperature by an end-over-end shaker at 30 rpm.
2. Supernatant were filtered by 0.45 μm filter + 10 ml 0.2 M BrCl after 20 min entrifugation at 3000 rpm.
3. All extracts and rinse extracts were collected as step 1.

F4: Elemental mercury (Hg-e)

Extractant: 12 M HNO_3

1. The residues from step 3 + 25 ml extractant in a 50 ml PET tube, and shaking for 18 h at room temperature by an end-over-end shaker at 30 rpm.
2. All extracts and rinse extracts were collected as step 1, excepet no filtration(the extractant of this step can destroy the filter paper).

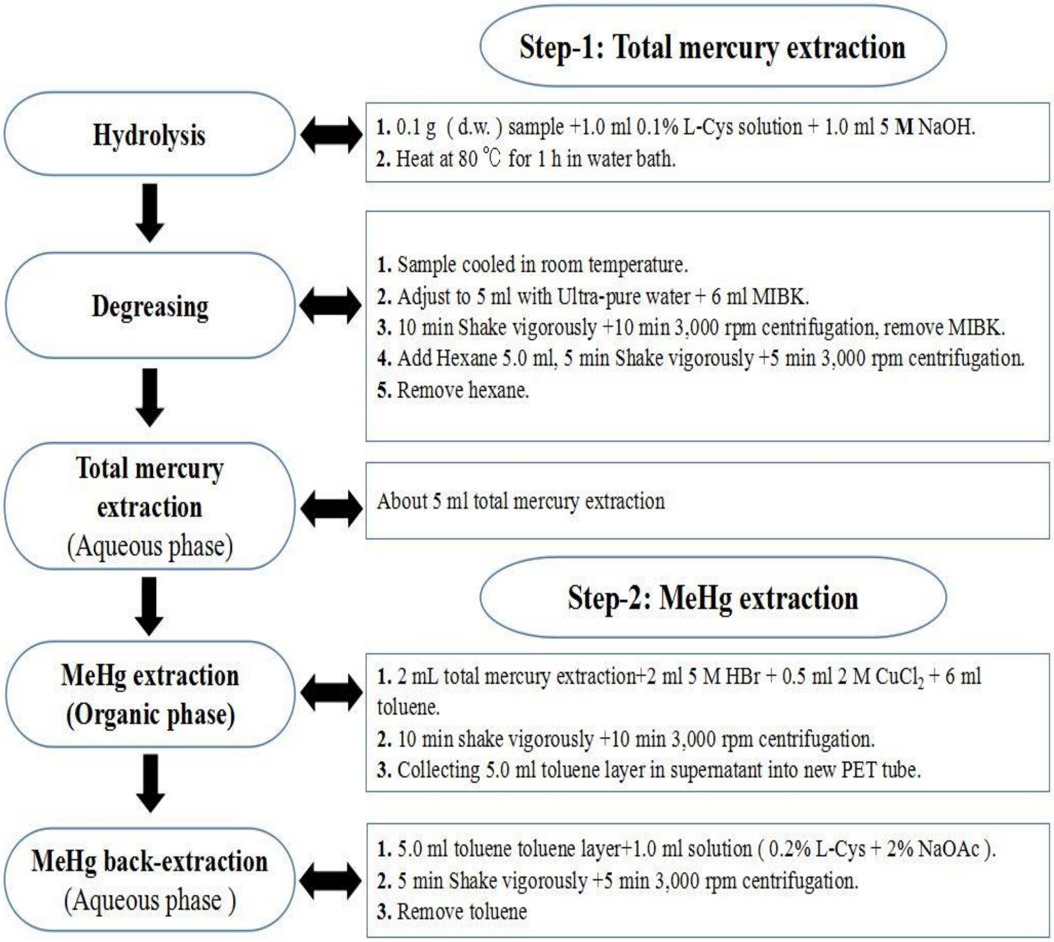
F5: Mercuric sulfide (Hg-s)

Extractant: Aqua regia

1. The residue from step 4 + 10 ml conc. HCl in a 50 ml PET tube, and swirling to dislodge the sample.
2. 3 ml of conc. HNO_3 was added slowly, keeping the tube were loosely capped at room temperature for 12 h.
3. Supernatant were colleced after 20 min entrifugation at 3000 rpm. and diluted to 50ml with Ultra-pure water (also this step no filtration, because the aqua regia of this step can destroy the filter paper).

Fig. S1-3 Flow diagram of the sequence extraction for soil mercury fractions

The earthworms were pre-treated in 15 mL polypropylene (PP) tubes to extract the T-Hg and MeHg, according to certain procedures as shown in Fig. S1-4 (Nakano et al., 2010; Yoshimoto et al., 2016). Each sample of an aliquot of approximately 0.1 g dw was weighed out in triplicate, and transferred to a 15 mL PP tube with screw caps. After adding 1.0 mL 0.1% L-Cysteine (L-Cys) solution and 1.0 ml 5 M NaOH, the sample was then heated at 80 °C for 1 h in a water bath and then cooled to room temperature. T-Hg in the earthworm samples was extracted using pure water, methyl isobutyl ketone (MIBK), and hexane (Step-1). Then, MeHg in the earthworm samples was extracted from the extraction product of step-1 using 5 M HBr, 2 M $CuCl_2$, toluene, L-Cysteine (0.2%), and NaOAc (2%) solution (Step-2).



1. 0.1 g （d.w.) sample +1.0 ml 0.1% L-Cys solution + 1.0 ml 5 **M** NaOH.
2. Heat at 80 ℃ for 1 h in water bath.

1. Sample cooled in room temperature.
2. Adjust to 5 ml with Ultra-pure water + 6 ml MIBK.
3. 10 min Shake vigorously +10 min 3,000 rpm centrifugation, remove MIBK.
4. Add Hexane 5.0 ml, 5 min Shake vigorously +5 min 3,000 rpm centrifugation.
5. Remove hexane.

About 5 ml total mercury extraction

1. 2 mL total mercury extraction+2 ml 5 M HBr + 0.5 ml 2 M CuCl$_2$ + 6 ml toluene.
2. 10 min shake vigorously +10 min 3,000 rpm centrifugation.
3. Collecting 5.0 ml toluene layer in supernatant into new PET tube.

1. 5.0 ml toluene toluene layer+1.0 ml solution (0.2% L-Cys + 2% NaOAc).
2. 5 min Shake vigorously +5 min 3,000 rpm centrifugation.
3. Remove toluene

Fig. S1-4 Flow diagram for extracting T-Hg and MeHg in *B. parvus* tissues

The concentrations of T-Hg in the soil extracts and the concentrations of T-Hg (Step-1) and MeHg (Step-2) in the earthworm extracts were determined in triplicate using a direct thermal decomposition Hg analyzer MA-3000 (Nippon Instruments Corporation, Tokyo, Japan). The MA-3000 uses the principle of thermal decomposition, gold amalgam collecting, and cold-vapor atomic absorption spectroscopy, the concentration of total mercury in sample can be determined, only directly prepared and put into sampling boats. Hg content of the sequential extract solutions in each step (F1-F5) were acidified using H_2SO_4 (1:1 in volume). $SnCl_2$ solutions (10%) were added to reduce Hg ion. After that, the contents were immediately determined by reducing-vaporization atomic absorption measurements of MA3000 (Fig. S1-5). The MA-3000 uses the principle of thermal decomposition, gold amalgam collection, and cold-vapor atomic absorption spectroscopy (wave-length 253.7 nm). The samples were directly put into sampling boats to measure the Hg contents. We confirmed 0.1 ng Hg as the detection limit of MA3000 in this experiment.

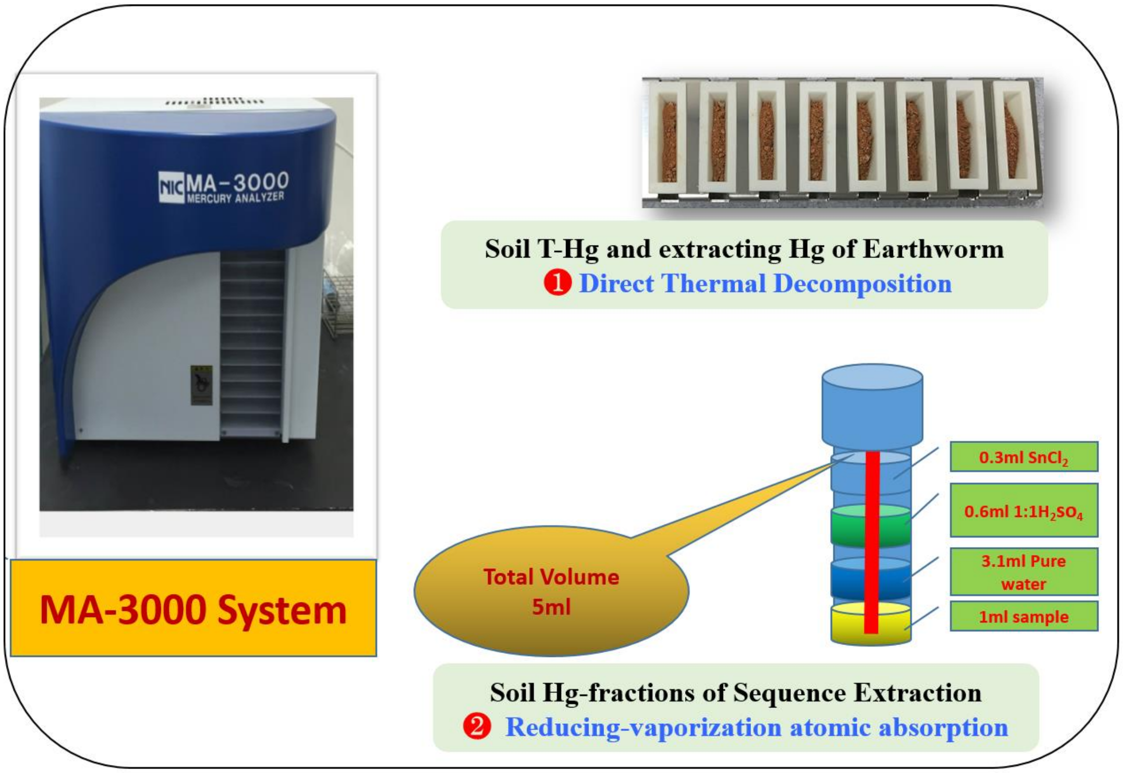


Fig. S1-5 Mercury analysis schematic of direct thermal decomposition and reducing-vaporization atomic absorption in MA 3000.

2.6 Quality assurance (QA) and quality control (QC)

Reagents of analytical grade were used for the analyses. Analytical operations (e.g., beaker, vessels, and tubes) were conducted with ultra-clean handling to avoid any possible contamination: soaked in Merck Extran detergent 5% for 24 h and immobilized with tap water 3 times, and finally soaked in 10% HNO_3 for 24 h. All items were rinsed 3 times with Milli-Q water, and then air-dried in a clean environment to avoid cross-contamination.

The accuracy and precision of analyses were validated by measuring the Hg content in duplicate of the subsamples, blanks, and verified recovery of certified reference materials (CRMs). All CRMs were purchased from the National Metrology Institute of Japan (NMIJ). The swordfish tissue (7403-a No.165) and codfish tissue (7402-a No.250) were used for the extraction analysis of T-Hg and MeHg. The marine sediments (7302-a No. A-0210) and estuarine sediments (7303-a No. E-0190) were used as the CRMs for the sequential extraction of soil T-Hg (Bloom et al., 2003; Zagury et al., 2006). Three replicates (n = 3) of the samples, and random intervals of CRMs and blanks were determined in each series of the sample analyses. Blanks were used to confirm the purity of the reagents and to correct the results of the analysis. Average values were used in statistical and modeling processing. In addition, the largest value of deviations was excluded in the calculation of mean values if the coefficient of variation (CV) of the replicates (n = 5) was more than 0.3. The analytical calibration curves were set up with different volumes of standard $HgCl_2$ solutions (0.1 mg L^{-1} and 1 mg L^{-1}), prepared using 1000 mg L^{-1} $HgCl_2$ standard solution. The range of calibration curve was from 0 to 200 ng (0, 2, 5, 10, 20, 50, 100, and 200 ng).

The expected recovery was within 95% confidence limits of the recommended standard T-Hg/MeHg values for the above certified materials (n = 6). The extraction recovery of T-Hg ranged between 90.2% and 105.4% (mean: 95.4%), and that of MeHg ranged between 93.9% and 113.8% (mean: 102.3%). Therefore, we used 102.3% as the correction factor for the calculation of MeHg concentration extracted from the earthworm samples (Nakano et al., 2010; Yoshimoto et al., 2016). The recovery of sequential extractions of Hg had a range of 96.7 – 103.3% in the sediment CRMs, and no statistically significant differences ($p > 0.05$) were found between the total Hg in the extracted fractions and the total Hg in the digest sediment CRMs of the sequential extraction. Hg concentrations in the reagents and blanks were always below the detection limit for the analysis. The accuracy and precision of the methods were larger than 95%.

The limit of detection (LOD) of instrument for T-Hg and MeHg were defined as 3 times the standard deviation (SD) signal-to-noise ratio and limit of quantitation (LOQ) as 10 times signal-to-noise ratio of the lowest detectable concentration of mixture of standards. The LOD was 0.2 ng/g d.w. (dry weight) and the LOQ was 0.6 ng/g d.w.. By reducing-vaporization atomic absorption measurements of MA3000, the LOD for T-Hg was 1 ng/L, and the LOQ was 3 ng/L in this study (Watanuki et al., 2015).

2.7 Statistical analysis

The differences of the average and normal distribution mean values of T-Hg were evaluated with Student’s *t*-test. In cases where the differences did not pass the t-test, Wilcoxon’s test was used. SPSS version 21.0, Graph pad Prim version 5.01, and Excel 2013 were used for statistical analysis. All data were presented as means ± standard deviation (SD). Spatial distribution treatment was modeled by Surfer Golden Software version 8.0 (Golden Software, Inc., Colorado), and the linear variogram interpolation method of universal kriging was used for the soil and earthworm samples to map the areal distribution to T-Hg, MeHg in *B. parvus*, and soil T-Hg, Hg fractions, PSD, and SOM (Teršič et al., 2011). Spearman’s correlation was used for assessing the relationships among the variables in soils and earthworms. Correlation coefficients (r) and values of significance probability (p) of less than 0.05 were applied as criterion for significance in the statistical analyses.

3. Results

3.1 Characteristics of soils and native earthworms

All soil samples had pH values between 4.08 and 5.57 (median: 4.82), indicating that the soils of the study zone were highly acidic. EC values were also large in the range of 66.67 – 110.00 mS/m (median 86.00 mS/m). The lowest SOM content was 4.82% (S1) and the highest was 17.59% (S3). The C/N ratio varied from 11.66 to 20.00 (Table 1-1). Abundant mineralogical components were similar in the soils. The contents of SiO_2, Al_2O_3, Fe_2O_3, and CaO in percentage ranged between 63.19 and 66.01 (median: 63.79), 20.38 and 21.93 (median: 20.99), 3.97 to 6.12 (median: 5.15), 0.40 to 0.89 (median: 0.6), respectively, except that of MnO, which had a relatively wider range of 0.08 – 1.7 (median: 0.3). The relative contents of sands, silts, and clays in the soils ranged from 28.27 – 60.73 % (median: 49.18%), 12.9 – 33.6% (median: 20.74%), and 13.76 – 48.59% (median: 32.66%), respectively (Table 1-1). From the spatial distribution of SOM and soil PSD, SOM and clays displayed similar spatial distributions. Their contents were high in the central, north-west, and southwest parts of the zone, and the distribution of sands was inverse to those of SOM and clay (Fig. 1-2).

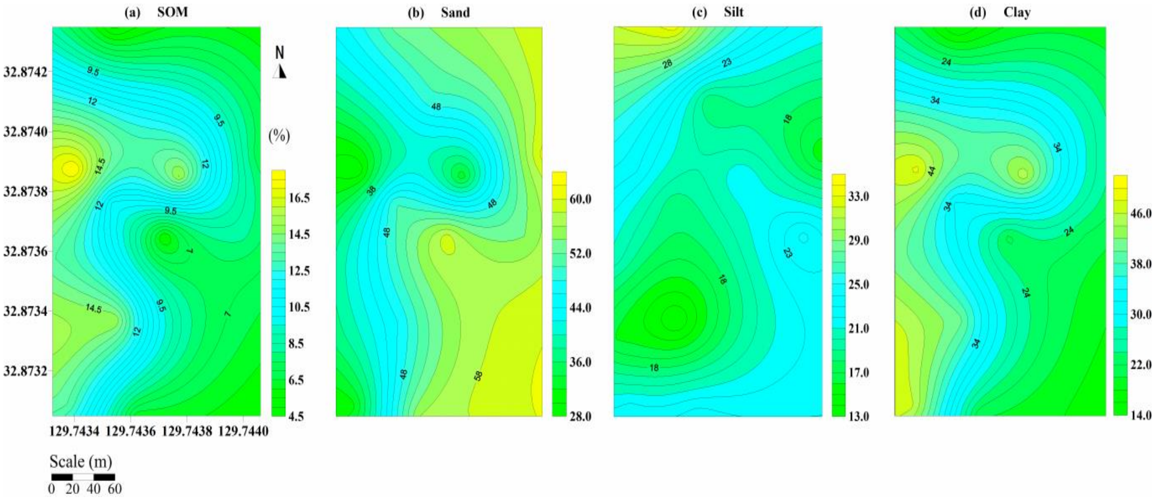


Fig. 1-2 Spatial distributions of (a) SOM, and soil particle sizes: (b) sands, (c) silts and (d) clays.

Two earthworm species were identified: *B. parvus* and *Dendrobaena octaedra* sp. (*D. octaedra*) (shown in Table 1-1). *B. parvus* (count 339) was abundant and found in all sites (S1-S13). There were merely three *D. octaedra*, and were found at 2 sites (S4 and S5) only. Therefore, earthworms belonging to *B. parvus sp.* were the dominant species and were treated as the native earthworm species in the leachate-contaminated zone of MSW landfill.

Table 1-1 Sampling locations, earthworm species, and geochemical properties of the soil samples.

Sites	Longitude (E)	Latitude (N)	Altitude (m)	Earthworm species (Count)	[1]SOM (%)	[2]C/N	SiO_2 (%)	Al_2O_3 (%)	Fe_2O_3 (%)	CaO (%)	MnO (%)	[3]EC (mS/m)	pH	[4]PSD		
														Sand (%)	Silt (%)	Clay (%)
S1	*129.7435*	*32.8743*	*350*	*B. parvus* (3)	4.82	11.66	n.a.[5]	n.a.	n.a.	n.a.	n.a.	73.67	*5.57*	52.64	33.60	13.76
S2	*129.7437*	*32.8741*	*345*	*B. parvus* (13)	8.82	19.04	66.01	20.99	3.97	0.60	0.16	78.67	*4.58*	49.18	18.16	32.66
S3	*129.7434*	*32.8739*	*339*	*B. parvus* (43)	17.59	12.96	n.a.	n.a.	n.a.	n.a.	n.a.	80.67	*4.55*	28.27	23.14	48.59
S4	*129.7438*	*32.8739*	*325*	*B. parvus* (60) *D. octaedra* (1)	9.15	15.29	63.35	21.20	6.12	0.42	0.08	78.00	4.82	32.62	21.93	45.45
S5	*129.7435*	*32.8738*	*325*	*B. parvus* (35) *D. octaedra* (2)	9.39	14.49	n.a.	n.a.	n.a.	n.a.	n.a.	86.00	4.91	49.94	18.37	31.69
S6	*129.7440*	*32.8737*	*326*	*B. parvus* (38)	8.98	15.87	63.19	21.93	6.03	0.71	0.35	93.33	4.94	53.92	24.32	21.76
S7	*129.7441*	*32.8739*	*326*	*B. parvus* (20)	6.99	18.60	n.a.	n.a.	n.a.	n.a.	n.a.	93.00	4.84	60.73	15.19	24.08
S8	*129.7435*	*32.8734*	*305*	*B. parvus* (21)	10.92	18.45	63.79	20.38	5.15	0.89	1.70	86.33	5.15	46.19	12.90	40.91
S9	*129.7433*	*32.8736*	*313*	*B. parvus* (22)	13.52	16.29	n.a.	n.a.	n.a.	n.a.	n.a.	110.00	4.82	38.30	20.74	40.96
S10	*129.7433*	*32.8733*	*280*	*B. parvus* (24)	15.29	15.10	n.a.	n.a.	n.a.	n.a.	n.a.	81.00	4.63	37.17	15.86	46.97
S11	*129.7434*	*32.8731*	*265*	*B. parvus* (18)	13.42	20.00	65.94	20.45	5.04	0.40	0.30	91.00	4.18	30.95	22.35	46.70
S12	*129.7436*	*32.8730*	*242*	*B. parvus* (17)	6.20	15.58	n.a.	n.a.	n.a.	n.a.	n.a.	105.67	4.08	55.99	23.84	20.17
S13	*129.7437*	*32.8736*	*323*	*B. parvus* (25)	5.41	12.08	n.a.	n.a.	n.a.	n.a.	n.a.	66.67	4.96	59.15	19.43	21.42
Control soil for natural Hg background levels in this study																
C1	*129.7533*	*32.8857*	*371*		9.78	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	46.03	4.58	4.58	45.37	25.32
C2	*129.7545*	*32.8840*	*342*		8.76	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	49.47	5.21	5.21	38.19	29.46
C3	*129.7551*	*32.8839*	*337*		10.55	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	41.23	4.76	4.76	37.93	28.31

Note:

1. SOM: Soil organic materials.
2. C/N: Total organic carbon / Total nitrogen.
3. EC: Electrical conductivity.
4. PSD: Soil particle size distribution (Sands: 0.050 < % < 2 mm, Silts: 0.002 < % < 0.050 mm, Clays: % <0.002 mm).
5. n.a.: Data not available.

Table 1-2 Concentrations (mean values ± standard deviation mg kg^{-1} dw) of T-Hg, Hg fractions in soil, and Hg (T-Hg, MeHg) in *B. parvus* earthworms.

No.	[1]Soil Hg						[2]Total Fractions	[3]Recovery%	Earthworms (*B. parvus*)	
	T-Hg	Hg-w	Hg-h	Hg-o	Hg-e	Hg-s			T-Hg	MeHg
S1	1.1410 ± 0.1540	0.0009 ± 0.0001	0.0010 ± 0.0001	0.1427 ± 0.0006	0.9433 ± 0.0123	0.0290 ± 0.0020	1.1169	97.89	[5]n.a.	n.a.
S2	0.2770 ± 0.0490	0.0007 ± 0.0001	0.0006 ± 0.0001	0.1717 ± 0.0050	0.0403 ± 0.0006	0.0493 ± 0.0012	0.2626	94.80	3.2260 ± 0.5130	0.0770 ± 0.0070
S3	2.7500 ± 0.2640	0.0014 ± 0.0001	0.0007 ± 0.0001	1.4307 ± 0.0541	1.1373 ± 0.0268	0.0793 ± 0.0012	2.6494	96.34	6.7750 ± 0.1710	0.2180 ± 0.0230
S4	0.4980 ± 0.0440	0.0009 ± 0.0001	0.0122 ± 0.0019	0.2960 ± 0.0053	0.1540 ± 0.0030	0.0210 ± 0.0010	0.4841	97.21	3.9310 ± 0.0580	0.0950 ± 0.0010
S5	0.4260 ± 0.0130	0.0009 ± 0.0001	0.0330 ± 0.0020	0.2380 ± 0.0079	0.1280 ± 0.0092	0.0210 ± 0.0010	0.4209	98.80	4.5000 ± 0.0430	0.0920 ± 0.0110
S6	1.2840 ± 0.0910	0.2494 ± 0.0153	0.0004 ± 0.0001	0.7880 ± 0.0121	0.2077 ± 0.0035	0.0257 ± 0.0006	1.2712	99.00	3.3630 ± 0.3280	0.0860 ± 0.0020
S7	0.2270 ± 0.0340	0.0173 ± 0.0030	0.0005 ± 0.0001	0.1513 ± 0.0184	0.0373 ± 0.0076	0.0233 ± 0.0042	0.2297	101.19	2.6210 ± 0.0660	0.0710 ± 0.0170
S8	2.9190 ± 0.2110	0.0022 ± 0.0001	0.0024 ± 0.0002	0.5347 ± 0.0177	1.9733 ± 0.0394	0.0620 ± 0.0114	2.5746	88.20	6.1680 ± 0.0650	0.1840 ± 0.0020
S9	1.8510 ± 0.2280	0.0167 ± 0.0021	0.0006 ± 0.0001	0.7893 ± 0.0194	0.8090 ± 0.0240	0.0803 ± 0.0047	1.6959	91.62	5.5340 ± 0.1550	0.1280 ± 0.0080
S10	2.8520 ± 0.1500	0.0205 ± 0.0047	0.0008 ± 0.0001	1.2923 ± 0.0236	1.0157 ± 0.0221	0.1347 ± 0.0090	2.4640	86.40	6.5510 ± 0.2680	0.1390 ± 0.0220
S11	1.3180 ± 0.1000	0.0011 ± 0.0001	0.0004 ± 0.0001	0.9500 ± 0.0085	0.2300 ± 0.0098	0.0603 ± 0.0071	1.2418	94.22	5.8010 ± 0.3440	0.1930 ± 0.0100
S12	1.0340 ± 0.0450	0.0284 ± 0.0036	0.0004 ± 0.0000	0.7570 ± 0.0038	0.1640 ± 0.0046	0.0458 ± 0.0060	0.9956	96.29	2.1060 ± 0.1990	0.0530 ± 0.0090
S13	0.2760 ± 0.0850	0.0004 ± 0.0001	0.0006 ± 0.0001	0.1940 ± 0.0075	0.0567 ± 0.0061	0.0197 ± 0.0015	0.2714	98.33	1.2420 ± 0.0670	0.0310 ± 0.0030
[6]Ave.	1.2964	0.0262	0.0041	0.5951	0.5305	0.0501		95.41	4.3182	0.1139
Natural Hg background levels in control soil										
C1	0.0740 ± 0.0010								0.3500 ± 0.0100	0.1180 ± 0.0090
C2	0.0840 ± 0.0030								0.4900 ± 0.0170	0.1060 ± 0.0230
C3	0.1030 ± 0.0060								0.4300 ± 0.0110	0.0890 ± 0.0150
Ave.	0.0870								0.4320	0.1040

Note:

1. Soil Hg: T-Hg (total mercury), Hg-w (water soluble Hg), Hg-h (human stomach acid soluble Hg), Hg-o (organic-chelated Hg), Hg-e (elemental Hg), Hg-s (mercuric sulfide Hg)
2. Total fractions=Hg-w + Hg-h + Hg-o + Hg-e + Hg-s
3. Recovery % = Total fractions / T-Hg of soil
4. MeHg % = MeHg concentration / T-Hg concentration of earthworm
5. n.a.: Data not available
6. Ave.: average

3.2 Hg contents in soils and *B. parvus* earthworms

As shown in Table 1-2 and Fig. S1-6, mean T-Hg concentration in the soils ranged from 0.2270 to 2.9190 mg kg^{-1} dw (median: 1.1410 mg kg^{-1} dw), which was much higher than that of the background soil in the control sites (ranged from 0.0740 to 0.1030 mg kg^{-1} dw with a median: 0.0870 mg kg^{-1} dw). The results indicated that Hg was rich in the soils, and had potential adverse effects on the biota in these areas. The concentrations of T-Hg in the samples was about one third of the level measured about 20 years ago in 1998 (ranged from 0.81 to 8.48 mg kg^{-1} dw). The decrease is the consequence of reducing rainwater leaching and filtering leachate-contaminated groundwater(SanPouZan Commission of Water Environment Protection).

T-Hg concentration in *B. parvus* tissues varied from 1.2420 to 6.7750 mg kg^{-1} dw (median: 4.2160 mg kg^{-1} dw), and MeHg concentration varied from 0.0310 to 0.2180 mg kg^{-1} (median: 0.0940 mg kg^{-1} dw). The percentages of MeHg when compared to T-Hg varied from 2.04 to 3.33% (median: 2.51%) in *B. parvus* tissues. The average concentrations of T-Hg and MeHg were 4.3180 mg kg^{-1} and 0.1140 mg kg^{-1} in all *B. parvus* tissues, respectively. Significant statistical differences in the Hg concentration were observed between the T-Hg and MeHg of *B. parvus* ($t = 7.869$, $p < 0.0001$), and between T-Hg and soil T-Hg of *B. parvus* from leachate-contaminated sites ($t = 4.975$, $p < 0.0001$) (Fig. S1-6). By contrast, the T-Hg concentration varied from 0.3500 to 0.4900 mg kg^{-1} dw (average: 0.4230 mg kg^{-1} dw), and MeHg concentration varied from 0.0890 to 0.1180 mg kg^{-1} (average: 0.1040 mg kg^{-1} dw) in *B. parvus* tissues from control sites (Table 1-2).

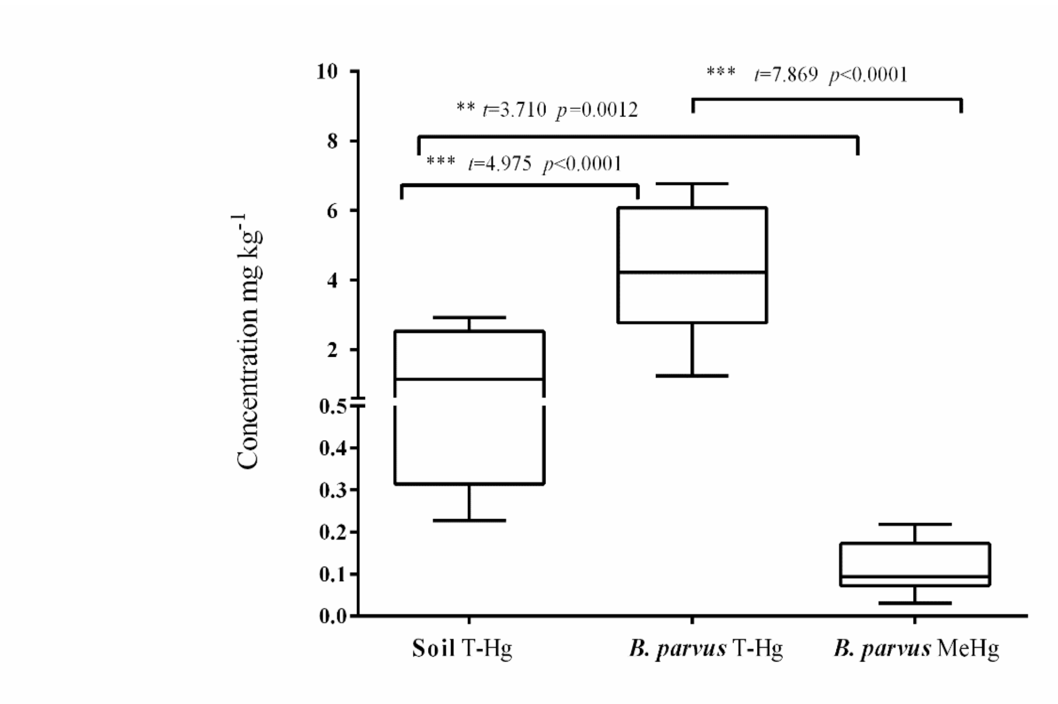


Fig. S1-6 Comparisons between the concentrations of soil T-Hg and *B. parvus* earthworm T-Hg and MeHg. The box extends from the 25th to the 75th percentiles and the line inside it is the median. The whiskers extend is the minimum and the maximum values.

The respective average concentrations of soil-Hg fractions of F1-F5 are shown in Table 1-2. F1/Hg-w ranged between 0.0004 and 0.2494 mg kg^{-1} (median: 0.0014 mg kg^{-1} dw), F2/Hg-h ranged between 0.0002 and 0.0330 mg kg^{-1} (median: 0.0006 mg kg^{-1} dw), F3/Hg-o ranged between 0.1427 and 1.4307 mg kg^{-1} (median: 0.3785 mg kg^{-1} dw), F4/Hg-e ranged between 0.0373 and 1.9733 mg kg^{-1} (median: 0.2077 mg kg^{-1} dw), and F5/Hg-s ranged between 0.0197 and 0.1347 mg kg^{-1} (median: 0.029 mg kg^{-1} dw). Maximum concentrations of F1/Hg-w, F2/Hg-h, F3/Hg-o, F4/Hg-e, and F5/Hg-s were observed in S6, S5, S3, S8, and S10, respectively. The percentages of the 5 Hg fractions in the soils were in the order of F3/Hg-o (12.78%–76.50%) > F4/Hg-e (15.35%–84.46%) > F5/Hg-s (2.02%–18.77%) > F1/Hg-w (0.05%–19.62%) > F2/Hg-h (0.03%–7.84%) at the 13 sites (Figs. 1-3a and 1-3b). The F3/Hg-o and F4/Hg-e were the dominant components in these samples, occupying 55.50% and 35.31% of the total Hg fractions, respectively (Fig. 1-3b).

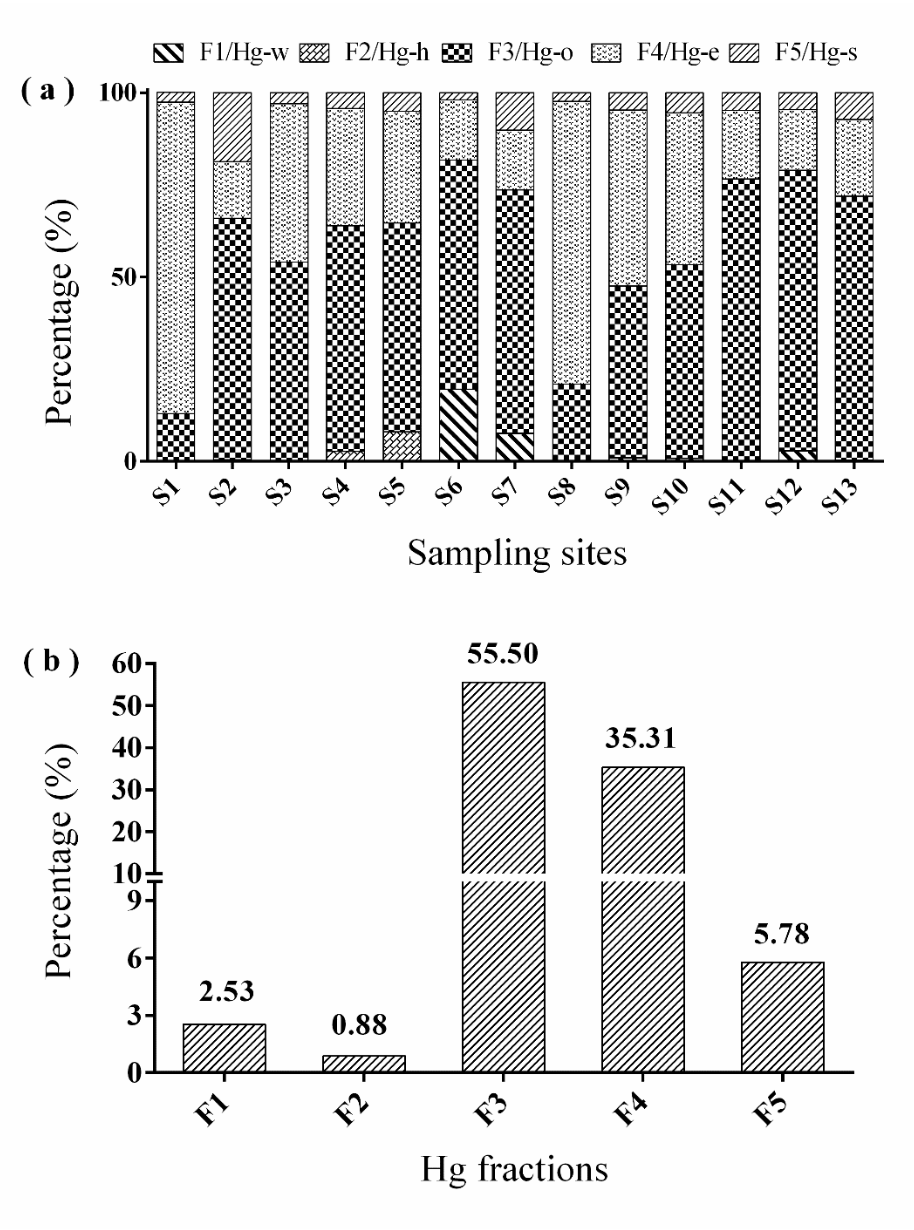


Fig. **1-3** Soil Hg fractions of sequence extraction, (a) Percentages of Hg fractions in each site, (b) Mean percentages of soil Hg fractions at all sites. Water soluble Hg (F1/Hg-w), Human stomach acid soluble Hg (F2/Hg-h), Organo-chelated Hg (F3/ Hg-o), Elemental Hg (F4/Hg-e), Sulfide Hg (F5/Hg-s)

The total amount of Hg fractions in each step of the sequential extraction was in good agreement with the soil T-Hg, and the recovery ratio was suitable for the analysis, which ranged between 86.40 and 101.19% (mean: 95.41%) in all the soil samples (Table 1-2). The recovery ratio was in line with the previously reported ratio of extracting Hg from contaminated soil, which ranged from 94.3 to 125.1% (Liu et al., 2006). The reason for the recoveries of more than 100% can be explained by the factors of soil heterogeneity. Hg presence in soils is not homogenous. It is likely that the aliquot soil does not contain similar Hg content as that of T-Hg. Recoveries lower than 100% can be attributed to two reasons: lacking homogeneity and losing as volatile during the process of experiment (Reis et al., 2010).

Hg-w and Hg-h constituted 2.53% and 0.88% of T-Hg in all the soils (Fig. 1-3b), respectively. Previous studies suggested that 0.13 mg kg^{-1} was a specifically critical limit for inorganic Hg (Hg^{2+}) content (Tipping et al., 2010). Below this value, Hg does not have harmful effects on soil organisms. The average concentrations of combined Hg-w and Hg-h ranged from 0.0010 mg kg^{-1} to 0.2498 mg kg^{-1}. About 7.7% (1/13) of the concentrations exceeded the critical limit. Therefore, the possibility of the effects of Hg-w and Hg-h on soil organisms was small in the study areas. F3/Hg-o (55.50%) and F4/Hg-e (35.31%) in the soils were abundant in all the samples (Fig. 1-3b). Similar results of dominant Hg fractions were also reported in the contaminated soil (Liu et al., 2006). However, this was different from F3/Hg-o, which was predominantly found in wetland as reported in other studies (Ramasamy et al., 2012). Hg-s, accounting for 5.78%, was the third highest, which included sulfide Hg and other stable forms associated with silicates (Bloom et al., 2003).

3.3 Spatial distributions of Hg in soils and *B. parvus* earthworms

The concentrations of T-Hg in the soils from the north-western area to the mid-west area were higher than that in the other areas (Fig. 1-4a). *B. parvus* earthworms in the north-western and south-western areas contained more T-Hg and MeHg than those in the north-central and south-eastern areas (Figs. 1-4b and 1-4c). According to the topography of the sampling sites (Fig. 1-1), soils and *B. parvus* having relatively higher T-Hg concentrations were found in the areas of low altitudes and downstream of the leachate ditches, indicating that soil T-Hg deposition was likely influenced by the altitude and surface ditches direction on spatial distribution (Fig. 1-1 and Fig. 1-4). Another possible reason for the higher concentrations of Hg in the soils and *B. parvus* was that the concentrations of SOM and clay in these areas were relatively high (Figs. 1-2a and 1-2d). Viewed from the topography of the concentration variation trends between the *B. parvus* earthworm Hg (T-Hg, MeHg) and soil T-Hg (Hg fractions). The *B. parvus* earthworm tissues Hg (T-Hg, MeHg) showed the similar spatial distributions with the soil T-Hg and stable soil Hg fractions (F3/Hg-o, F4/Hg-e and F5/Hg-s). However, they were different from those of the soil fractions F1/Hg-w and F2/Hg-h (Fig. 1-2 and Fig. 1-4). Moreover, T-Hg and MeHg in *B. parvus* also showed similar distributions of increase to those of SOM, and clays, and decrease with the sands content of soil particle size parameters. However, dissimilarities with silts were also observed in their spatial distributions (Fig. 1-2c and Fig. 1-4).

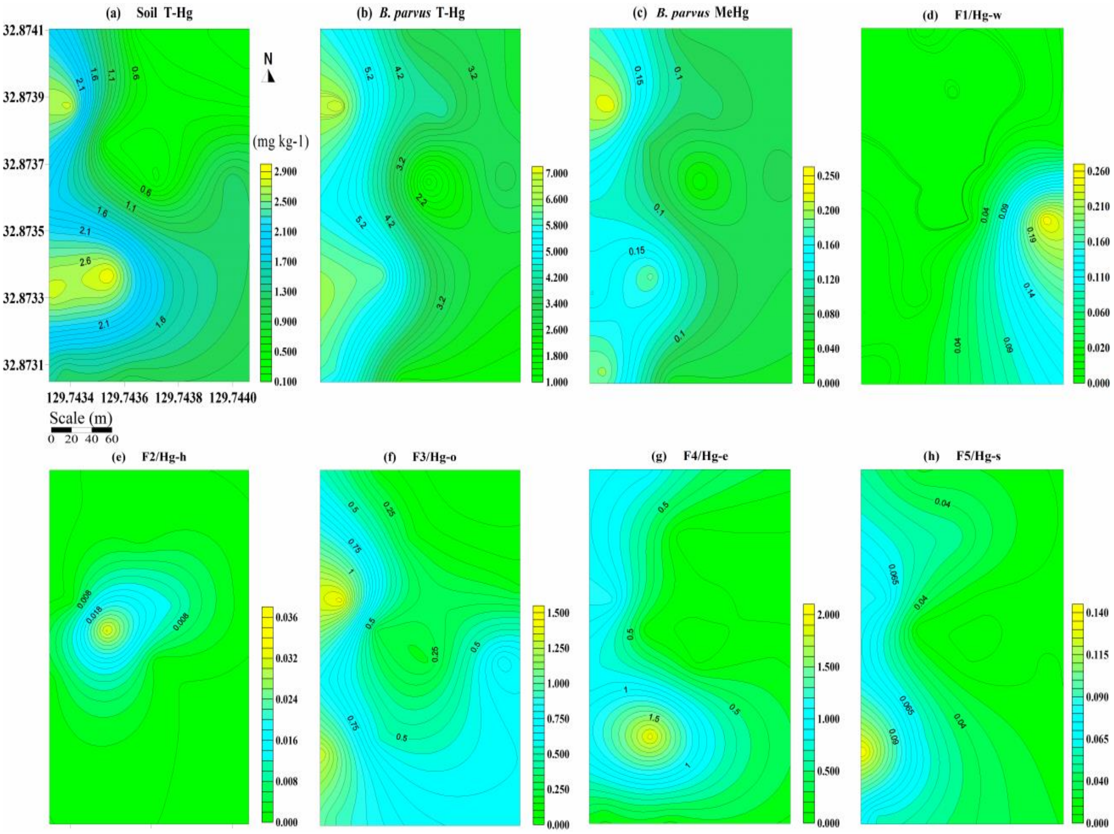


Fig. 1-**4 S**patial distribution of (a) soil T-Hg, (b) *B. parvus* T-Hg, (c) *B. parvus* MeHg, and soil Hg fractions: (d)Water soluble Hg (F1/Hg-w), (e)Human stomach acid soluble Hg (F2/Hg-h), (f) Organo-chelated Hg (F3/ Hg-o), (g) Elemental Hg (F4/Hg-e), (h) Sulfide Hg (F5/Hg-s).

3.4 Correlation analyses

Significant correlations were observed between the contents of *B. parvus*-Hg (T-Hg/MeHg) and soil-Hg fractions (Hg-o, Hg-e, Hg-s). However, no correlations were found among easily available soil-Hg fractions (Hg-w, Hg-h) and *B. parvus*-Hg (T-Hg/MeHg), (Table 1-3). These statistical correlations were supported by those findings that similar spatial distributions were observed between the concentration variation trends of *B. parvus* earthworm Hg (T-Hg, MeHg), soil T-Hg and stable Hg fractions (F3/Hg-o, F4/Hg-e and F5/Hg-s) viewed from the topography (Fig. 1-4).

The correlations of stable soil-Hg fractions were associated with SOM and particle size (sand, clay). Soil particles (sand, silt, and clay) settled as the main monitoring factor upon good shaking or stirring rate in extracted solutions of Hg selective extractions (Bloom et al., 2003). The coefficient of correlation (*r*) between SOM, F3/Hg-o, F4/Hg-e, and F5/Hg-s obtained in this study was consistent with the result that they showed similar trends in spatial distribution (Fig. 1-2a and Figs. 1-4f; 1-4g; 1-4h). Moreover, the content of sands had negative correlations with F3/Hg-o, F4/Hg-e, F5/Hg-s, SOM, respectively (Table 1-3), and their spatial distributions were also similar (Fig. 1-2d and Figs 1-4f, 1-4g, 1-4h). Soil T-Hg was positively correlated with clays and SOM, because their Hg binding ability was better than that of sands (Obrist and Johnson, 2009). These results suggested that SOM was involved in the processes of Hg cycling within Hg fractions in soils, which was in consistence with the strong correlation ($r^2 = 0.83$) found at a forest site (Obrist and Johnson, 2009; Ravichandran, 2004). The coefficient of correlation (*r*) between SOM, F3/Hg-o, F4/Hg-e, and F5/Hg-s obtained in this study appeared to be similar with regards to their spatial distribution (Fig. 1-4a and Figs. 1-4f, 4g, and 1-4h). Moreover, the content of sands had negative correlations with F3/Hg-o ($r = -0.670$, $p < 0.01$), F4/Hg-e ($r = -0.582$, $p < 0.05$), F5/Hg-s ($r = -0.591$, $p < 0.05$), SOM and ($r = -0.819$, $p<0.01$), respectively (Table 1-3), and their spatial distributions were also similar (Fig. 1-4d, 1-4f, 1-4g, and 1-4h). Apart from the first two easily available fractions F1/Hg-w and F2/Hg-h, soil T-Hg and most Hg fractions had good correlations with the SOM and clay contents.

pH values showed no significant correlation with all the Hg speciation fractions. This result is not in agreement with the reported literature. In general, the organic matter is effective sorbent for inorganic Hg retention in soil. It has been observed that improving pH decreased inorganic mercury (IHg) adsorption to soils. The possible reason may be led by pH values (4.08-5.57) which was the comparatively low and proximate composition. Also, can decreases the sorption of Hg in SOM because of acidic condition, and exchangeable Hg would be easily dissolved and released into environment and disturb the relationship between pH and those Hg speciation fractions. They maybe lose the tight combining capacity with mineral matrix into mobile and bio-accessible Hg phases. Especially can be released and migrated to other places with rainwater constantly, because of leachate infiltration zone is a slope. Besides, there is another explanation that Hg under the acid natural environment, migrate through “soil-to-plant” constantly transferred Hg to above-ground.

Soil T-Hg and most Hg fractions had good correlations with the SOM and clay contents, apart from the first two easily available fractions F1/Hg-w and F2/Hg-h in the extraction procedures. However, a few studies have also observed different phenomena in sediments (Ramasamy et al., 2012), where Hg fractions had a negative correlation with clay contents and positive correlation with sand. This may be attributed to the relatively low contents of SOM, which will help the Hg to dissolve with the reagents in sequential extraction, such as different solutions, extraction ratio and extraction time (Biester et al., 2002). In addition, the soil EC correlated only with soil F1/Hg-w and C/N. The pH values did not correlate with any soil-Hg fractions and soil properties (Table 1-3).

Table 1-3 Spearman's correlation coefficients (*r*) between the Hg contents in soils with earthworms (*B. parvus*), and soil general geochemical properties variables

Soil (n=13)	^{1}Soil Hg						Earthworm		^{2}SOM	^{3}C/N	^{4}EC (mS/m)	pH	^{5}PSD		
	T-Hg	Hg-w	Hg-h	Hg-o	Hg-e	Hg-s	THg	MeHg					Sand (%)	Silt (%)	Clay (%)
T-Hg	1.000														
Hg-w	.431	1.000													
Hg-h	.133	-.447	1.000												
Hg-o	**.747****	.492	-.194	1.000											
Hg-e	**.956****	.315	.239	**.610***	1.000										
Hg-s	**.812****	.415	-.086	**.669***	**.718****	1.000									
Ear. $_{\text{T-Hg}}$	**.839****	.123	.426	**.741****	**.825****	**.739****	1.000								
Ear. $_{\text{MeHg}}$	**.804****	.053	.319	**.734****	**.804****	**.683***	**.965****	1.000							
SOM	**.714****	.260	.189	**.830****	**.571***	**.696****	**.972****	**.937****	1.000						
C/N	.016	.254	-.478	.088	-.198	.264	-.028	.098	.181	1.000					
EC (mS/m)	.407	**.702****	-.333	.368	.236	.407	.224	.238	.396	**.626***	1.000				
pH	-.096	-.219	.423	-.550	.044	-.409	-.147	-.207	-.391	-.360	-.066	1.000			
Sand (%)	**-.648***	.094	-.306	**-.670***	**-.582***	**-.591***	**-.832****	**-.895****	**-.819****	-.088	-.011	.429	1.000		
Silt (%)	.066	.094	-.300	.148	.176	-.107	-.098	.007	-.225	-.363	-.209	-.118	-.044	1.000	
Clay (%)	.549	.044	.217	**.709****	.418	**.594***	**.881****	**.895****	**.918****	.231	.148	-.476	**-.890****	-.286	1.000

Note:

1. Soil Hg fractions: T-Hg (total mercury), Hg-w (water soluble Hg), Hg-h (human stomach acid soluble Hg), Hg-o (organic-chelated Hg), Hg-e (elemental Hg), Hg-s (mercuric sulfide Hg)
2. SOM: Soil organic materials.
3. C/N: Total organic carbon / Total nitrogen.
4. EC: Electrical conductivity.
5. PSD: Soil particle size distribution (Sands: 0.050 < % < 2 mm, Silts: 0.002 < % < 0.050 mm, Clays: % <0.002 mm).

4. Discussion

4.1 Hg in soils

Currently, there are no common understandings on the environmental toxicity limit standard for Hg levels in soils. It was hypothesized that soil T-Hg concentration exceeding 0.25 mg kg^{-1} was the consequence of anthropogenic activities (Nakagawa and Yumita, 1998). Among the 13 soil samples, T-Hg concentration in 12 samples exceeded 0.25 mg kg^{-1}. Besides, it is recommended that the probable effect concentration (PEC) of Hg should be 1.06 mg kg^{-1}; Hg pollution exceeding this limit is very harmful to organisms (MacDonald et al., 2000). There were 8 of the 13 soil samples wherein PEC exceeded its limit. The USA Environmental Protection Agency (USEPA 2013) recommended that inorganic Hg (IHg) limit [guidelines for Soil Screening Levels (SSL)] is 23 mg kg^{-1} for residential soils and 310 mg kg^{-1} for industrial soils (United States Environmental Protection Agency (USEPA), 2013). The SSL limits were also established for MeHg and elemental Hg in industrial soils (MeHg:100 mg kg^{-1}; elemental Hg: 43 mg kg^{-1}) and in residential soils (MeHg: 7.8 mg kg^{-1}; elemental Hg: 10 mg kg^{-1}) (Rimondi et al., 2012; United States Environmental Protection Agency (USEPA), 2013). The concentrations of the predominant soil-Hg fractions (F3/Hg-o, F4/Hg-e) in soils were much lower than that of the above limit levels. It must be noticed that the PEC of Hg is established to access sediments and is not directly applicable to soils in vicinities of MSW's landfill. The soil T-Hg levels in the landfill of this study met the USEPA industrial SSL. However, the present soil T-Hg levels were very high in comparison to the concentrations of the natural background soils (Table 1-2).

The distribution of T-Hg can be explained by the content of MnO in soils. It has been reported that soil-Hg are associated with Fe and Mn oxides in soils (Liu et al., 2006). The results of XRD analysis revealed that the contents of MnO had a relatively wider range (0.08 to 1.70 %). As expected, significantly high T-Hg concentrations were also found in S6 (1.284 ± 0.091 mg kg^{-1}), S8 (2.919 ± 0.211 mg kg^{-1}), and S11 (1.265 ± 0.100 mg kg^{-1}). Soils from those sites contained relatively high concentrations of MnO (0.35% in S6; 1.7% in S8; 0.3% in S11) than in the samples from other sites, which was probably related to the abundance of Fe/Mn minerals in S6, S8, S11 (Table 1-1).

Previous studies have reported that there was a good positive correlation between the concentration of metal ions and the EC in groundwater (Liu et al., 2003). High inorganic Hg contents coexist with high EC in soils (Rodríguez Álvarez et al., 2014). Our results also showed a good relationship between EC and F1/Hg-w (r = 0.702, $p < 0.01$). However, there was no correlation between EC and F2/Hg-h, which might be caused by the stable pH values (4.08 to 5.57) of the soils (Table 1-3). The EC of a sample is determined by the amount of soluble ion concentrations in the solution. The contents of F1/Hg-w in S6 (19.62%), S7 (7.53%), and S12 (2.85%) were high, and the EC values of the samples were also high (93.33 mS/m, 93.00 mS/m, 105.67 mS/m). Moreover, the EC values of the three soils with high F1/Hg-w contents were larger than those of the three soils with high F2/Hg-h contents (S5: 7.84%; S4: 2.52%; S2: 0.23%); the EC values of which were 86.00, 78.00, and 78.67 mS/m, respectively (Table 1-1). Unfortunately, the reasons for the relationships of EC with Hg-w and Hg-h and EC, as well as for the correlation between C/N and EC ($r = 0.626$, $p < 0.05$) are not clear.

Soil pH values did not show a significant correlation with any geochemical properties of the soils or *B. parvus* earthworms. This result was different from that of the previous studies (Dang et al., 2015). In general, organic matters are effective sorbents for inorganic Hg retention in soils (Teršič et al., 2011). It has been observed that large pH can decrease Hg adsorption to soils (Yin et al., 1996), and the efficiency of extracting Hg can be reduced because of high contents of clays minerals (Biester et al., 2002). However, our results did not have correlation between F4/Hg-e and clays (Table 1-3). This was possibly caused by the stable pH (4.08–5.57), which was relatively low, and similar mineral compositions of the soil samples. Hg-e may easily lose the tight capability of sorption and combination to mineral matrixes (e.g., SOM) into exchangeable Hg (mobile and bio-accessible phases) in acidic soils. Because of the slope of the leachate-contaminated zone, Hg can be easily dissolved and released into the environment, and in particular, can migrate to other places by rainwaters or floods. Besides, Hg-e can also migrate via “soil-to-plant” paths to move out of the soils (Reis et al., 2016). These processes could have resulted in the reduction of soil T-Hg concentrations in the past years.

4.2 Hg in native *B. parvus*

Previous investigations on T-Hg and MeHg in earthworms are relatively limited. Most studies did not measure MeHg in the earthworm tissues (Zhang et al., 2009). Many researches focused on other earthworm species (e.g., *Eisenia fetida, Lumbricus terrestris,* and *Lumbricus rubellus*) to determine soil T-Hg content at the polluted sites. Some studies were designed with laboratory experiments to measure Hg uptake (i.e., breeding earthworms with Hg spiked soil) or to predict the quantitative Hg relationships with body burden of earthworms (Le Roux et al., 2016; Nahmani et al., 2007; Rieder et al., 2011). We observed a strong positive correlation ($r = 0.965$; $p < 0.01$) between T-Hg and MeHg in *B. parvus*. This result was in accordance the observation on uptake of inorganic T-Hg and MeHg by *Lumbricus terrestris* (*L. terrestris*) in Hg (including stable isotopes) spiked soils (Rodríguez Álvarez et al., 2018; Rodríguez Álvarez et al., 2014). The average T-Hg concentration of *B. parvus* (4.318 mg kg^{-1}) was higher than those in *Epigeic* (0.49 mg kg^{-1}), *Endogeic* (1.63 mg kg^{-1}), *Anecic* (0.69 mg kg^{-1}), *Alolobophora* sp. (2.80 mg kg^{-1}) and *Drawida* sp. (1.43 mg kg^{-1}). MeHg concentration of *B. parvus* (0.114 mg kg^{-1}) was lesser than those in *Endogeic* (0.153 mg kg^{-1}) and *Alolobophora* sp. (0.34mg kg^{-1}); however, it was higher than that of *Epigeic* (0.025mg kg^{-1}), *Anecic* (0.015mg kg^{-1}), and *Drawida sp.* (0.04 mg kg^{-1}). The percentages of MeHg to T-Hg in *B. parvus* were low and varied from 2.04 to 3.33% with a mean of 2.59% of the T-Hg, which was much less than those in other earthworm species, such as between *Aporrectodea onga* (5.7%), *L. terrestris* (10.1%), and *Allolobophora* sp. (12%), excluding *Drawida* sp. (about 3%) (Rieder et al., 2011; Zhang et al., 2009). Be limited from the MeHg concentration of soil which not be measured in our study due to the lack of laboratory conditions. We proposed that the low proportion of MeHg to T-Hg might indicate the relatively low Hg accumulation or methylation levels in *B. parvus*, which also implied that most Hg in *B. parvus* might be present in inorganic or other Hg forms. This is mainly accepted that some other species of earthworms presented higher bioaccumulation for MeHg than T-Hg, and positive correlation with MeHg concentration in soils (Dang et al., 2015; Rieder et al., 2011), and ingested inorganic Hg can be readily assimilated and methylated in vivo of earthworms (Dang et al., 2016; Rodríguez Álvarez et al., 2014). This differences of MeHg bioaccumulation were possibly resulted the species-specificity of earthworms, owing to feeding behavior, soil geochemical properties (Ernst and Frey, 2007) and the bacterial methylation ability associated communities in gut of earthworms (Rieder et al., 2013), or different constituents of soil-Hg fractions in leachate-contaminated zone (see below).

4.3 Spatial distribution characteristics of Hg

Few studies have been conducted to investigate the relationships among Hg in soils. Simultaneous occurrence of soil-Hg fractions and Hg in earthworms has been rarely reported. In this study, significant correlation was observed between soil T-Hg and *B. parvus* T-Hg ($r = 0.839$, $p < 0.01$), which is consistent with that found in earthworm species of *Aporrectodea caliginosa*, *Aporrectodea rosea*, *Octolasion cyaneum*, *Dendrobaena rubidus* and *L. terrestris* (Rieder et al., 2011); however, it was different from that of the earthworm species of *Pheretima guillemi* (Dang et al., 2015) and *Eisenia andrei* (Zagury et al., 2006). Spearman's coefficient analysis also indicated significant association between T-Hg/MeHg of *B. parvus* and soil-Hg fractions, which was consistent with the morphology of the contour line of Hg in their spatial distributions (Table 1-3, Fig. 1-2, Fig. 1-4). Both aspects of the above results suggested that the relatively high soil-Hg contents distribute in areas where the altitudes were relatively low, and were ditched downstream. This supported our hypothesis that Hg contents diffuse along with the leachate ditches and scatter in the leachate pool zone on the surface soils.

F1/Hg-w and F2/Hg-h were important fractions of soil-Hg as they were of high mobility and potential bioavailability in comparison to other soil-Hg fractions. Hg-w is weakly absorbed by soils, and able to diffuse into deep soils and groundwater, including more easily transported and methylated Hg compounds compared to other Hg fractions (e.g., $HgCl_2$) (Bloom et al., 2003; Pinedo-Hernández et al., 2015; Zagury et al., 2006). F2/Hg-h was defined as potentially bioavailable inorganic Hg, as simulating soluble Hg by human stomach acid for absorption in the digestive system. F2/Hg-h is a susceptible reactive Hg fraction to environmental conditions, especially to pH change (Bloom et al., 2003). Obvious correlations between Hg of earthworms and Hg-w or Hg-h in soils were reported in short-term experiments in laboratories (Zagury et al., 2006). In contrast, no significant correlation between Hg in *B. parvus* and Hg-w or Hg-h in soil was observed in this study. The possibly reason was that the positive effect of Hg-w or Hg-h in soils to the concentration of Hg of earthworms were mostly observed in laboratory experiments. However, for environmental investigations, the concentrations of Hg-w or Hg-h in soils may be influenced and disturbed by the change in the environmental factors (e.g., temperature, rainwater, and sunshine). As the concentrations of Hg-w and Hg-h in soils altered frequently, it was difficult to find good statistical correlations between Hg-w or Hg-h and T-Hg in *B. parvus*. We proposed that Hg-w and Hg-h in soils were highly bioavailable and attention should be paid on their adverse effects. However, their concentrations were too low to show statistical correlation with Hg of *B. parvus* in leachate-contaminated zone of MSW landfill. This also supports that physicochemical characteristics of soil parameters had very small or even no influence on Hg accumulation in earthworms (Dang et al., 2016; Kennette et al., 2002; Nannoni et al., 2011).

F3/Hg-o and F4/Hg-e were the dominant components of soil T-Hg. The proportion of MeHg to T-Hg in all *B. parvus* samples was less than 4%, indicating that the accumulation of Hg in earthworms was mostly in inorganic forms. Both Hg-o and Hg-e in soil bound mainly to SOM with organic ligands (for F3/Hg-o) or in association with amorphous organosulfur, crystalline iron oxides, and amalgams of T-Hg (for Hg-e) (Bloom et al., 2003; Pinedo-Hernández et al., 2015; Reis et al., 2016). F3/Hg-o as a major soil-Hg fraction is related to potential methylation. Similar studies suggested that organic acids of SOM (e.g., humic and fulvic acids) were related to Hg-o, which may influence the ability of Hg desorption and the soil parameters to promote Hg methylation in both biotic and abiotic conditions (Rodríguez Álvarez et al., 2018; Rodríguez Álvarez et al., 2014). Another main fraction of F4/Hg-e represented all free Hg^0, and other slightly soluble Hg compounds such as Hg (I) (Bloom et al., 2003). Although Hg-e is released from landfills with relatively low solubility and mobility, the main environmental damage is because of its oxidation to divalent mercury and vaporization to the atmosphere. Elemental Hg was also transformed into other forms mediated by organic matter (Biester et al., 2002). As *B. parvus* feeds on soil and absorbs nutrients mainly from SOM for growth, the correlation of Hg-o with *B. parvus* Hg (T-Hg and MeHg) was reasonable. Although both Hg-o and Hg-e were reported to have limited mobility and bioavailability (Bloom et al., 2003).

F5/Hg-s was reported as a hardly soluble compound in the soil-Hg fractions in previous studies, and some short-term laboratory experiments suggested that Hg-s was difficult to accumulate and exert biological functions in soil (Bloom et al., 2003; Liu et al., 2006). However, other studies had observed that Hg-s appeared to convert into methylated forms at a low level in laboratory incubations (Barkay and Wagner-Döbler, 2005; Drott et al., 2007; Holloway et al., 2009). In addition, it has also been observed that, to some extent, mercury sulfide could be dissolved and transformed into bioavailable forms by plants in soil (Han et al., 2008; Han et al., 2012). We observed a statistically significant positive correlation between Hg (T-Hg, MeHg) in *B. parvus* and soil F5/Hg-s in our study which supported the speculation that F5/Hg-s could also be dissolved and changed to bioavailable transformation in field environments. It reminds us that long-term biological effect of soil F5/Hg-s should not be ignored. Besides, we observed the variation in correlation coefficients when the portion of F5/Hg-s was deducted from soil T-Hg in correlation analysis between T-Hg in *B. parvus* and soils, which from $r = 0.839$ ($p < 0.01$) to $r = 0.790$ ($p < 0.01$). This indicates that F5/Hg-s probably had a substantial contribution to the Hg bioavailability of *B. parvus*.

Previous study have shown that the anaerobic conditions were existed in the digestive tracts of earthworm (Drake and Horn, 2007). Especially, the sulfur-reducing bacteria (SRB) was found to play an important role in the methylation of inorganic Hg under the anaerobic conditions (Drott et al., 2007; Holloway et al., 2009). Some studies observed that earthworms have the ability of Hg bio-methylation in their digestive tract, comparison study found that the active methylated bacteria (e.g., SRB) did not live in soils but lived only in the digestive tracts of earthworms (Rieder et al., 2013). Considering the correlation analysis which shows the mutual correlations between the concentrations of T-Hg and MeHg in *B. parvus* tissues (Table 1-3), and the high enrichment ability of the *B. parvus* (more than 5 times) for soil T-Hg in our results (Fig. 1-S6), it is possibly that MeHg in *B. parvus* may not completely from the soils, and the ability of *B. parvus* in processing MeHg in vivo could not be ignored in our study. Therefore, we have further determined the concentration of MeHg in soils and native earthworms both leachate-contaminated and uncontaminated control soil around this traditional landfill, to explore the characteristics of bioaccumulation on mercury and methylmercury in native *B. parvus* earthworms especially (those materials under consideration for publication elsewhere). On the other hand, from the perspective of ecology, the habitat of earthworms was kept in a relatively fixed activity scope in soils, which combined with previous results that the similar distributions and positive significant correlations between the *B. parvus* earthworm tissues Hg (T-Hg, MeHg) and stable soil Hg (Hg-o, Hg-e, Hg-s), (Fig. 1-4 and Table 1-3), this means that those observed relationships of the similar distributions are stability between *B. parvus* Hg and soil stable Hg fractions (Hg-o, Hg-e, Hg-s) at a certain extent. Alternatively, these positive relationships apparently support the gut uptake of soil-bound mercury during soil consumption of earthworms, as proposed by (Rieder et al., 2013), recently. Meanwhile, this also supports our hypothesis that Hg in earthworm tissues was accumulated via the gut uptake from the surface soil in the leachate-contaminated sites.

5. Summary

In this study, spatial distributions on fractionation of soil-Hg and Hg speciation (T-Hg and MeHg) of native *B. parvus* earthworms were investigated in the leachate-contaminated zone around a Hg polluted traditional MSW landfill. Average soil T-Hg concentration (0.227 to 2.919 mg kg^{-1} dw) was about one-third of the level measured 20 years ago (0.81 to 8.48 mg kg^{-1} dw). Hg fractions were sequentially in the order of Hg-o > Hg-e > Hg-s > Hg-w > Hg-h, and dominated by the relative low mobility and bioavailability fractions of Hg-o (55.50%) and Hg-e (35.31%) in leachate-contaminated soils.

Similar spatial distributions and close correlations were observed between the Hg (T-Hg / MeHg) content of *B. parvus* tissues and soil F3/Hg-o, F4/Hg-e and F5/Hg-s, which were also associated with the soil organic matter (SOM) and particle size (sand, clay). However, there were no correlations with soil F1/Hg-w or F2/Hg-h. These results indicated that easily bioavailable and soluble Hg fractions (Hg-w, Hg-h) of soil are not suitable variables to evaluate the bioaccumulation and distribution of Hg in *B. parvus*. Instead, the stable soil-Hg fractions (Hg-o, Hg-e, Hg-s) showed good relationships between the bioaccumulation and spatial distributions in the leachate-contaminated soils. Therefore, it is advisable to avoid the evaluation of Hg bioaccumulation and distribution using soluble Hg fractions only. Instead, stable Hg fractions in leachate-contaminated soil should be included along with soluble Hg fractions for the evaluation of Hg bioaccumulation and distribution in field or outdoor conditions. In addition, native *B. parvus* earthworms with a high enrichment ability for soil T-Hg could be act as bio-indicators for the assessment of dominated stable Hg fractions in leachate-contaminated soils.

However, because of relative narrow concentration range of soil T-Hg in this study and limitations of the extraction procedures of soil Hg, the applicability of our findings might be limited under field conditions (i.e., waste tape and composition, seasonal weather variation, age, precipitation) in leachate-contaminated soils. More laboratory experiments are necessary to quantitatively examine the potential influencing factors for *B. parvus*, such as long-term stability, mobility, transformation of different soil-Hg fractions, and natural factors.

Supplementary data of " Spatial distribution characteristics of mercury in the soils and native earthworms (*Bimastos parvus*) of the leachate-contaminated zone around a traditional landfill "

Table S1-1 Initial data used for spearman's correlation coefficients (*r*) between the Hg contents in soils with earthworms (*B. parvus*), and soil general geochemical properties variables

[1]Soil Hg fractions average (mg kg^{-1} dw)						Ear. THg	Ear. MeHg	[2]SOM (%)	[3]C/N	[4]EC (mS/m)	pH	[5]PSD		
THg	F1 (Hg-w)	F2 (Hg-h)	F3 (Hg-o)	F4 (Hg-e)	F5 (Hg-s)							Sand (%)	Silt (%)	Clay (%)
1.141	0.0009	0.001	0.1427	0.9433	0.0290	[6]ND	ND	4.82	11.66	73.67	5.57	52.64	33.6	13.76
0.277	0.0007	0.0006	0.1717	0.0403	0.0493	3.226	0.077	8.82	19.04	78.67	4.577	49.18	18.16	32.66
2.75	0.0014	0.0007	1.4307	1.1373	0.0793	6.775	0.218	17.59	12.96	80.67	4.553	28.27	23.14	48.59
0.498	0.0009	0.0122	0.2960	0.1540	0.0210	3.931	0.095	9.15	15.29	78	4.82	32.62	21.93	45.45
0.426	0.0009	0.0330	0.2380	0.1280	0.0210	4.5	0.092	9.39	14.49	86	4.913	49.94	18.37	31.69
1.284	0.2494	0.0004	0.788	0.2077	0.0257	3.363	0.086	8.98	15.87	93.33	4.94	53.92	24.32	21.76
0.227	0.0173	0.0005	0.1513	0.0373	0.0233	2.621	0.071	6.99	18.6	93	4.837	60.73	15.19	24.08
2.919	0.0022	0.0024	0.5347	1.9733	0.0620	6.168	0.184	10.92	18.45	105.67	5.15	46.19	12.90	40.91
1.851	0.0167	0.0006	0.7893	0.8090	0.0803	5.534	0.128	13.52	16.29	110	4.82	38.30	20.74	40.96
2.852	0.0205	0.0008	1.2923	1.0157	0.1347	6.551	0.139	15.29	15.1	81	4.627	37.17	15.86	46.97
1.318	0.0011	0.0004	0.9500	0.2300	0.0603	5.801	0.193	13.42	20	91	4.183	30.95	22.35	46.70
1.034	0.0283	0.0004	0.7570	0.1640	0.0457	2.106	0.053	6.2	15.58	86.33	4.077	55.99	23.84	20.17
0.276	0.0004	0.0006	0.1940	0.0567	0.0197	1.242	0.031	5.41	12.08	66.67	4.963	59.15	19.43	21.42

Note:

1. Soil Hg fractions: T-Hg (total mercury), Hg-w (water soluble Hg), Hg-h (human stomach acid soluble Hg), Hg-o (organic-chelated Hg), Hg-e (elemental Hg), Hg-s (mercuric sulfide Hg)
2. SOM: Soil organic materials.
3. C/N: Total organic carbon / Total nitrogen.
4. EC: Electrical conductivity.
5. PSD: Soil particle size distribution (Sands: 0.050 < % < 2 mm, Silts: 0.002 < % < 0.050 mm, Clays: % <0.002 mm).
6. ND: Data not available.

Table S1-2 Initial data used for soil T-Hg concentrations (n=6) in sampling sites (mg kg^{-1} dw^{1}).

Detection replicates for soils (n=6)	Location number of sampling sites												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.220	0.354	2.560	0.547	0.432	1.415	0.244	3.011	1.623	2.812	1.459	1.003	0.244
2	0.908	0.229	2.916	0.531	0.419	1.183	0.243	3.126	2.270	2.912	1.335	1.053	0.278
3	1.058	0.299	2.576	0.487	0.405	1.247	0.269	2.706	1.675	3.081	1.319	1.105	0.237
4	1.366	0.231	2.480	0.473	0.442	1.212	0.211	3.073	1.807	2.913	1.291	1.025	0.244
5	1.163	0.296	3.175	0.428	0.421	1.372	0.222	2.679	1.856	2.738	1.352	0.974	0.445
6	1.128	0.250	2.795	0.521	0.434	1.273	0.172	[2]ND	1.873	2.657	1.151	1.041	0.207
[3]Aver.	1.141	0.277	2.750	0.498	0.426	1.284	0.227	2.919	1.851	2.852	1.318	1.034	0.276

Note:

1. dw: dry weight.
2. ND: Data not available.
3. Aver.: average.

Table S1-4 Hg in traditional Municipal Solid Waste (MSW) landfill worldwide.

Landfill sites	**Period**	**Hg Aver. Con. (mg kg^{-1})**	**Materials**	**Reference**
Guiyang, China	2004	0.17-46.22	Covered soil	*Li et al., 2010*
USA	2005	13	Covered soil	*US EPA: NEI, 2005*
Europe	2005	10.1	Covered soil	*AMAP/UMEP, 2008*
Seoul, Korea	2002	3.45-2952 ng m^{-3}	Surface air	*Kim and Kim, 2002*
Wuhan, China	2003-2006	0.24-1.271	Covered soil	*Wang et al., 2012*
Florida, USA	1994	0.03-16.08	Covered soil	*Celia DA et al., 1999*
India	2005	77.4	Covered soil	*Mukherjee AB et al., 2009*
China	2005	14.1	Covered soil	*Feng X et al., 2009*
Nagasaki, Japan	1999	0.81-8.48	Covered soil	*http://www.sanpouzan.jp/report.html*
Japan	2013	11-24 tons/year	Into landfill annually	*Masaki Takaoka, 2015*

Table S1-3 Initial data of earthworm Hg species (THg and MeHg, n=3) and 5-steps sequential extraction for soil Hg fractions (F1-F5, n=6) in sampling sites (mg kg^{-1} dw^{1}).

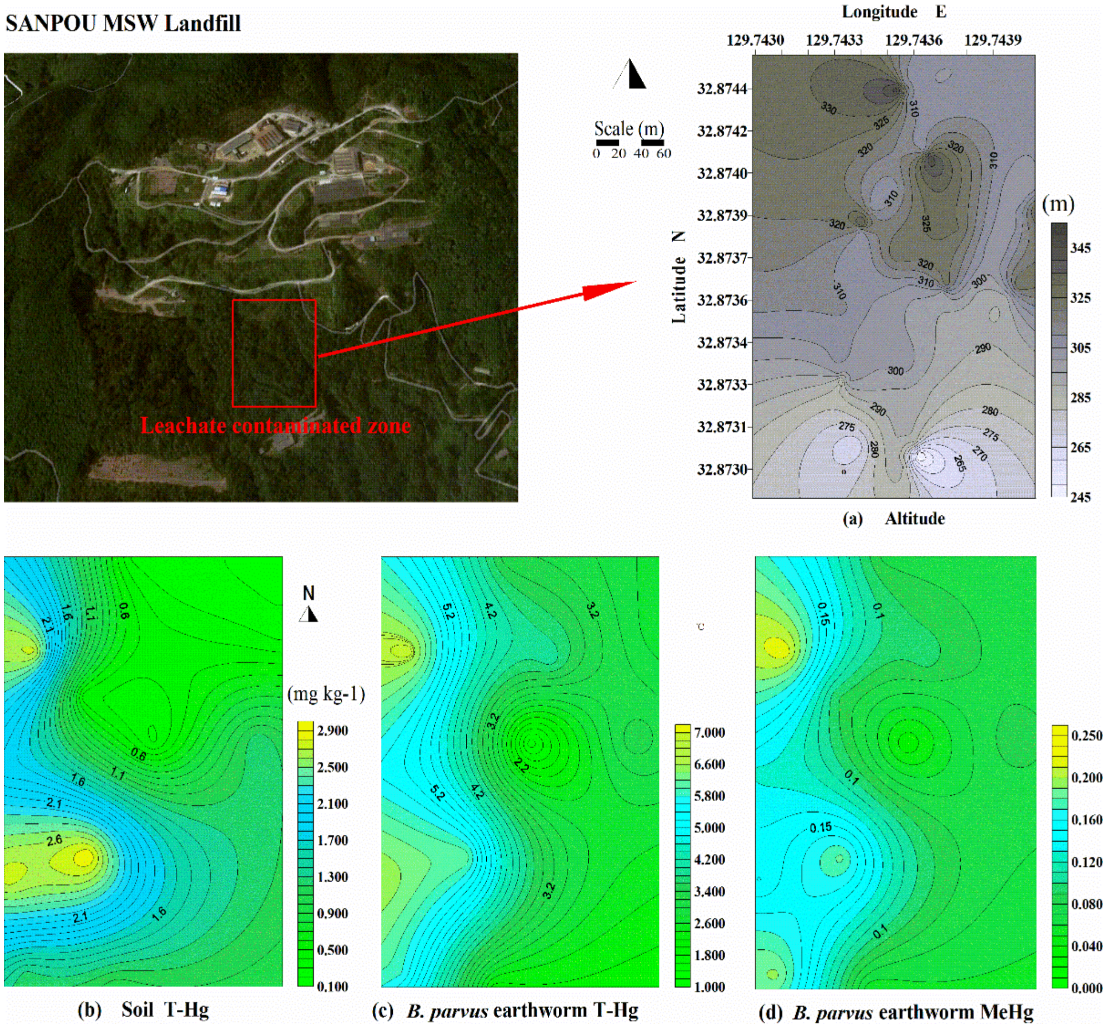
*Sampling sites	**Soil Hg fractions					Total Hg fractions	Earthworm	
	F1 (Hg-w)	F2 (Hg-h)	F3 (Hg-o)	F4 (Hg-e)	F5 (Hg-s)		T-Hg	MeHg
S1-1	0.0008	0.0009	0.1430	0.9570	0.0310	1.1327	***NA	NA
S1-2	0.0009	0.0010	0.1430	0.9400	0.0290	1.1139	NA	NA
S1-3	0.0009	0.0010	0.1420	0.9330	0.0270	1.1039	NA	NA
S2-1	0.0007	0.0005	0.1670	0.0410	0.0500	0.2592	2.8780	0.0690
S2-2	0.0007	0.0007	0.1710	0.0400	0.0480	0.2604	3.8150	0.0830
S2-3	0.0008	0.0007	0.1770	0.0400	0.0500	0.2685	2.9840	0.0800
S3-1	0.0014	0.0007	1.3840	1.1130	0.0800	2.5791	6.5840	0.2410
S3-2	0.0014	0.0006	1.4900	1.1660	0.0780	2.7360	6.9160	0.2180
S3-3	0.0014	0.0008	1.4180	1.1330	0.0800	2.6332	6.8240	0.1950
S4-1	0.0009	0.0111	0.3020	0.1510	0.0210	0.4860	3.8640	0.0940
S4-2	0.0009	0.0144	0.2920	0.1540	0.0220	0.4833	3.9690	0.0960
S4-3	0.0008	0.0110	0.2940	0.1570	0.0200	0.4828	3.9590	0.0950
S5-1	0.0010	0.0352	0.2290	0.1180	0.0210	0.4042	4.4610	0.0860
S5-2	0.0008	0.0312	0.2410	0.1300	0.0220	0.4250	4.5460	0.1040
S5-3	0.0010	0.0326	0.2440	0.1360	0.0200	0.4336	4.4940	0.0850
S6-1	0.2670	0.0005	0.7750	0.2040	0.0260	1.2725	3.0440	0.0870
S6-2	0.2389	0.0004	0.7990	0.2080	0.0260	1.2723	5.0990	0.0840
S6-3	0.2424	0.0004	0.7900	0.2110	0.0250	1.2688	1.9450	0.0870
S7-1	0.0179	0.0006	0.1670	0.0340	0.0200	0.2395	2.5470	0.0800
S7-2	0.0140	0.0006	0.1560	0.0460	0.0220	0.2386	2.6730	0.0520
S7-3	0.0199	0.0004	0.1310	0.0320	0.0280	0.2113	2.6420	0.0820
S8-1	0.0022	0.0025	0.5550	1.9930	0.0540	2.6067	6.1050	0.1830
S8-2	0.0023	0.0021	0.5260	1.9990	0.0570	2.5864	6.2340	0.1860
S8-3	0.0022	0.0025	0.5230	1.9280	0.0750	2.5307	6.1640	0.1840
S9-1	0.0150	0.0007	0.7940	0.7850	0.0820	1.6767	5.4500	0.1290
S9-2	0.0161	0.0006	0.7680	0.8330	0.0840	1.7017	5.7130	0.1350
S9-3	0.0191	0.0005	0.8060	0.8090	0.0750	1.7096	5.4400	0.1190
S10-1	0.0177	0.0008	1.3170	0.9970	0.1290	2.4615	6.5410	0.1210
S10-2	0.0260	0.0007	1.2900	1.0400	0.1300	2.4867	6.8240	0.1340
S10-3	0.0179	0.0009	1.2700	1.0100	0.1450	2.4438	6.2880	0.1630
S11-1	0.0011	0.0003	0.9410	0.2220	0.0590	1.2234	5.5650	0.2040
S11-2	0.0011	0.0004	0.9580	0.2410	0.0680	1.2685	5.6430	0.1880
S11-3	0.0010	0.0004	0.9510	0.2270	0.0540	1.2334	6.1960	0.1860
S12-1	0.0246	0.0005	0.7510	0.1560	0.0430	0.9751	2.0800	0.0492
S12-2	0.0238	0.0004	0.7650	0.1620	0.0590	1.0102	1.9210	0.0631
S12-3	0.0366	0.0004	0.7550	0.1740	0.0350	1.0010	2.3160	0.0475

Table S1-3 (*Continued*)

*Sampling sites	**Soil Hg fractions						Earthworm	
	F1 (Hg-w)	F2 (Hg-h)	F3 (Hg-o)	F4 (Hg-e)	F5 (Hg-s)	Total Hg fractions	T-Hg	MeHg
S13-1	0.0004	0.0007	0.1870	0.0500	0.0200	0.2581	1.1750	0.0040
S13-2	0.0004	0.0005	0.2020	0.0620	0.0210	0.2859	1.3080	0.0020
S13-3	0.0005	0.0007	0.1930	0.0580	0.0180	0.2702	1.2430	0.0080
Reference 1	NA	NA	NA	NA	NA	NA	0.3602	0.1150
Reference 1	NA	NA	NA	NA	NA	NA	0.3396	0.1283
Reference 1	NA	NA	NA	NA	NA	NA	0.3504	0.1103
Reference 2	NA	NA	NA	NA	NA	NA	0.4846	0.0960
Reference 2	NA	NA	NA	NA	NA	NA	0.5090	0.1328
Reference 2	NA	NA	NA	NA	NA	NA	0.4756	0.0902
Reference 3	NA	NA	NA	NA	NA	NA	0.4409	0.0824
Reference 3	NA	NA	NA	NA	NA	NA	0.4187	0.1056
Reference 3	NA	NA	NA	NA	NA	NA	0.4298	0.0782

Note:
* n=3 Detection replicates for sampling soils.
** Soil Hg fractions: T-Hg (total mercury), F1/Hg-w (water soluble Hg), F2/Hg-h (human stomach acid soluble Hg), F3/Hg-o (organic-chelated Hg), F4/Hg-e (elemental Hg), F5/Hg-s (mercuric sulfide Hg).
*** ND: Data not available.

Fig. S1-7 GRAPHICAL ABSTRACT



(a) Altitude

(b) Soil T-Hg

(c) *B. parvus* earthworm T-Hg

(d) *B. parvus* earthworm MeHg

Fig. S1-7 Spatial distribution of mercury in the soils and native earthworm (*Bimastos parvus*) of the leachate-contaminated zone.

HIGHLIGHTS

· Hg was fractionated into 5 fractions in the leachate-contaminated soil.

· Main components of the Hg fractions were Hg-o (55.50%) and Hg-e (35.31%).

· Hg distribution in *B. parvus* and soil showed a dependence on SOM and clays.

· Distributions of *B. parvus* Hg and soil stable Hg fractions were similar.

· Soluble Hg fractions cannot associate with Hg distribution in native *B. parvus*.

CHAPTER 2

Comparison of total mercury and methylmercury bioaccumulation in earthworms (*Bimastos parvus*) native to landfill-leachate-contaminated forest soil (LLCFS)

Citation:

Changhua He, **Koji Arizono, Hezhe Ji, Yuka Yakushiji, Daizhou Zhang, Kuangwei Huang, Yasuhiro Ishibashi.** Comparison of mercury and methylmercury bioaccumulation in earthworms (*Bimastus parvus*) native to landfill-leachate-contaminated forest soil. ***The Joural of Toxicological Sciences*** **2018; Vol.43, No. 7 , 459-471.**

★ Chapter Profile - 2

Total mercury (THg) and methylmercury (MeHg) bioaccumulation was explored in the *Bimastus parvus* species of earthworm (*B. parvus*) native to the leachate-contaminated forest soils around a Hg-polluted traditional landfill in Japan. General soil properties and concentrations of THg and MeHg in forest soils and in *B. parvus* were determined.

The results indicated that the average THg concentrations in *B. parvus* and in forest soils in the leachate-contaminated sites were 10.21 and 14.90 times higher than those in the reference sites, respectively, whereas similar average MeHg concentrations were observed in forest soils (< 0.01 mg kg^{-1}) and in *B. parvus* (0.100–0.114 mg kg^{-1}) across all sampled sites. The average bioaccumulation factors of THg in *B. parvus* (BAF_{THg}) in forest soil were similar between the leachate-contaminated sites and the reference sites. Cluster and regression analyses demonstrated that the *B. parvus* Hg (THg and MeHg) and soil THg were positively correlated with each other and with soil organic matter (SOM) and clays, but were negatively correlated with sand and hardly correlated with silts and pH in leachate-contaminated forest soils.

From these results, it was proposed that Hg exposure to food chains is possible through *B. parvus*, because *B. parvus* showed a high ability to accumulate THg and MeHg in both leachate-contaminated and reference forest soils. Together, these findings indicated that the role of *B. parvus* in MeHg production is not clear, and it is possible that the MeHg in *B. parvus* was firstly formed within forest soils and then accumulated in their tissues.

Keywords:
Bioaccumulation, Mercury, Forest soil, Earthworms, Landfill

1. Introduction

Mercury can be accumulated and amplified in predatory animals and humans through the food chain, causing many hazardous health effects, such as neurotoxicity, mortality, and reproductive toxicity (Scheuhammer et al., 2007). It is also considered one of the most toxic nonessential contaminants to human health and the environment (Buch et al., 2017). Hence, the level of Hg in the environment has been controlled by many countries and international organizations, such as the World Health Organization (WHO), United Nations Environment Programme (UNEP), and Food and Agriculture Organization (FAO) (Ding et al., 2007).

Earthworms are considered useful bio-indicators for assessing soil health and quality in numerous studies of contaminated sites and in laboratory experiments (Nannoni et al., 2011; Teršič and Gosar, 2012). They spend most of their life in soil and play an important role in soil structure maintenance; approximately 80% of soil fauna biomass is constituted by earthworms in terrestrial ecosystems (Buch et al., 2017). Earthworms can be sampled easily, have proven to be a good biological model, and can accumulate trace metals from soil through direct dermal contact and/or diet uptake by gut ingestion from soils. Also, Hg accumulation in earthworms has been demonstrated in many studies (Burton et al., 2006). It has been generally accepted that inorganic forms of Hg can be methylated by soil bacterial activity (mostly under anaerobic conditions); methylation enhances bioavailability and bio-toxicity and increases potential threats to soil ecosystem functions (Shao et al., 2012). However, recent studies have found that inorganic Hg is methylated within the guts of earthworms and not by soil bacterial action, based on the fact that sulfate-reducing bacteria were detected in the gut tract of earthworms (Elisabeth Kaschak, 2014; Rieder et al., 2013).

Globally, up to 70% of municipal solid waste (MSW) is disposed of in landfills, and Hg is inevitably and unavoidably deposited in traditional landfills via a variety of Hg-containing MSW, such as household batteries, devices, lamps, mirror coatings, and thermometers (Li et al., 2010; Zhu et al., 2013). About 1010–4070 tons of Hg are discharged into the environment every year from natural sources, such as volcano eruptions, forest fires, and re-emission from surface soils and oceans, and from anthropogenic sources, such as ore mining, fossil fuel combustion, and MSW (United Nations Environment Programme, 2013). In 2005, it was estimated that approximately 8% of total anthropogenic Hg emissions have been contributed by disposal procedures in landfills and waste utilization (Gworek et al., 2015; Zhu et al., 2013). Hg-containing components in MSW can enter and impact surrounding groundwater, soil, and air through Hg-contaminated leachates and gas emissions after traditional burial (Cheng and Hu, 2012). Large amounts of Hg have been found in inorganic forms, such as Hg, $HgCl_2$, HgS, and HgO, in leachates and gases from MSW landfills. Furthermore, inorganic Hg can be methylated by soil bacterial action, which causes high toxicity to surrounding ecosystems under different conditions (Cheng and Hu, 2012; Earle et al., 1999; Lee et al., 2016). Similar to landfills in other countries over the last century, about 70% of MSW landfills in Japan were built into mountainsides using the natural space between ridges to limit land usage (Tanaka et al., 2005). It has been reported that between 1976 and 1979, only 40% of traditional landfills were constructed with a liner system for preventing leachate leaks; this increased to 100% for landfills constructed in 1990 (Tanaka et al., 2005). Meanwhile, the double-liner system for preventing leachate leaks became mandatory for new Japanese landfills after 1997 (Tanaka et al., 2005). Therefore, the potential leachate pollution caused by those traditional landfills without anti-leaching systems have been a societal concern (Moody and Townsend, 2017).

Forest soil is essential in biological cycling of forest ecosystems. Previous studies have shown that forests can deposit large amounts of trace metals, including Hg, through canopies from atmospheric aerosols; Hg can reach the forest soil through litterfall or rain (Driscoll et al., 2007; Gong et al., 2014). Atmospheric Hg accumulation in litter and soil in tropical forests has been reported in recent research (Luo et al., 2015; Siudek et al., 2016). Generally, Hg is relatively immobile because it binds with organic matter and accumulates in the top layers of soil (Ravichandran, 2004). To our knowledge, limited studies have focused on the assessment of risks from total mercury (THg) bioaccumulation by earthworms in forest soil environments, and only a few reports determined the bioaccumulation of methylmercury (MeHg) (Ernst et al., 2008; Rieder et al., 2011). There have been no studies on Hg accumulation in earthworms from Hg-containing leachate-contaminated soils around traditional municipal landfills, particularly forest soils. Therefore, exploring the Hg (THg and MeHg) accumulation in native earthworms in forest soils will improve our understanding of the risks of secondary Hg poisoning to predators, such as birds and mammals, from earthworms in forest ecosystems (Dang et al., 2015), especially in Hg-containing leachate-contaminated terrestrial ecosystems.

This study investigated the current status of Hg (THg and MeHg) accumulation in native earthworms and forest soils around a traditional Hg-polluted MSW landfill compared to that of non-contaminated reference sites. The focus was on the characteristic relationships and impacts of Hg (THg and MeHg) levels in *Bimastus parvus* earthworms and general soil properties of Hg-containing leachate-contaminated forest soils compared to Hg background levels in uncontaminated forest soils.

2. Materials and methods

2.1 Sampling sites and sample preparation

A traditional MSW landfill was built on forested mountainsides of Mt. Sanpou (32.876°N, 129.743°E) in suburban Nagasaki (north-west of Kyushu, Japan) in 1975. Large amounts of traditional MSW that might have contained Hg, such as incinerated ash, industrial wastes, and municipal sludge, were buried in this landfill between 1975 and the early 1990s (Earle et al., 1999; Jung et al., 2004). A large flood in Nagasaki in 1982 enhanced the Hg pollution in the area by bringing in mixed Hg-contaminated municipal garbage, such as damaged fluorescent lights, switch devices, consumer electronics, and household products. A leachate-contaminated forest zone gradually formed about 500 m below the landfill. Previous investigations indicated that THg concentrations in the leachate–contaminated forest soil ranged from 0.81 mg kg^{-1} to 8.48 mg kg^{-1}, which was about 13–140 times higher than natural background levels of Hg (0.06 mg kg^{-1}) in 1998 (Arisawa et al., 2000; Commission of Water Environment Protection and SanPouZan, 2017).

A series of environmental remedial measures have been implemented for this unqualified management MSW since 1998. specific measures include treatment facility to central MSW pollution and filter leachate from MSW vicinity, restructuring storm drain and sewers along the MSW landfill for reducing rainfall leaching to landfill. Formulating regulations and enforced to control illegal dumping. On the other hand, a classifying Hg filter tower was introduced for filtering of groundwater which pumped from landfill site.

All sampling sites for earthworms and soils were located in the surface of the landfill-leachate-contaminated soil along the ditches and in small, scattered puddles formed by leachate and rainwater, which were in relatively soft and wet areas (Fig. 2-1). Earthworms and 2 kg soil samples (S1-S13) were collected from each site. The earthworms were found after digging in a 0.25 m^2 area at a depth of 0–15 cm. Another mountainside with similar natural environmental properties, which was about 1.6 km from the contaminated study site, was selected as the reference site (R1-R3) for natural background Hg levels. The contaminated and reference sites were densely covered by a canopy of local deciduous forest.

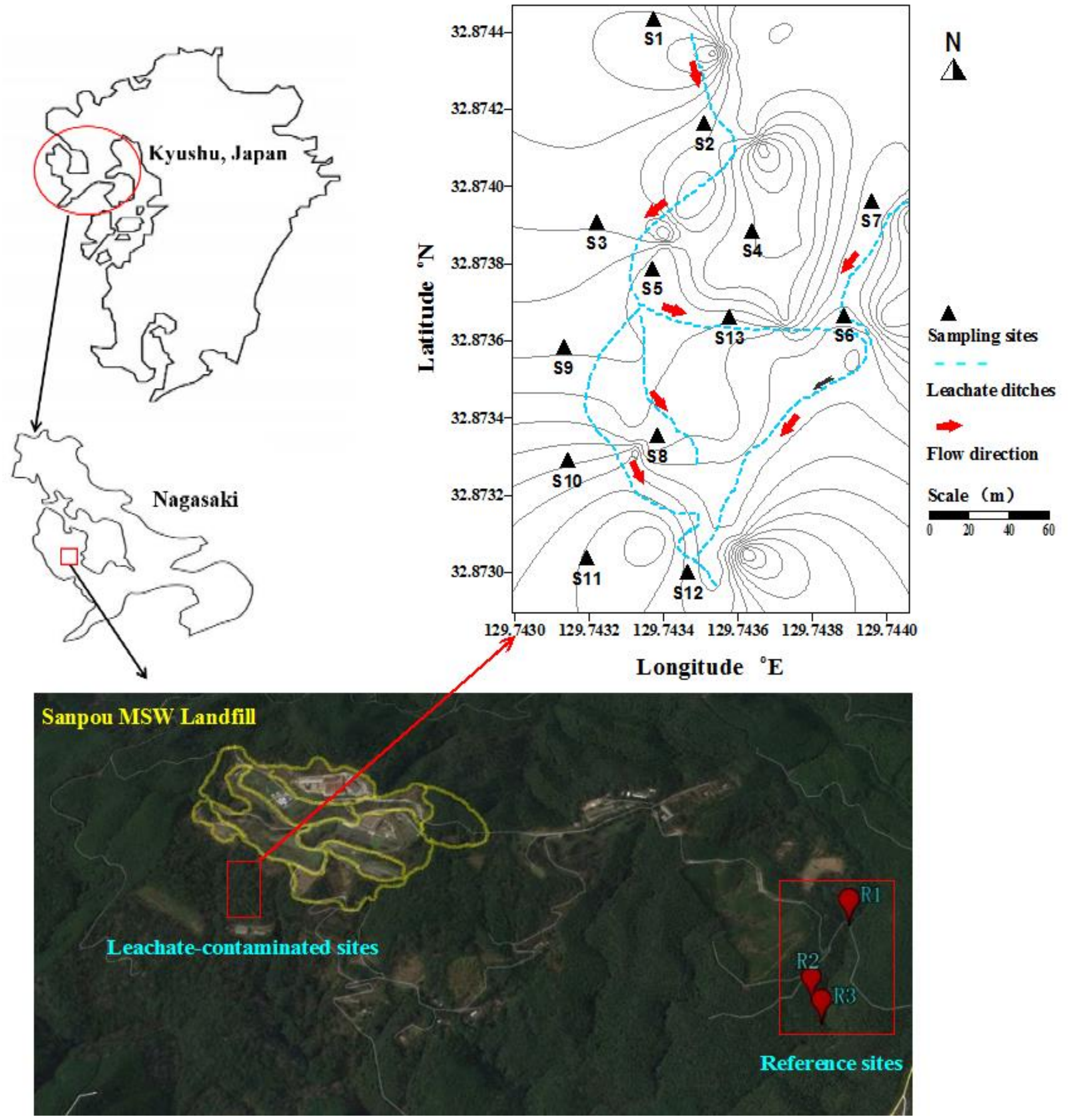


Fig. 2-1 Location of sampling forest soils in landfill leachate-contaminated zone

Earthworms were maintained in the dark at 22 ± 2 °C without feeding for 3 days, until they emptied their gut contents (Fig. 2-2). Mature earthworms with obvious developed clitella were classified by site and species, and the identification was combined with morphologic characteristics (e.g., clitellum shapes, tubercula pubertatis, genital, ventral view, tail, setal pairing arrangements, color, size, etc.) and ecological distribution information in accordance with earthworm monographs (Minamiya, 2014; Sims and Gerard, 1985). Thereafter, the earthworms, with lengths ranging between 4.5 cm and 5.5 cm, were lyophilized and crushed into a fine powder using an agate pestle, and then stored at -20 °C until analysis. The soil samples were air-dried to constant dry weight (dw) at laboratory conditions of 25 ± 3 °C and approximately 40% humidity.

Earthworms and soil samples were gently ground into a fine powder with an agate mortar and pestle, and soil powder was sieved through a 2 mm nylon mesh.

2.2 Analyses

2.2.1 General soil properties

General soil properties that might affect Hg accumulation in *B. parvus* were analyzed. Soil organic matter (SOM) was analyzed by determining the loss on ignition (LOI) at 550 °C for 2 h (ISUZU AT-S13) in a muffle furnace (ISUZU AT-S13), as a proxy for estimating soil organic matter (SOM) content in sampling soil (Heiri et al., 2001). Soil pH was measured in 0.01 M $CaCl_2$ with an extractant ratio of 1:2 v/v (soil-liquid) using a pH meter (Eutech instruments Cyberscan, pH 1100) (Xu et al., 2014). The soil particle size distributions (PSD) were classified as sands (0.050 mm < % < 2 mm), silts (0.002 mm < % < 0.050 mm), and clays (% < 0.002 mm) according to USDA classification standard for soil texture. This method is a combination for evaluating soil particle-size distribution, using the steps on sieving and precipitation procedures. With the characteristics of rapid and simple operation designed from an analysis of particulate organic matter (POM). Only using a 0.053-mm sieve for collecting the sand fraction of soil. The detailed steps are as follows: A certain amount of soil mass 15 g was used for evaluating soil PSD. The dispersion soil particle is prepared by adding sodium hexametaphosphate [HMP, (NaPO3) n] at the 3% of aqueous mass concentration by weight, add a certain volume dispersion liquid, and then shaking the suspension of solid-liquid for 2h on an oscillating shaker at the frequency of oscillation 120 reciprocations per minute, after using the 0.053-mm sieve for rinsing the silt and clay particles into a beaker big enough, this residual of sieved by the 0.053-mm sieve is the mass of sand fraction in evaluated soil, and then to be dried with an oven at the condition of 105 °C to constant weight. Contrast, the smaller volume of solution can be flow in beaker. The collected solution is the mixing suspend of silt and clay from evaluated soil mass (15g) and stirred thoroughly, then keep this suspend of silt and clay solution at room temperature (18-24°C), after the sedimentation period of static duration 1.5-6h. The supernatant is the clay suspend to be decanted and discarded, and the residual from this step is the mass of silt fraction in evaluated soil, the part of silt residual is then dried with an oven at the condition of 105 °C to constant weight. The sand% and silt% are calculated as the followed math equation based on their original mass of fraction (T. A. Kettler, 2001). The Clay% is determined by calculating in total 100% minus the sum of the Sand% and Silt% derived the evaluated soil mass = 100% = Clay% + Sand% + Silt%.

a. Sand% = (dry sand mass by over **μg** / original dry soil mass **μg**) × 100%

b. Silt% = (dry silt mass by over **μg** / original dry soil mass **μg**) × 100%

c. Clay% = 100 – (Sand% + Silt%).

All analyses were tested in triplicate at minimum.

2.2.2 Extraction of mercury and methylmercury

THg and MeHg extractions from earthworms were performed based on the reported protocol for biological tissue (Nakano et al., 2010; Yoshimoto et al., 2016). For THg extraction, an aliquot of approximately 0.1 g (dw) of each earthworm sample was weighted in 15 mL Polypropylene (PP) tubes with screw caps, in triplicate. First, the samples were hydrolyzed by adding 0.1% L-Cys solution (1.0 mL) and 5 M NaOH (1.0 mL). Samples were placed in a water bath (80 °C) for 1 h with vortexing shaker every 10 min until incubating hydrolysis was completed. Samples were then cooled in the hydrolyzed tubes to room temperature (22 ± 2 °C) in tap water. Second, the hydrolyzed sample solution was adjusted to 5 mL with ultrapure water and degreased for 10 min with 6 mL methyl isobutyl ketone (MIBK) and a shaking machine (220 rpm). The upper MIBK suspension was removed after 10 min of centrifugation (3000 rpm); the residual MIBK was eliminated by adding and removing 5 mL of hexane after shaking (5 min, 220 rpm) and centrifugation (5 min, 3000 rpm), and the final liquid was used for THg determination in earthworms.

For MeHg extraction, a 2 mL solution of the THg extraction was added to 2 mL HBr (5 M), 0.5 mL $CuCl_2$, and 6 mL toluene, followed by shaking (10 min, 220 rpm) and centrifuging (10 min, 3000 rpm). Then, 5 mL of the upper toluene supernatant was collected into a new 15 mL PP tube; 1 mL of the mixed solution was added (0.2% L-Cys and 2% NaOAc) to the toluene extract and then shaken (5 min, 220 rpm) and centrifuged (5 min, 3000 rpm). After removing the upper toluene, the final liquid was used for MeHg determination in earthworms.

2.3 Reagents and materials

All reagents used for analyzing Hg in soil and earthworm were analytical grade or higher in this study. The $HgCl_2$ standard solution ($HgCl_2$ 1,000mg/L) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Glacial acetic acid, hydrochloric acid (HCl), 5M sodium hydroxide (NaOH), nitric acid (HNO_3), hydrogen bromide, methyl-isobutyl ketone (MIBK, atomic absorption spectrometry grade), copper chloride dehydrates ($CuCl_2 \cdot 2H_2O$), hexane and toluene (HPLC, analysis grade), L-Cysteine (L-Cys) were purchased from Kanto Chemical Co. (Tokyo, Japan). Sodium acetate trihydrate (NaOAc: $CH_3COONa \cdot 3H_2O$) were purchased from Merck

(Darmstadt, Germany). Milli-Q water was prepared using an Ultra-pure water system (Merck Millipore, Tokyo, Japan). Fresh 0.1% L-Cysteine solution and 0.2% L-Cysteine +2%NaOAc solution were prepared for each experiment. All analytical procedure and extraction materials (beaker, vessels, centrifuge tubes, etc.) were conducted ultra-clean handling to avoid cross-contamination and then soaking and washing with a detergent (Extran MA02, Merck, Ltd.) and Milli-Q water rinsing 3 times. And then following with soaking into 20% HNO_3 24h and Milli-Q water rinsing 3 times. Finally, all materials were air-dried in clean environment.

All containers (beaker, vessels, centrifuge tubes, etc.) used in the analytical procedures were soaked in 10% HNO_3 for 24 h, rinsed three times in deionized water, and dried before use. Hg concentrations in all reagents and blanks were always below the detection limits for each relevant analytical procedure.

2.4 Mercury determination, quality control (QC), and quality assurance (QA)

A direct thermal decomposition Hg analyzer (MA-3000, Nippon, Japan) was used to analyze soil samples for THg concentration and earthworm extracts for THg and MeHg concentrations. The instrument uses thermal decomposition, gold amalgam collection, and cold vapor-atomic absorption spectroscopy. The concentration of total Hg in the sample was determined by direct preparation and placement into sample determining boats. Soil MeHg was detected using the electron capture detector gas chromatography (GC-ECD) method used by Japan Food Research Laboratories.

All recovery tests for the extraction procedures for THg and MeHg were conducted using certified reference materials (CRM) from swordfish 7403-a (CRM165) and codfish 7402-a (CRM250). Digestion of CRM using this extraction procedure on replicates (n = 6) showed a MeHg recovery of 98.2 ± 3.7% (i.e., 4.910 ± 0.190 mg kg^{-1}) for CRM165 (i.e., 5.000 ± 0.220 mg kg^{-1}) with a coefficient of variation (CV) of 3.79%, and 96.9 ± 2.2% (i.e., 0.550 ± 0.010 mg kg^{-1}) recovery for CRM250 (i.e., 0.580 ± 0.020 mg kg^{-1}) with a CV of 2.32%. CRM165 (5.340 ± 0.140 mg kg^{-1}) showed a THg recovery of 97.6 ± 4.3% (i.e., 5.210 ± 0.170 mg kg^{-1}) with a CV of 4.4%, and CRM250 (i.e., 0.610 ± 0.020 mg kg^{-1}) showed a recovery of 98.01 ± 2.9% (i.e., 0.580 ± 0.020 mg kg^{-1}) with a CV of 2.95%. Therefore, we used 97.5% (the mean CRM MeHg recovery for swordfish and codfish) as the correction factor for calculating the concentration of MeHg extracted from earthworm samples (Nakano et al., 2010; Yoshimoto et al., 2016). The recovery tests for soil THg were measured using CRM lake sediments 7303-a (CRM E-0190) and marine sediments 7302-a (CRM A-0210). The mean (n = 6) THg recovery was 96.8 ± 5.3% (i.e., 0.065 ± 0.004 mg kg^{-1}) for CRM E-0190 (i.e., 0.067 ± 0.006 mg kg^{-1}) with a CV of 5.51%, and 96.7 ± 3.1% (i.e., 0.503 ± 0.160 mg kg^{-1}) for

CRM A-0210 (i.e., 0.520 ± 0.030 mg kg^{-1}) with a CV of 3.19%. All CRM for this study were purchased from the National Metrology Institute of Japan (NMIJ).

2.4 Data statistical analysis

Bioaccumulation factors (BAFs) were calculated as Hg (THg and MeHg) concentrations in earthworm tissues divided by the corresponding Hg (THg and MeHg) concentrations in forest soils (BAF_{THg} and BAF_{MeHg}). All data were presented as mean ± standard deviation (SD), except for the general forest soil properties, which were only presented as means. Data were analyzed using Statistical Product and Service Solutions (SPSS) version 21.0, and Excel 2016 was used for data statistical treatment. Statistical analyses included one-way ANOVA tests, or the non-parametric Kruskal-Wallis test if the data had a non-normal distribution. Hierarchical cluster, correlation, and linear regression analyses were used for evaluating the relationships between general soil properties and Hg (THg and MeHg) concentrations in earthworm tissues and forest soils. Less than 0.05 was considered the level of statistical significance (p value) in all statistical treatments of the data. Figures were generated using GraphPad Prism version 6.04 and SPSS 21.0.

3. Results

3.1 Characteristics of forest soils and native earthworms

3.1.1 Soil physicochemical characteristics

The physicochemical characteristics of forest soil for all samples are summarized in Table 2-1 and Fig. S2-1. The measured pH ranged from 4.08 to 5.57 (mean: 4.77) in leachate-contaminated soils, which were similarly acidic to the reference soils, whose pH ranged from 4.58 to 5.21 (mean: 4.85). SOM was slightly higher in the leachate-contaminated soils, which varied between 4.82% and 17.59% (mean: 10.04%), compared to the reference soils, which varied between 9.78% and 10.55% (mean: 9.7%). The investigated forest soil PSD contained a wide range of sands, silts, and clays. The PSD percentages of sands ranged from 28.27% to 60.73% (mean: 45.77%), that of silts ranged from 12.90% to 33.60% (mean: 20.76%), and that of clays ranged from 13.76% to 48.59% (mean: 33.47%) in leachate-contaminated sites, which were generally similar to the uncontaminated sites; the latter had sands that ranged from 37.93% to 45.37% (mean: 40.50%), silts that ranged from 25.32% to 29.46% (mean: 20.27%), and clays that ranged from 29.31% to 33.76% (mean: 31.81%).

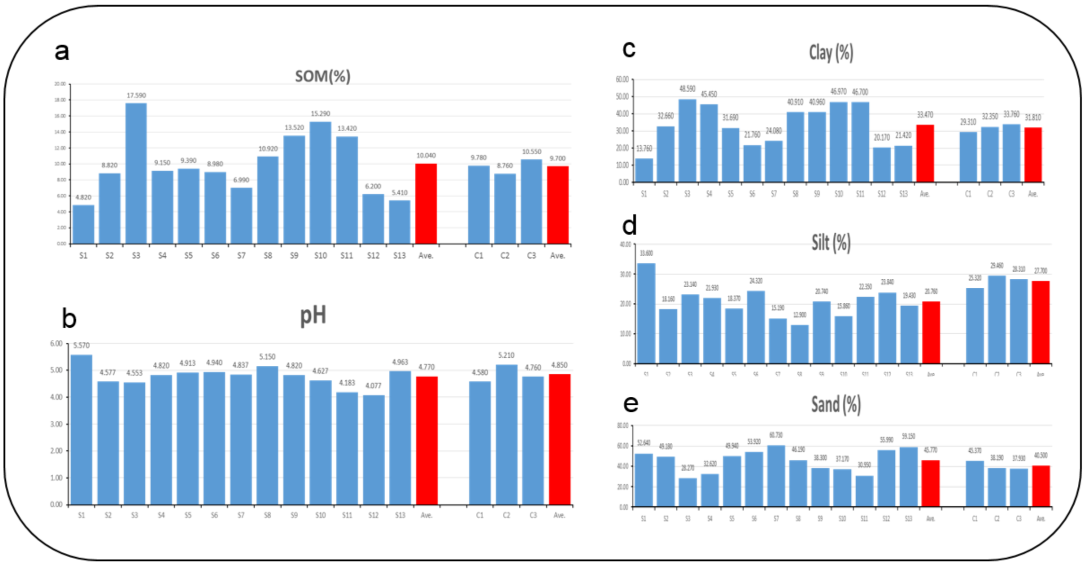


Fig. S2-1. Comparisons of the (a) soil organic materials (SOM), (b) soil pH, and soil particle size distribution (PSD, (c) clay (d) silt (e) sand between the leachate contaminated sites and the reference forest site.

3.1.2 Identification of native earthworm

Two different species of earthworms were collected from the surface soil (0–15 cm) at the leachate-contaminated sites and the reference sites (Table 2-1). The two species were *Bimastus parvus* (Fig. S2-2) and *Dendrobaena octaedra* (Fig. S2-3). *B. parvus* was the dominant species, and were sampled in all sites (total count of 339 in leachate-contaminated sites and 63 in uncontaminated reference sites). In comparison, *D. octaedra* was relatively abundant at the reference sites; approximately fifteen were found across three sites, compared to the leachate-contaminated sites, where only three were found across two sites. Therefore, the native *B. parvus* earthworms were sufficient for analyses, and were selected in this study as the subject for Hg accumulation comparisons.

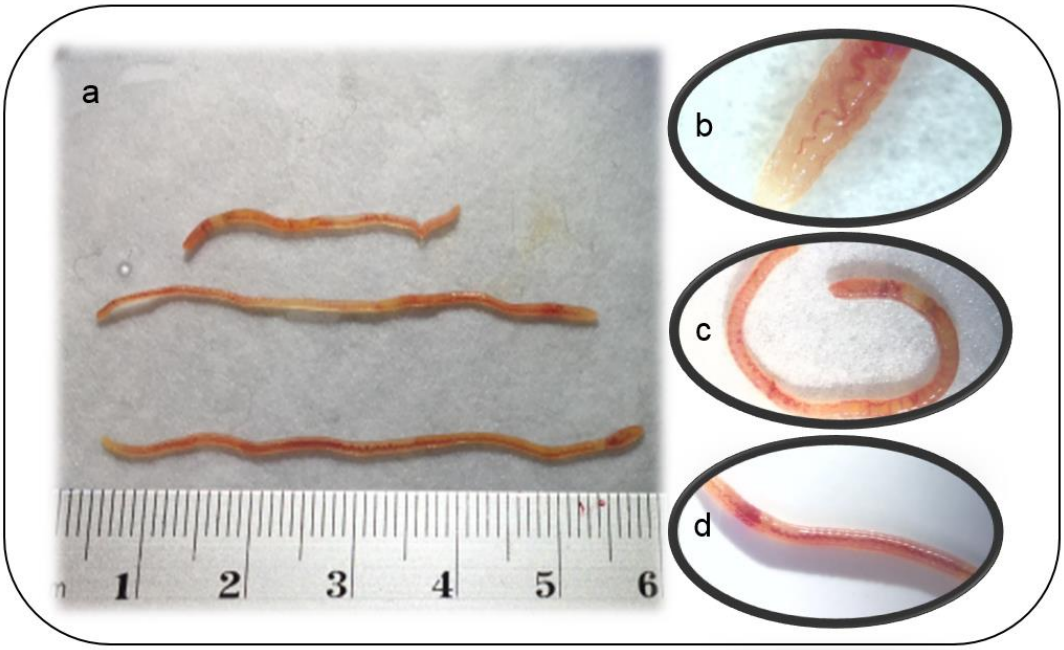


Fig. S2-2 Images of the dominant earthworm species: *Bimastos parvus* (*B. parvus*) (a) body size, (b) head and prostomium, (c) clitella and segment, (d) genital tumescence in landfill-leachate-contaminated forest soils.

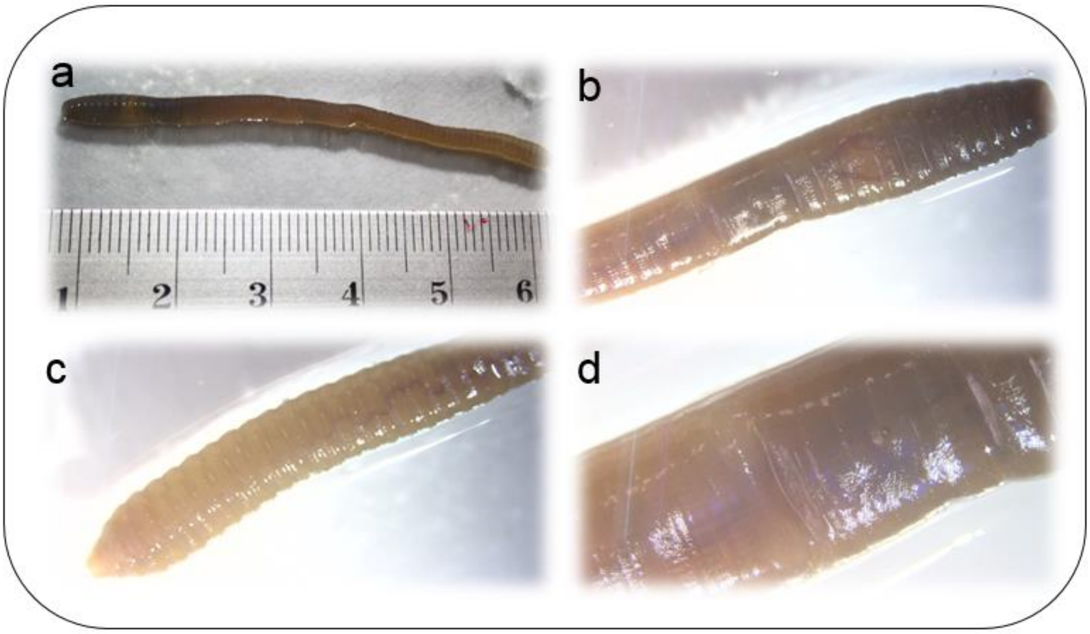


Fig. S2-3 Images of the earthworm species: *Dendrobaena octaedra* (*D. octaedra*): (a) body size, (b) head and prostomium, (c) segment and tail, (d) clitella and genital tumescence in landfill-leachate-contaminated forest soils.

Table 2-1

Sampling locations, earthworm species, and general soil properties in the study.

Site	Longitude (°E)	Latitude (°N)	Earthworm species (Count)	pH	[1]SOM (%)	[2]Soil PSD		
						Sand (%)	Silt (%)	Clay (%)
Leachate contaminated sites								
S1	*129.7435*	*32.8743*	*B. parvus* (3)	5.57	4.82	52.64	33.60	13.76
S2	*129.7437*	*32.8741*	*B. parvus* (13)	4.58	8.82	49.18	18.16	32.66
S3	*129.7434*	*32.8739*	*B. parvus* (43)	4.55	17.59	28.27	23.14	48.59
S4	*129.7438*	*32.8739*	*B. parvus* (60), *D. octaedra* (1)	4.82	9.15	32.62	21.93	45.45
S5	*129.7435*	*32.8738*	*B. parvus* (35), *D. octaedra* (2)	4.91	9.39	49.94	18.37	31.69
S6	*129.7440*	*32.8737*	*B. parvus* (38)	4.94	8.98	53.92	24.32	21.76
S7	*129.7441*	*32.8739*	*B. parvus* (20)	4.84	6.99	60.73	15.19	24.08
S8	*129.7435*	*32.8734*	*B. parvus* (21)	5.15	10.92	46.19	12.90	40.91
S9	*129.7433*	*32.8736*	*B. parvus* (22)	4.82	13.52	38.30	20.74	40.96
S10	*129.7433*	*32.8733*	*B. parvus* (24)	4.63	15.29	37.17	15.86	46.97
S11	*129.7434*	*32.8731*	*B. parvus* (18)	4.18	13.42	30.95	22.35	46.70
S12	*129.7436*	*32.8730*	*B. parvus* (17)	4.08	6.20	55.99	23.84	20.17
S13	*129.7437*	*32.8736*	*B. parvus* (25)	4.96	5.41	59.15	19.43	21.42
[3]Ave.				4.77	10.04	45.77	20.76	33.47
Reference sites								
R1	129.7533	32.8857	*B. parvus* (27), *D. octaedra* (6)	4.58	9.78	4.58	45.37	25.32
R2	129.7545	32.8840	*B. parvus* (17)	5.21	8.76	5.21	38.19	29.46
R3	129.7551	32.8839	*B. parvus* (19), *D. octaedra* (9)	4.76	10.55	4.76	37.93	28.31
Ave.				4.85	9.70	40.50	27.70	31.81

Note:

1. SOM: soil organic materials.
2. Soil PSD: soil particle size distribution (Sands: 0.050 < % < 2 mm, Silts: 0.002 < % < 0.050 mm, Clays: % <0.002 mm).
3. Ave.: average.

3.2 Total mercury and methylmercury in soils and earthworms

The THg concentrations in leachate-contaminated soils varied between 0.227 mg kg^{-1} and 2.919 mg kg^{-1} dw (mean 1.296 mg kg^{-1} dw), and were significantly higher than those of the reference soils (t = 3.464, $p < 0.0001$), which varied between 0.074 mg kg^{-1} and 0.103 mg kg^{-1} dw (mean 0.087 mg kg^{-1} dw), as expected (Figs. 2-2a and 2-2b). The average THg concentrations in leachate-contaminated sites were higher than those in the reference sites, and were up to 10.21 (4.318 mg kg^{-1} versus 0.423 mg kg^{-1}) and 14.90 (1.296 mg kg^{-1} versus 0.087 mg kg^{-1}) times higher in *B. parvus* and forest soils, respectively. Three sites (S3, S8, and S10) showed the highest THg concentrations (2.750 ± 0.264 mg kg^{-1} dw, 2.919 ± 0.211 mg kg^{-1} dw, and 2.852 ± 0.150 mg kg^{-1} dw, respectively) in leachate-contaminated forest soils compared to the other study sites (Fig. 2-2a), whereas similar average MeHg concentrations in forest soils (< 0.01 mg kg^{-1}) and *B. parvus* (0.100–0.114 mg kg^{-1}) were widespread across all sampled sites. No significant differences were found between the concentrations of MeHg in the forest soil samples of leachate-contaminated sites and the reference sites, and they were all below the detection limits (< 0.01 mg kg^{-1} dw) of the testing methodology (Fig. 2-2).

As the *B. parvus* earthworms from S1 died during pre-treatment for depuration of gut contents, only the tissues of *B. parvus* earthworms from 12 of the 13 leachate-contaminated sites were analyzed for Hg (THg and MeHg). The THg concentrations in earthworm tissues varied between 1.242 mg kg^{-1} dw and 6.775 mg kg^{-1} dw, with an average value of 4.318 mg kg^{-1} dw in leachate-contaminated sites. These values were significantly higher than the earthworm tissue THg values measured from the reference soil (t = 6.273, $p < 0.0001$), which varied between 0.350 mg kg^{-1} dw and 0.490 mg kg^{-1} dw (mean: 0.423 mg kg^{-1} dw), also as expected (Figs. 2-2a and 2-2b).

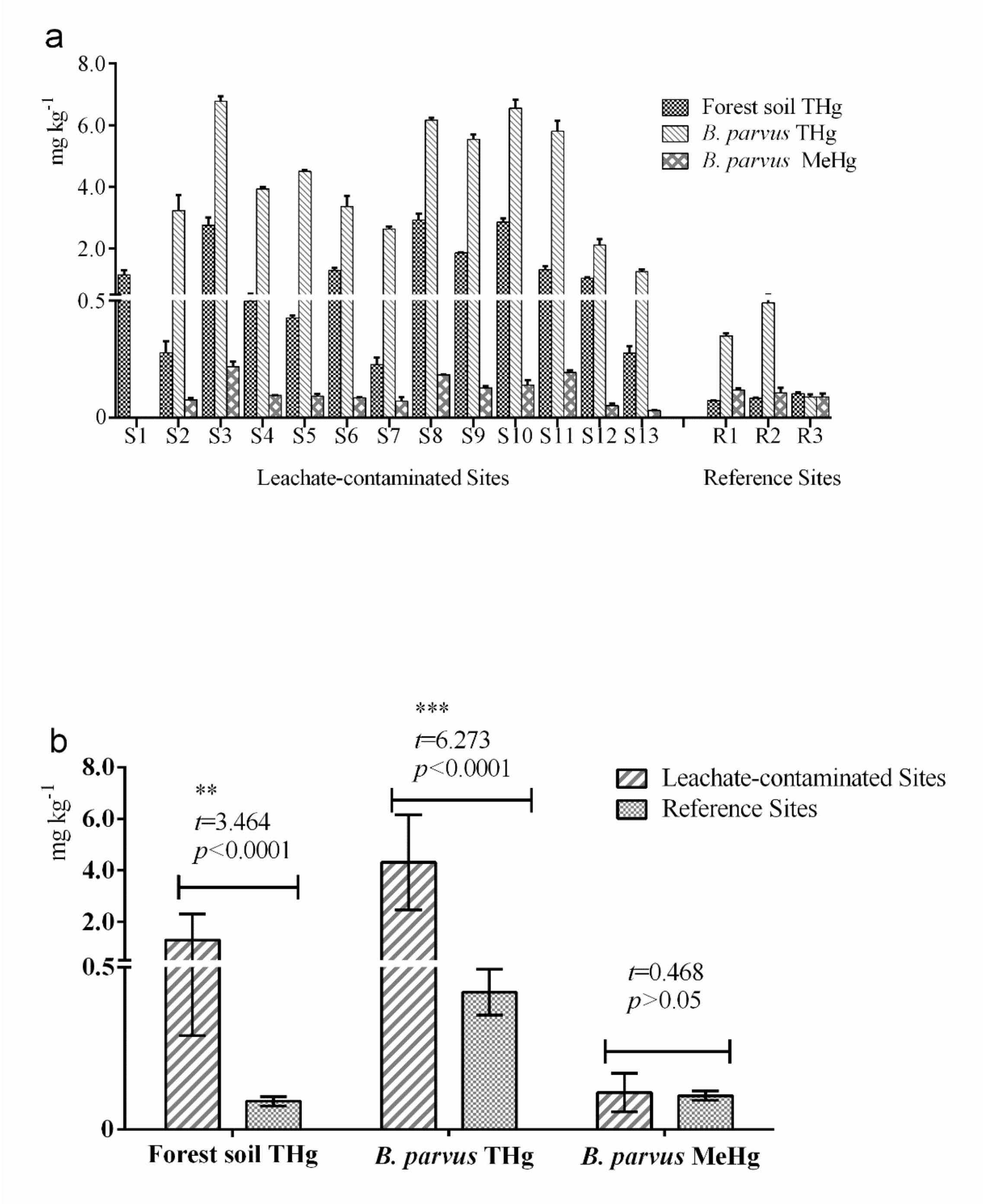


Fig. 2-2 Concentration (values are means ± standard deviation (SD) mg kg^{-1} dw) comparisons of THg and MeHg in (a) each sampling sites, (b) soils and native *B.parvus* earthworms, both leachate contaminated and control forest sites.

No statistically significant differences in the mean MeHg concentrations (*t* = 0.468, *p* > 0.05) in *B. parvus* tissues were found between the leachate-contaminated soils (0.031–0.218 mg kg^{-1} dw, mean: 0.114 mg kg^{-1} dw) and reference soils (0.089–0.118 mg kg^{-1} dw, mean: 0.104 mg kg^{-1} dw) (Figs. 2-2a and 2b). The top three highest *B. parvus* tissue THg concentrations were found in the soils with the corresponding maximum THg concentrations (6.775 ± 0.171 mg kg^{-1} dw for S3, 6.168 ± 0.065 mg kg^{-1} dw for S8, and 6.551 ± 0.268 mg kg^{-1} dw for S10) in the leachate-contaminated forest sites (Fig. 2-2a). The dendrogram branches in the cluster analysis indicated that the THg and MeHg concentrations in *B. parvus* tissues were correlated with each other and with soil THg, SOM, and clays, and were not correlated with the soil pH, sands, and silts in leachate-contaminated forest soil (Fig. 2-3). Further linear regression results also indicated that there were close relationships between earthworm Hg (THg and MeHg) and forest soil THg from the leachate-contaminated sites (Figs. 2-4a, 2-4b, and 2-4c).

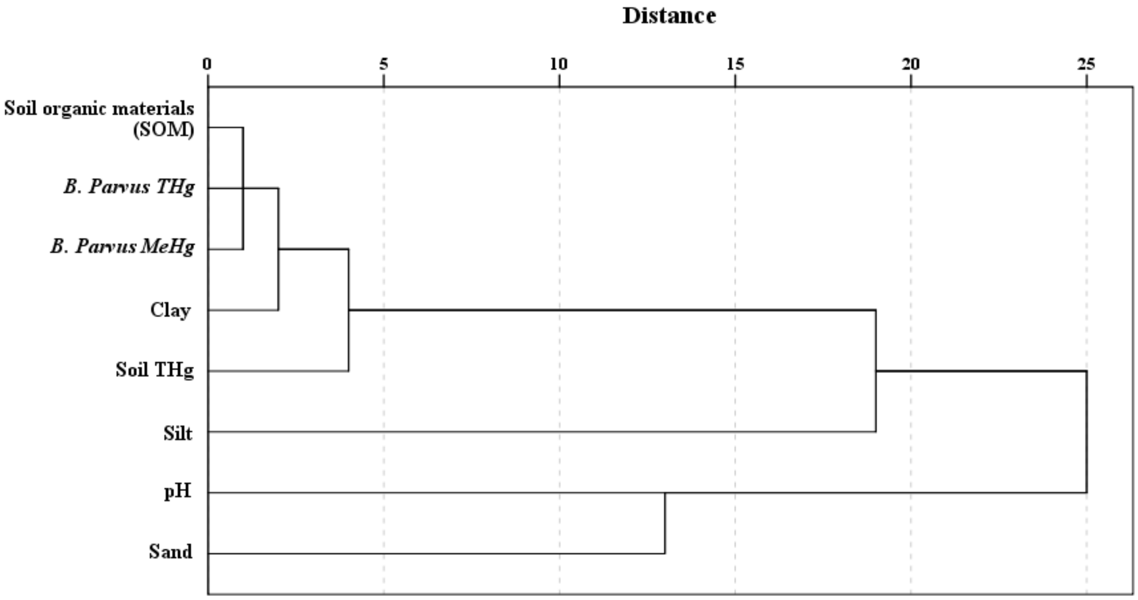


Fig. 2-3 Hierarchical cluster analysis between the determined soil THg, Hg (THg and MeHg) concentrations of *B. parvus* earthworms and soil properties (Soil organic materials (SOM), Clay, Silt, Sand, pH) in the leachate contaminated forest soils.

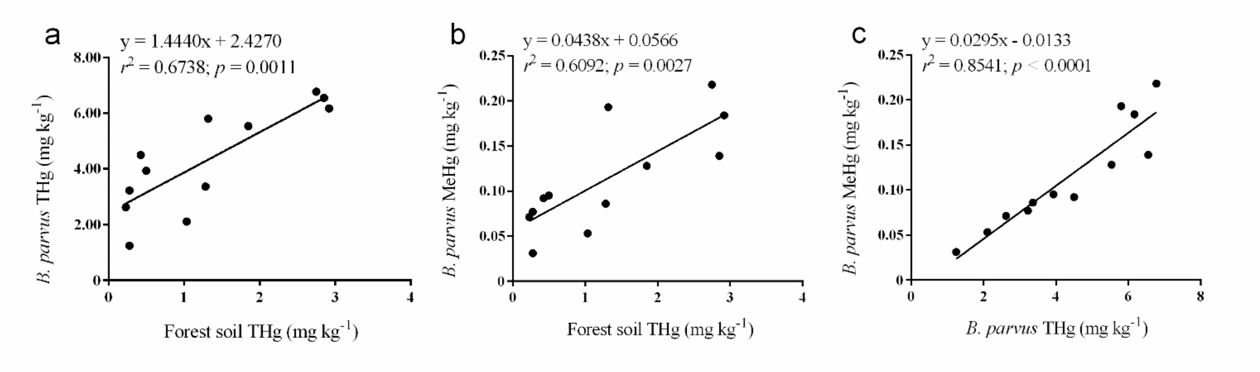


Fig. 2-4 Bivariate relationships of regression analyzing among Hg concentration in soil and tissue of native earthworm, (a) Between soil THg and *B.parvus* earthworm THg, (b) Between soil THg and *B.parvus* earthworm MeHg, (c) Between THg and MeHg in *B.parvus* earthworms.

3.3 Mercury accumulation in earthworm tissues

The measured *B. parvus* BAF for forest soil THg contained a wide range of values, varying between 2.04 and 11.65 in leachate-contaminated soils (Fig. 2-5) and between 4.17 and 5.83 in reference soils. Sites S2, S5, and S7 had the highest earthworm BAF_{THg} values of 11.65, 10.56, and 11.55, respectively, in the leachate-contaminated forest soils compared to the values of other study sites (Fig. 2-6a). The mean BAF_{THg} of *B. parvus* in the leachate-contaminated forest sites (5.42) was slightly higher than that of the reference sites (mean: 4.91) (Fig. 2-6a). The ratio of MeHg to THg (MeHg/THg %) in *B. parvus* ranged between 2.04% and 3.33% (mean 2.59%) in leachate-contaminated soils, which was significantly lower than the range (MeHg/THg %) in reference soils, which was between 20.70% and 33.71% (25.35%) (Fig. 2-6b).

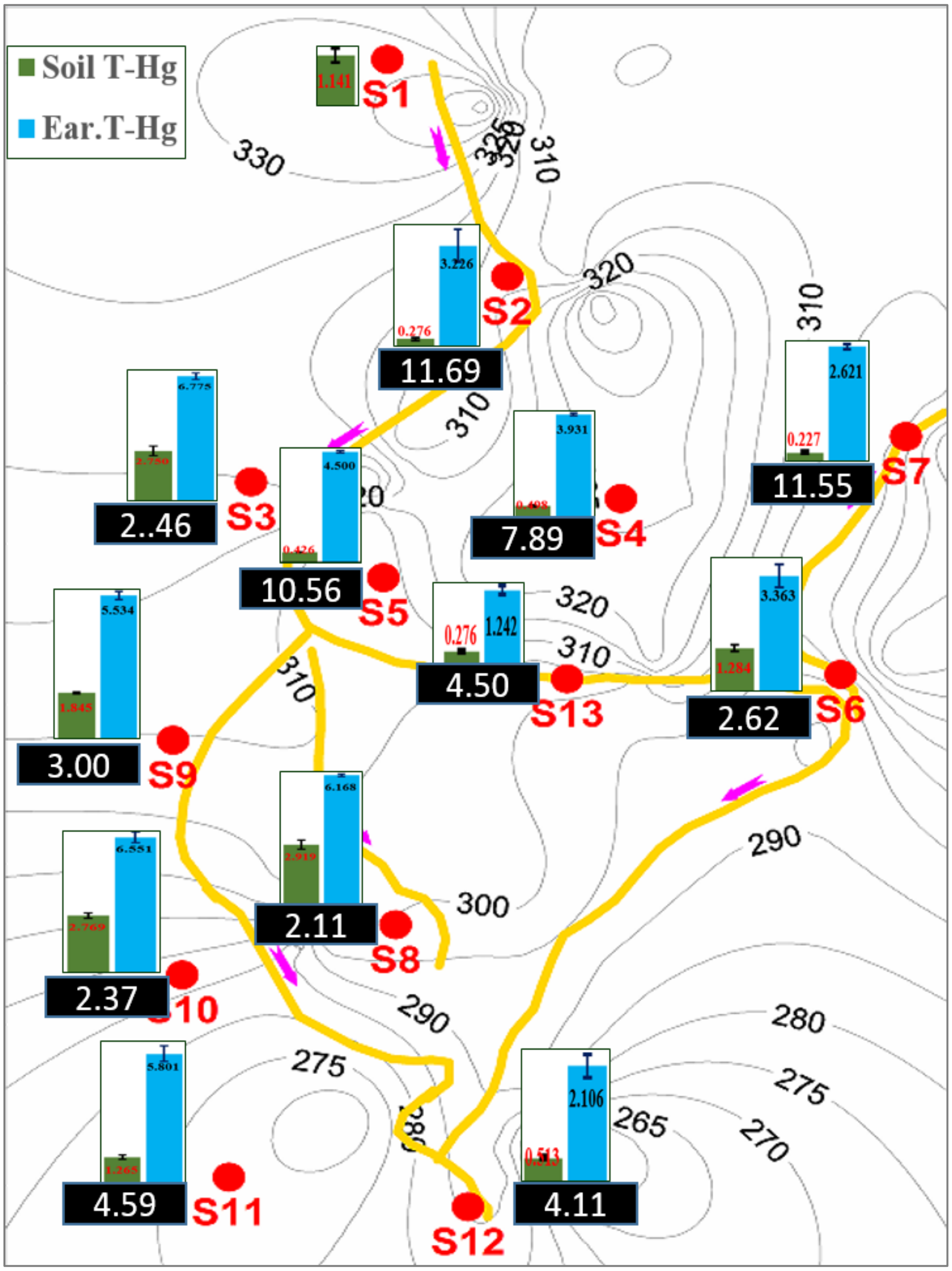


Fig. 2-5 The variation of total mercury bioaccumulation factors (BAF$_{THg}$) in *B. parvus* earthworm tissues at the landfill-leachate-contaminated soils

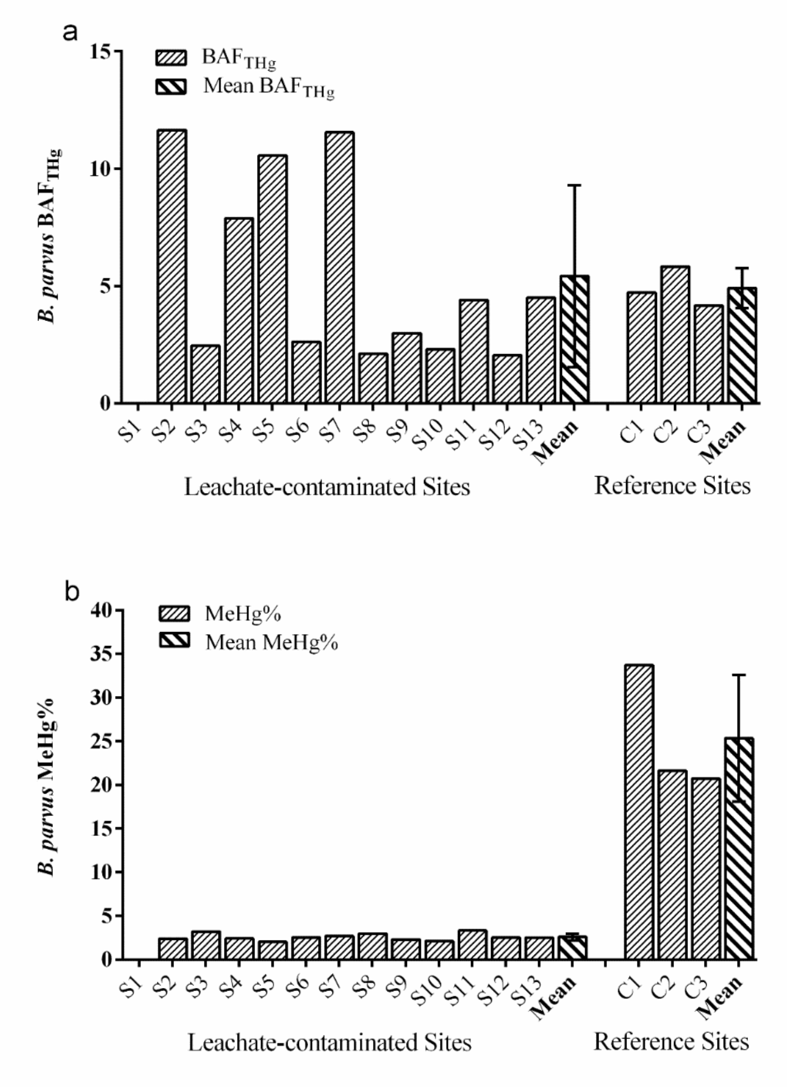


Fig. 2-6 Comparisons of the (a) BAF_{THg} and (b) MeHg% of THg in native *B.parvus* earthworm both the leachate contaminated sites and the control forest sites.

4. Discussion

4.1 Mercury concentrations in forest soils and native earthworms

Most studies on soil THg have been conducted in polluted sites, and have found a wide range in concentration of THg, from a few milligrams to several thousand milligrams per kilogram of soil (Fernández-Martínez et al., 2015; Reis et al., 2009). According to a survey of environmental Hg levels conducted in 18 prefectures of Japan, the residual soil THg concentrations varied between 0.002 mg kg^{-1} and 78.6 mg kg^{-1} (Nakagawa, 2008). However, it is difficult to find studies analyzing THg and MeHg concentrations in earthworms from MSW leachate-contaminated forest soils in the literature; the general consensus of previous studies is that atmospheric Hg deposition is the major source of Hg in forest soils (Buch et al., 2017; Rieder et al., 2011).

Our results indicated that THg concentrations in the reference forest soil surface varied between 0.074 mg kg^{-1} dw and 0.103 mg kg^{-1} dw, with a mean value of 0.087 mg kg^{-1} dw. These were similar to measurements from forest soils in Switzerland, which varied between 0.07 mg kg^{-1} dw and 0.55 mg kg^{-1} dw (Rieder et al., 2011), and soils in Brazil, which varied between 0.02 mg kg^{-1} and 0.15 mg kg^{-1} (Buch et al., 2017; Buch et al., 2016). The concentrations were lower than those from soils in French Guiana, which were between 0.25 mg kg^{-1} and 0.55 mg kg^{-1} (Da Silva et al., 2016). THg concentrations in landfill-leachate-contaminated forest soils varied between 0.227 mg kg^{-1} dw and 2.919 mg kg^{-1} dw, and were significantly higher (approximately 15 times higher) than the background values of THg in uncontaminated forest soils. Higher THg concentrations were correlated with higher SOM content in leachate-contaminated forest soils, which was consistent with other reports (Buch et al., 2017; Rieder et al., 2011). This observation may be explained by SOM having a strong affinity for binding with Hg, especially for SOM containing thiol groups (Skyllberg and Drott, 2010). In addition, soil clays were correlated with THg concentrations and SOM, and these relationships have also been found in previous reports (Buch et al., 2017; Soares et al., 2015). In contrast, the pH value had no significant correlation with soil THg contents in leachate-contaminated forest sites, potentially because of the relatively narrow and proximate pH range (4.08–5.57); previous reports showed that SOM had better affinity to Hg when the pH values ranged between 4 and 5 in tropical forest soils (Buch et al., 2017). This acidity might cause most Hg to accumulate in organic matter and clays of the surface forest soils, which also represents the main food source for most soil dwelling fauna, including earthworms (Meili et al., 2003).

However, there has been limited understanding of the influence of Hg (THg and MeHg) from leachate-contaminated soils on soil dwelling organisms. Hg limits of 0.13 mg kg^{-1} in soils and 3.3 mg kg^{-1} in organic matter have been recommended as guidelines; it is assumed that Hg poses no harm to soil organisms below these critical levels (Tipping et al., 2010). Based on this critical limit, THg concentrations of all leachate-contaminated forest soils (13) exceeded this guideline, while THg concentrations at three reference sites were below this reference guideline.

THg, especially MeHg, in earthworms has the potential for bio-magnification in natural food chains of earthworm predators, such as birds, reptiles, and other invertebrates (Han et al., 2012). The results in this study indicated that the THg concentrations in *B. parvus* tissues in leachate-contaminated forest soils (1.242–6.775 mg kg^{-1} dw) were significantly higher than those in reference forest soils (0.350–0.490 mg kg^{-1} dw), and mean concentrations of MeHg in *B. parvus* tissues were similar to those in both the leachate-contaminated and uncontaminated sites (0.114 mg kg^{-1} dw and 0.104 mg kg^{-1} dw, respectively). Standards have not been published for Hg in earthworm tissues. Hg transfer coefficients in the food chain are 0.88 and 2.35 in herbivorous/omnivorous and carnivorous invertebrates, respectively, as proposed by Canadian soil quality guidelines (Buch et al., 2017). Accordingly, the acceptable earthworm Hg limit would be below approximately 0.2 mg kg^{-1}, referencing the fish Hg limit of 0.5 mg kg^{-1} (WHO, 2008) Therefore, it cannot be ignored that Hg will have potential effects on the food chain via earthworms native to leachate-contaminated forest soil ecosystems.

4.2 Mercury accumulation in native earthworms

The current results demonstrated that *B. parvus* can accumulate Hg in their tissues from leachate-contaminated or uncontaminated forest soil, as the average BAF values for soil THg were similar in all sites, which were 4.91 (reference forest soils) and 5.42 (leachate-contaminated soils). The BAF_{THg} varied between 2.04 and 11.65 in leachate-contaminated forest soils, which was close to the BAF_{THg} found for four endogeic species of earthworm (between 3 and 15) reported in Swiss forest soils (Rieder et al., 2011). However, it is generally accepted that the assimilated accumulation of soil inorganic Hg is much lower in earthworms and varies across species in many studies. The BAF_{THg} of *Diplocardia* earthworm species varied between 0.47 and 1.75 in floodplain soil (Han et al., 2012); the BAF_{THg} of three earthworm species (*Drawida*, *Allolobophora*, and *Limnodrilus*) varied between 0.040 and 0.539 in soils polluted by chlor-alkali and smelting industries (Zhang et al., 2009); and *Eisenia fetida* in dredge spoil soils had a BAF value of 0.4 (Edwards et al., 1998). These previously measured BAF_{THg} values were different from our results, most likely due to different geochemical characteristics and earthworm species in forest soils. For example, Hg accumulation in earthworms is influenced by SOM contents, the soil components for binding Hg, and earthworm exposure time or life expectancy (Buch et al., 2017; Dang et al., 2015; Han et al., 2012).

Regarding MeHg accumulation, previous studies have found that BAF_{MeHg} values for earthworms were higher than BAF_{THg} values (Rieder et al., 2011). Unfortunately, we were not able to accurately calculate the value of BAF_{MeHg} in *B. parvus* because all soil MeHg concentrations were below the detection limit (< 0.01 mg kg^{-1} dw). Therefore, MeHg accumulation in *B. parvus* in this study cannot be quantitatively compared with other reports. MeHg concentrations in soils were low, and the relative contents ranged from 0.5% to 1.5% of soil THg in previous studies (Cristol et al., 2008; Rieder et al., 2011). Referencing this ratio of MeHg, we extrapolated from soil THg to estimate MeHg (0.001 mg kg^{-1} to 0.044 mg kg^{-1}) and BAF_{MeHg} (between 3 and 62) of *B. parvus* in this study. These estimated results were consistent with the BAF_{MeHg} values measured in other earthworm species (between 10 and 249) for soil MeHg (Burton et al., 2006; Rieder et al., 2011; Zhang et al., 2009), and were about 20 to 150 times higher than the BAF_{THg} in earthworms (Zhang et al., 2009). This difference may be due to MeHg being highly lipophilic, which makes it easier than inorganic Hg to be absorbed into earthworm bodies (Rieder et al., 2011). MeHg also has a strong adsorption tendency with organic matter, maintains high solubility for accumulation by invertebrates, and can be magnified along food chains (Ernst et al., 2008). In addition, the long lifespan of earthworms, usually from a few months to eight years, provides conditions for biological enrichment of soil Hg in their bodies (Han et al., 2012). The role of *B. parvus* as a bioindicator in Hg-contaminated environmental surveillance was proposed in this study. This study also showed that *B. parvus* has a high ability to accumulate MeHg (or THg) while reducing soil MeHg (or THg) contents in both leachate-contaminated and reference forest soils. Considering earthworms can be as an important protein source for predators in the ecosystem (e.g., birds, fish, reptiles, insects, and many mammals)(Dang et al., 2015). Thus, it was proposed that *B. parvus* can be a significant source of MeHg (or THg) exposure for food chains, which cannot be ignored.

4.3 Mercury methylation characteristics

It is normally accepted that inorganic forms of Hg can be methylated by soil bacterial activities, which enhances the bioavailability and bio-toxicity of Hg, and increases the potential threat of Hg to terrestrial organisms in soil ecosystems (Dang et al., 2015). However, recent research has indicated that this may not be universally true. Earthworms (*Eisenia foetida*) were found to potentially methylate Hg in their digestive tract (Hinton and Veiga, 2002). In addition, earthworms (*Lumbricus terrestris*) showed MeHg synthesis in vivo due to the sulfate-reducing bacteria found in earthworms but not in soils (Rieder et al., 2013). In our study, MeHg concentrations in the forest soil and native *B. parvus* earthworms were expected to increase due to the higher soil THg concentrations in the leachate-contaminated forest sites compared to the reference sites. Unexpectedly, no significant differences in MeHg concentrations were found between the leachate-contaminated soils and the reference soils (< 0.01 mg kg^{-1} dw in all sampled forest soil), or between the average MeHg concentrations in *B. parvus* tissues (0.114–0.104 mg kg^{-1}).

In addition, the ratio of MeHg to THg in *B. parvus* varied between 2.04% and 3.33% in leachate-contaminated forest soils, which was significantly lower than that of *B. parvus* in the uncontaminated forest soils, which ranged between 20.70% and 33.71%, because of the different THg concentrations in the forest soils. The ratio of MeHg to THg in *B. parvus* from leachate-contaminated forest soils was similar to the reported value of 3.01% in *Drawida* earthworms (Zhang et al., 2009), but was below the range of 5.7% to 10.1% found in seven different forest earthworm species (Rieder et al., 2011) and below the ratio of 12.02% found in *Allolobophora* earthworms (Zhang et al., 2009). The similar MeHg levels found among *B. parvus* tissues in the contaminated forest soils and reference forest soils might have been due to the similar MeHg levels in the forest soil of them (< 0.01 mg kg^{-1}

dw). The results support the proposal that MeHg was firstly formed by inorganic Hg being methylated in the forest soil, which was then ingested and accumulated in *B. parvus* via the lipophilic action, rather than that the MeHg was fromed in the digestive tracts of *B. parvus* containing MeHg synthesizing bacteria (e.g., sulfate-reducing bacteria) (Lee et al., 2016; Rieder et al., 2013; Shao et al., 2012). The later will lead to more MeHg synthesized and accumulated in *B. parvus* as the average THg concentrations in forest soils and earthworms in leachate-contaminated soils were significantly higher than those in uncontaminated forest soils (about 14.9 times and 10.2 times higher, respectively). Another possibility was that the *B. parvus* had similar bioaccumulation factors for soil MeHg between the contaminated and reference sites. The similar BAF_{THg} of *B. parvus* between the contaminated forest sites and reference forest sites supported this possibility (Fig. 2-5a), even if we could not calculate the BAF_{MeHg} of *B. parvus* accurately. It was believed that *B. parvus* accumulated similar MeHg levels from both leachate-contaminated and reference forest soils in our study. Therefore, we reasoned that the in vivo MeHg biosynthesis of *B. parvus* was not obvious in our current investigation results.

But inescapable problem is that the Hg methylation process within *B. parvus* could not be completely ruled out, and further studies are needed to address the potential sources of MeHg accumulation in *B. parvus*. Also, it is worth to investigate the underlying reasons of some of the uncertainties. First, there were uncertainties about the amount of mobile Hg (Hg^+) both in the leachate-contaminated soils and the reference forest soils. Ideally, Hg exists in soils in different chemical forms with different mobility and biotoxicity to organisms (Issaro et al., 2009). Previous reports indicated that THg concentration in soil cannot completely represent the biological behavior in ecosystems (Issaro et al., 2009; Pinedo-Hernández et al., 2015), and Hg biotoxicity effects are mainly determined by the actual amount of soluble Hg (e.g., $HgCl_2$) (Zagury et al., 2006), because the soluble soil-Hg fractions were high mobility and potential bioavailability comparing other stable or low soluble soil-Hg fractions (Bloom et al., 2003). That is why soluble Hg (e.g., $HgCl_2$) is often used for testing Hg methylation in earthworm digestive tracts in laboratory studies (Shao et al., 2012; Stephan Raphael Rieder, 2013). Second, comparing with other study, *B. parvus* is a different earthworm species sampled from different forest soils in contaminated and reference sites, and some uncertainties arose from potential differences in anaerobic bacterial communities which associated with the Hg methylation in earthworm digestive tracts or in soils of sampling sites from the previous reports (Elisabeth Kaschak, 2014; Lee et al., 2016; Rieder et al., 2013). The long-term effects of landfill leachate can greatly influence Hg speciation through changing the associated microbial communities under anaerobic conditions (Lee et al., 2016). It is difficult to determine the distribution of bacterial Hg methylation in the forest soils and in *B. parvus*, especially considering that the landfill leachate might differently affect the activity of bacterial Hg methylation in different sampling areas of leachate-contaminated forest soils. Third, the results from our cluster and linear regression analyses indicated that the THg and MeHg concentrations in *B. parvus* tissues were correlated with soil THg, SOM, and clays (Fig. 2-4). Based on previous studies, this suggested that the Hg forms were affected by multiple aspects of soil geochemical properties in forest soil because Hg specific compounds can combine with the soil matrix (e.g., SOM) to codetermine the Hg mobility and bioavailability in the natural environment (Bloom et al., 2003). The Hg combination formed by Hg compounds and the soil matrix was likely variable under the complex environment of the leachate-contaminated forest zone. The leachate Hg speciation, biogeochemical transformations, and mobilization were affected by changes in the redox potential and microbial communities of anaerobic conditions associated with Hg methylation during the long-term changes in landfill-leachate-contaminated soil (Lee et al., 2016).

5. Summary

Most Hg accumulation studies have focused on aquatic ecosystems; however, the role of Hg (THg or MeHg) accumulated and transferred into food chains of terrestrial ecosystems has not attracted enough attention, and little academic knowledge is available on this topic. The present work showed that Hg deposited in leachate-contaminated forest soils around traditional Hg-polluted landfills and background levels of Hg in uncontaminated soils deposited via natural mechanisms (e.g., atmospheric deposition) can be accumulated by *B. parvus* over time. *B. parvus* showed similar BAF_{THg} in both leachate-contaminated (mean 5.42) soils and uncontaminated (mean 4.91) forest soils. Although THg concentrations in the leachate-contaminated forest soils (mean 2.919 mg kg^{-1}) were significantly higher than those in uncontaminated forest soils (mean 0.087 mg kg^{-1}), the average MeHg concentrations were similar in all sampled forest soils and in *B. parvus*. Based on the current results, the role of *B. parvus* in MeHg production is not clear; MeHg in *B. parvus* could have first formed in forest soils, and then accumulated into their tissues. Nonetheless, potential Hg exposure of food chains through *B. parvus* in leachate-contaminated forest soils cannot be ignored because *B. parvus* shows a high ability to accumulate THg and MeHg from forest soils. To better understand the ecological risks of secondary Hg poisoning to organisms in terrestrial ecosystems, further studies of forest ecosystems around Hg-contaminated traditional landfills are necessary to confirm the possibility of an associated bacterial community for Hg methylation in *B. parvus* and soil.

Supplementary data of " Comparison of total mercury and methylmercury bioaccumulation in earthworms (*Bimastos parvus*) native to landfill-leachate-contaminated forest soil (LLCFS) "

Table S2-1 Hg (THg / MeHg) concentrations (values are means ± S.D. mg kg-1 dw) in soils and *B. parvus* earthworms of the study.

Site	Soil		*B. parvus* earthworms (mg kg^{-1} dw)			
	THg (mg kg^{-1} dw)	MeHg (mg kg^{-1} dw)	THg	MeHg	[1]MeHg (%)	[2]$BAF_{T\text{-}Hg}$
Leachate contaminated sites						
S1	1.141 ± 0.154	< 0.01	[3]n.a.	n.a.	n.a.	n.a.
S2	0.277 ± 0.049	< 0.01	3.226 ± 0.513	0.077 ± 0.007	2.39	11.65
S3	2.750 ± 0.264	< 0.01	6.775 ± 0.171	0.218 ± 0.023	3.22	2.46
S4	0.498 ± 0.044	< 0.01	3.931 ± 0.058	0.095 ± 0.001	2.42	7.89
S5	0.426 ± 0.013	< 0.01	4.500 ± 0.043	0.092 ± 0.011	2.04	10.56
S6	1.284 ± 0.091	< 0.01	3.363 ± 0.328	0.086 ± 0.002	2.56	2.62
S7	0.227 ± 0.034	< 0.01	2.621 ± 0.066	0.071 ± 0.017	2.71	11.55
S8	2.919 ± 0.211	< 0.01	6.168 ± 0.065	0.184 ± 0.002	2.98	2.11
S9	1.851 ± 0.228	< 0.01	5.534 ± 0.155	0.128 ± 0.008	2.31	2.99
S10	2.852 ± 0.150	< 0.01	6.551 ± 0.268	0.139 ± 0.022	2.12	2.3
S11	1.318 ± 0.100	< 0.01	5.801 ± 0.344	0.193 ± 0.010	3.33	4.4
S12	1.034 ± 0.045	< 0.01	2.106 ± 0.199	0.053 ± 0.009	2.52	2.04
S13	0.276 ± 0.085	< 0.01	1.242 ± 0.067	0.031 ± 0.003	2.50	4.5
[4]Ave.	1.296	< 0.01	4.318	0.114	2.59	5.42
Reference sites						
R1	0.074 ± 0.001	< 0.01	0.35 ± 0.010	0.118 ± 0.009	33.71	4.73
R2	0.084 ± 0.003	< 0.01	0.49 ± 0.017	0.106 ± 0.023	21.63	5.83
R3	0.103 ± 0.006	< 0.01	0.43 ± 0.011	0.089 ± 0.015	20.70	4.17
Ave.	0.087	< 0.01	0.423	0.104	25.35	4.91

Note:

1. MeHg % = MeHg / THg of earthworm.
2. Bioaccumulation factor (BAF): BAF_{THg} = THg concentration of earthworm / THg concentration of soil.
3. n.a.: data not available.
4. Ave.: average.

CHAPTER 3

Exploration of mercury (Hg) washing removal effects from landfill-leachate-contaminated soils (LLCS) using saturated sodium sulfide solution (ss-Na_2S): A laboratory study

★ Chapter Profile - 3

The objective of this laboratory study is to investigate the effects of washing remediation for leaching Hg of mercury (Hg)-containing landfill-leachate-contaminated soils (LLCS) using saturated sodium sulfide solution (ss-Na_2S) through considering the concentration changes of soil total mercury (THg), Hg-fractions in a validated 5-steps sequential extraction, soil Hg-static leaching test as a function of pH (1-12) and equilibrium time (0-4 weeks) before and after washing treatment of ss-Na_2S. The variation trends and relationships between the leaching concentration of Hg, electrical conductivity (EC) and total organic carbon (TOC) of leaching liquid from untreated Hg-containing LLCS were explored during equilibrium time in pH-dependent leaching test. The behavior of leaching Hg was tried and explored by selected Visual MINTEQ modeling according to the mineralogical composition analysis.

From an overall perspective of our experimental results, the exchangeable Hg (F1/Hg-w, F2/Hg-h) and F5/Hg-s to be washing removed by the treatment of ss-Na_2S from Hg-containing LLCS are significantly. Although the averaged THg concentration of LLCS reduced by 22.02% only (from 1.8266 mg kg^{-1} down to 1.4244 mg kg^{-1}) before and after washing treatment by ss-Na_2S. But even so, the concentration composition of some soil Hg fractions in LLCS have been significantly reduced before and after washing treatment of ss-Na_2S, including the average concentration of F1/Hg-w was reduced by 96.15%, F2/Hg-h was reduced by 95.58%, F3/Hg-o was reduced by 23.85%, F4/Hg-e was reduced by 5.92%, F5/Hg-s was reduced by 99.22%, respectively.

Our study shows that the ss-Na_2S as a Hg immobilization and washing removal application is an effective method to minimize the amount of leaching Hg from Hg-containing LLCS, those results were proved via a validated Hg five-steps sequential extraction procedure and pH dependent of Hg static-leaching test. Meanwhile, its washing effectiveness for the leaching Hg of LLCS are relatively high and stable checked by pH-dependent Hg leaching test with a wide range of equilibrium time (6h - 4w) and pH value (1 - 12) in this laboratory study. The main minimizing mechanism of the amount of leaching Hg is to reduce the concentration of exchangeable (i.e., Hg-w, Hg-h) and stable non-mobile Hg (i.e., Hg-s) of LLCS, that more than 95% of exchangeable Hg-w (96.15%) and Hg-h (95.58%) and stable Hg-s (99.22%) to be immobilized and washing removed by the excessive volume of ss-Na_2S from LLCS, However, it was found that the trial Visual MINTEQ modeling according to

mineralogical composition analysis is not suitable to predict the variation trends of leaching Hg in pH-dependent leaching test both in control and ss-Na_2S-washing treated Hg-containing LLCS.

Considering the exchangeable Hg fraction (i.e., Hg-w, Hg-h) were the high mobility and potential bioavailability than other Hg fraction in soil, the ss-Na_2S ex situ treatment may be developed a cost-effective Hg immobilization and washing process of soil remediation contaminated by Hg, for removing or reducing exchangeable Hg fraction (Hg-w, Hg-h) and non-mobile stable Hg fraction (Hg-s) from Hg-containing LLCS simultaneously for reducing or eliminating the Hg potential environmental risks of leaching Hg form LLCS.

Keywords: Mercury (Hg); Landfill-leachate-contaminated soil (LLCS); pH-dependent static leaching; Sodium sulfide solution (ss-Na_2S); Washing removal

1. Introduction

Mercury (Hg) is one of the most toxic heavy metal contaminants in the environment (water, food, air, soil etc.) (Canuel et al., 2009; Hissler and Probst, 2006). Due to its high risks of adverse effects for human health and ecosystem directly and indirectly, So far, Hg contamination is always a serious environmental problem worldwide (Canuel et al., 2009). No related information were reported to date that Hg has the beneficial biological function in biosphere (Ernst et al., 2008).

Soil Hg from natural processes, anthropogenic inputs and re-emitted of old environmental deposition are the major originating sources (Wang et al., 2012), and it is a relatively common phenomenon that the soil natural Hg concentration have been increased and led to environmental polluted issues were by anthropogenic activities (e.g., mining, industry, MSW landfilling etc.) (Gworek et al., 2015; Hylander and Meili, 2003; Zhu et al., 2013).

Municipal solid waste (MSW) (also called garbage or trash) is defined as wastes consisting of everyday items such as food scraps, newspapers, appliances, product packaging, clothing, bottles and furniture, cans, consumer electronics, fluorescent lamp and batteries (Sharholy et al., 2008). These MSW come from homes, industry such as factories, schools, hospitals, restaurants and small businesses. Once generated, MSW must be collected and managed methods including combustion for resulting energy used to generate electricity or steam in some cases, recovery for recycling or composting, and disposal in landfill (Diaz et al., 1993). MSW landfill have still been used for managing approximately 70% MSW disposal worldwide (Lee et al., 2016; Peter et al., 2002; Zhu et al., 2013). Mercury (Hg) containing garbage items goes into MSW landfill inevitable, especially in traditional MSW landfill is one of historical environmental heavy metal pollution (Gworek et al., 2015).

Landfill leachate was gradually formed by the internal biological processes and infiltration precipitation of the landfill (Duggan, 2005), and the Landfill-leachate-contaminated soil (LLCS) was found in surrounding soils of traditional landfill constructed without better anti-leaching system of leachate, the environmental hazardous pollutants can be released into the soil through leachate from landfill, especially in traditional landfill, therefore, the LLCS was contained higher contaminant levels than the acceptable limit for environmental soil safety (Liu et al., 2013). It has been reported that the complex landfill leachate contaminants including many dissolved macro organic matter, inorganic, xenobiotic organic compounds and heavy metals (e.g., Hg, Pb, Fe, Cu, Cd etc.) (Lee et al., 2016; Peter et al., 2002). Hg, in contrast to other toxic heavy metals, cycles in the environment between the water, land, and atmosphere, because environmental Hg can exist in different phases (gas, liquid, and solid), several studies have monitored the Hg contaminants in landfill leachate and in landfill gases (Kim and Kim, 2002; Kjeldsen and Christophersen, 2001; Nguyen et al., 2008). From the previous reports, Hg concentration in large MSW landfills ranged between 0.03 to 16.8 mg kg-1 in Florida, USA(Wang et al., 2012), and 0.17 and 46.22 mg kg^{-1} in Guiyang and 0.24 and 1.271 mg kg^{-1} in Wuhan City, China (Li et al., 2010), and Hg^0 concentration varied between 3.45 and 2952 ng m^{-3} in surface air, which was observed at the Nan-Ji-Do landfill in Korea (Kim and Kim, 2002).

It is well-known that Hg causing adverse biological effects are intimately related to the chemical speciation and complexed binding forms with organic materials, ligands, primary or secondary minerals (Fe, Mn etc.) in soil, which will control mobility, bioavailability, and toxicity to organisms in public health (Gleyzes et al., 2002; Issaro et al., 2009; Reis et al., 2015). Hence, many studies have proved that soil THg determination could not completely explain the details of biological behavior and risk to environmental organisms in soil (Issaro et al., 2009; Pinedo-Hernández et al., 2015). Particularly, it is hard to predict that the Hg alterations are variety for speciation or binding forms afforded by THg concentration in complex environmental conditions of MSW landfill or LLCS (Issaro et al., 2009; Lee et al., 2016; Wang et al., 2012).

Also, it is currently well known in previous studies that only THg determination is insufficient for establishing better remediation procedures and evaluating of remediation effects (Wang et al., 2012). The information concerning soil Hg speciation or binding forms can be used to predicate and understanding the hazardous of Hg, and assess or manage the quality of remediation for Hg contaminated soil, effectively (Serrano et al., 2012).

On the other hand, it is common understanding that methylmercury (MeHg) can accumulate along the aquatic food chain to produce toxins of ecosystem (Haris et al., 2017). Hazardous Hg methylation is the primary concern undergoing a number of Hg environmental transformation processes on chemical forms and biological speciation (Stein et al., 1996). Recent research reported that the sulfur reducing bacteria (SBR) seem to be an active and particular factor for Hg methylation under the anaerobic conditions in soils and gut of some animals (Barkay and Wagner-Döbler, 2005; Holloway et al., 2009; Rieder et al., 2013). Study also indicated that SBR can directly methylate the inorganic ($HgCl_2$) and the neutral soluble Hg sulfide complexes (HgS^0 and $Hg(SH)_2^0$) in the natural change of anaerobic environmental conditions (Benoit et al., 2001; Drott et al., 2007; Rieder et al., 2013). Therefore, it is a possibility on Hg in soil that to control those formation of soluble Hg species or fractions is effective measures for reducing the risks of Hg methylation to influence the ecosystems and human health (Serrano et al., 2012; Xiong et al., 2009). From existing studies and applications, the commonly employed and available techniques of the remediation for Hg contaminated soil including the stabilization/solidification (S/S), immobilization, soil washing, phyto-stabilization, thermal-desorption, nanotechnology, phytoextraction, phytovolatilization, electro-remediation and vitrification (Wang et al., 2012). Generally, the most commonly used remediation of heavy metal contaminated sites were to be used the solidification/stabilization (S/S) technology to immobilize metal contaminants, and disposal the treated materials into landfill, these management of heavy metals covering mercury (Hg), arsenic (As), cadmium(Cd), lead (Pb), chromium (Cr), copper (Cu), nickel (Ni) and zinc (Zn) in contaminated soil, because of this S/S technology can be fast scale applied to large area and the treated site by stabilization can be revegetated (Wang et al., 2012; Waste and Response, 2007). However, some disadvantages were found on S/S in the field application for remediation of soil heavy metals pollution. one hand, considering the metal contaminants were permanent in soil, the remedial measures base on S/S technology are not enough to soil environmental safety under the volume of waste increased by the regent of S/S treatment. On the other hand, the longevity of the solidified/stabilized materials are questionable, existing the uncertainty ecological risks of the long-term stability for S/S stabilized waste products, need to long-term monitor and manage those potential environmental risk of S/S treated materials on long-term environmental risk management in the future (Arisawa et al., 2000; Dermont et al., 2008; Ray and Selvakumar, 2000; Wang et al., 2012). There is always a risks of heavy metal leaching from S/S treated which can be happened under the changes in the environmental conditions. Therefore, using a permanent solution to remove the bulk of contaminant of heavy metals from soils so that the ecological risks can be drastically reduced in environment. To do so, soil washing is one of the few ex situ treatments using chemical extraction regents to remove metal contaminants from soils permanently (Tokunaga and Hakuta, 2002). This is an integrated processes through combing chemical and physical methods, including the chemical extraction, physical separation technologies (Dermont et al., 2008). The physical separation (PS) is to exploit the certain characteristic differences of soils and contaminants which influencing the metal contaminants to be removed, such as soil particles (clays, silts, sands), polluted metal-bearing particles, this general approach is relatively simple and fast operate application, the processes and equipment involved have been described in previous study (Bouchard, 2001; Wills and Finch, 2015). The applicability of physical particles can be predicted by the knowledge of mineralogical phase in remedial soils (Mercier et al., 2001; Sierra Fernández, 2013). Chemical extraction (CE) is related to extract the metal contaminants using chemical regents (chelating agents, acids/bases, surfactants, salts, or redox agent) transferring into aqueous solution. This CE technology is commonly used in extractive metallurgy for recovering, concentrating, and recycling the metals from ores or residual materials (Gupta, 2017). During the actual CE operation, the leaching solutions were using to enhance the solubility for dissolving the metal contaminants. Certainly, the removing of the metal contaminants by CE were depended on the soil geochemistry (e.g. buffering capacity, soil texture, organic matter content, cation exchange etc.), processing conditions (e.g. pH, equilibrium time, sequence of extraction steps, solid/liquid ratios, reagent addition etc.) and the dosage or chemical properties of extracting agent (Dermont et al., 2008).

On the status of soil washing technology which to be employed worldwide, many applicated examples of soil washing project were found in the European Netherland (Honders et al., 2003; Van Deuren et al., 1997), ARCADIS (Sogorka, 1998; Vik and Bardos, 2003), Germany (Vik and Bardos, 2003), Sweden (Griffiths, 1995), Norway (USEPA, 1997), and Belgium (Sogorka, 1998); for the Pb removal and remediation of a battery-manufacturing site in USA (Cunningham and Berti, 1993; Mann and Groenendijk, 1996; Nichols, 2016; Williford and Bricka, 2000), and ex situ removal of chromium, lead, form contaminated soil (Khan et al., 2004; Mann and Groenendijk, 1996; Pichtel and Pichtel, 1997); and some examples of soil washing systems performed in Canada (Bergeron, 2005; Mercier et al., 2007; Veetil et al., 2014). Generally, the physical separation is more popular employed processes than the chemical extraction because of the chemical extraction procedure for soil washing is more expensive ($ 358-1717m^{-3}) than physical separation ($ 70-187m^{-3}) (Van Deuren et al., 1997).

The soil metal speciation (chemical species) and fractions (fractionation forms bonding with specific soil substrates) in soil are very important parameters for assessing the removal efficiency of metal contaminants by chemical extraction treatment. However, to understand and quantify metal speciation or chemical forms in soils can be complicated and is an elusive goal, especially when the soil is contaminated by a complex mixture of organic and inorganic metal compounds, meanwhile dissolved a variety of organic matter, inorganic, and xenobiotic organic compounds in landfill-leachate-contaminated soils (Peter et al., 2002). It is most common that the soil metal speciation or fractions to be partitioned by sequential extraction procedures (Dahlin et al., 2002; Reis et al., 2016; Venditti et al., 2000). The soil metal fractions were generally involved five fractions in the analytical protocols (i.e. exchangeable fraction, acid soluble/carbonate bound fraction, reducible/Fe-Mn bound fraction, oxidizable/organic matter/ sulfide bound fraction, and residual fraction) (Finžgar and Leštan, 2007; Hintikka et al., 2001; Moutsatsou et al., 2006; Tessier et al., 1979; Wasay et al., 2001), also the metal partitioning up to 10 fractions have been reported (Benschoten et al., 1997). From the related reports, the monitoring of the metal fractions was amenable for metal removal from soil by chemical extraction (Peters, 1999), but the removal efficiency of metal from soil also depends on other parameters (e.g. metal contaminant concentration, soil geochemistry) (Ko et al., 2005).

Although numerous studies focused on soil washing for metal contaminants removal in field applications under assessing the removal efficiency of metal speciation or fraction by physical/chemical technologies. To our knowledge, these researches or mainly focused on other metal contamination removal, these heavy metals including (e.g., Pb, Fe, Cu, Zn, Ni, Cd etc.) have been successfully washed using soil washing technologies (Ehsan et al., 2006; Ko et al., 2005; Lee et al., 2016; Sun et al., 2001; Venditti et al., 2000; Wasay et al., 2001). Contrary, there are a small studies have been performed focusing on the remediation of THg contaminated soils using soil washing, these methods of the studies are mainly from the perspective of the soil's THg, the toxicity characteristic leaching procedure (TCLP) and leaching THg removed by the measures of remediation to assess the effectiveness of Hg removal (Wang et al., 2012).

It has been reported that many environmental hazardous pollutants can be released into the soil through leachate from landfill. The Hg specific compounds (speciation or fraction) in bonding with their interaction with the soil native substrates will determine the Hg mobility and bioavailability together under different native environmental conditions (Bloom et al., 2003). Particularly, the alterations of Hg physical or chemical compounds in complex environmental conditions of traditional MSW landfill was hard to predict and control (Lee et al., 2016). Soil-Hg fractions were performed by the sequential extractions to better understand the different characteristics of Hg geological solid phase (e.g., possible mobility, eco-toxicological) in soils (Bloom et al., 2003). For getting and assessing the changes of specific "make sense" Hg biogeochemical fraction will better understand the application effect of soil washing technologies for remediating to Hg contaminated soil.

Various sulfur compounds have been used for remediating Hg-contaminated soils (Ray and Selvakumar, 2000; Stepan et al., 1993; Wang et al., 2012). Sulfur has the high affinity capacity for bonding Hg, and the Hg sulfide compounds with low solubility will precipitates in water solution. However, the leachability of Hg sulfide compound precipitations will be increased with the time changes of incubation or hydration (Conner, 1990; Meng et al., 1998). Because the solubility of formed Hg sulfide compounds can be dissolved in the condition of excess amount of sulfide solution (e.g. saturated Na_2S) (Meng et al., 1998; Wang and Driscoll, 1995; Zhu et al., 2013). To our knowledge, there are have no studies on the changes of Hg fraction composition before and after soil washing treated by saturated Na_2S solution for Hg containing landfill-leachate-contaminated soils (LLCS). Therefore, it is essential to investigate the changes of different Hg fractions in LLCS by washing with saturated Na_2S solution treatment in order to accurately demonstrate the removal efficiency of the different Hg fraction by saturated Na_2S solution. Furthermore, the subsequent stable effect of treated LLCS under the controlled condition of the different pH values and equilibrium times in leaching test, and then judge the removal efficiency of exchange leaching Hg in Hg-containing LLCS from traditional landfill.

In this study, Hg containing LLCS were sampled at the leachate infiltration zone of an unlined large-scale traditional municipal solid waste (MSW) landfill used for more than 40 years. which is located at North-West of Nagasaki (32.876°N, 129.743°E), Kyushu inland, Japan. From the 1970s to the 1990s the landfill was dumped lots of the Hg-containing MSW (e.g., incinerated ash, industrial waste, and municipal sludge etc.), especially, including a large amount of high Hg materials (e.g., damaged fluorescent light, switch device, etc.) also be buried after big flood of Nagasaki in 1982. The THg concentrations of the LLCS around this MSW landfill were found ranged from 0.81 to 8.48 mg kg^{-1} in previous investigation, and this THg values were extremely higher than Hg natural background levels 0.06 mg kg^{-1} (Arisawa et al., 2000; He et al., 2018).

The study is to explore the efficiency of Hg extraction from LLCS by saturated Na_2S solution (ss-Na_2S), in order to minimize the risks of Hg leaching from LLCS to human health and to the environment both short-term and long-term equilibrium time in pH dependent of static-leaching test. In the present laboratory study, ss-Na_2S were utilized for the immobilization and removing of leaching Hg in Hg-containing LLCS. Under considering the changes of soil total mercury (THg) concentration, soil Hg-fractions, THg-static leaching test as a function of pH and equilibrium time, and the varied trends of leaching THg concentration were simulated by Visual MINTEQ modeling according to the mineralogical composition analysis. To test the trends of Hg concentration-time in Hg static-leaching test, as a function of different equilibrium time and pH values. For better understanding the Hg long-term leaching behavior and potential environmental risks on LLCS before and after treating by saturated Na_2S solution. Exploring and improving the capabilities of management and governance for controlling the environmental risks of leaching Hg from Hg-containing LLCS around the traditional landfill.

2. Materials and methods

2.1 Sample analysis

Soil properties including the soil pH value was measured in 0.01 mol/L $CaCl_2$ with

1:2.5 (soil: extractant solution ratio) using a pH meter (Cyberscan pH 1100, Eutech instruments), and the electrical conductivity (EC) was determined in Milli-Q water with 1:2 (soil: Milli-Q water ratio) using a conductivity meter (B-771 Compact conductivity Meter, HORIBA Ltd., Kyoto, Japan) (Pannu et al., 2014). The classification of soil particle size distribution (PSD) followed the USDA Texture Classes, and the particles were divided into three classifications by using a 0.053-mm sieve. The particles were characterized according to their size as: sands (2.0-0.05 mm); silts (0.05-0.002 mm); and clays (<0.002 mm), following the simplified method for soil sample PSD analysis (Kettler and Doran, 2001).

The total organic carbon (TOC) and total nitrogen (TN) in the soils were measured using an elemental analyzer (Flash Elemental analyzer EA1112Series). The TOC and TN were measured according to previously reported methods (Tsutsumi et al., 2015). Glycine expression was used as a standard to calculate the content of TOC and TN. Dissolved organic carbon (DOC) was measured using TOC-Vcsh/csn (SHIMADZU CORPORATION, KYOTO, JAPAN) The soil organic matter (SOM) content and the loss on ignition (LOI) was determined by igniting the dry soil for 2 h at 550 °C in a muffle furnace (ISUZU AT-S13) (Guédron et al., 2013; Haris et al., 2017; Heiri et al., 2001). The major chemical components of SiO_2, Al_2O_3, Fe_2O_3, CaO, and MnO in the powdered soil were analyzed in the lab of Asahi Environment Analysis Center in Kumamoto (Japan) for X-Ray Diffraction (XRD) (Fernández-Martínez et al., 2015).

2.2 LLCS collection and characterization

The landfill-leachate-contaminated soil (LLCS) used in this study was collected from the leachate infiltration zone of an unlined large-scale traditional municipal solid waste (MSW) landfill used for more than 40 years. which is located at North-West of Nagasaki (32.876°N, 129.743°E), Kyushu inland, Japan. From the 1970s to the 1990s the landfill was dumped lots of the Hg-containing MSW (e.g., incinerated ash, industrial waste, and municipal sludge etc.), especially, including a large amount of high Hg materials (e.g., damaged fluorescent light, switch device, etc.) also be buried after big flood of Nagasaki in 1982. The THg concentrations of the LLCS around this MSW landfill were found ranged from 0.81 to 8.48 mg kg^{-1} in previous investigation, and this THg values were extremely higher than Hg natural background levels 0.06 mg kg^{-1} (Arisawa et al., 2000; He et al., 2018).

Hg-containing LLCS was obtained by composite sampling of a 15 × 15 m^2 area (0-15 cm) in way of five points sampling at the Hg leachate polluted zone of traditional MSW landfill (He et al., 2018). Soil-Hg was fractionated into 5 categories: F1/water soluble Hg (Hg-w), F2/human stomach acid soluble Hg (Hg-h), F3/organic-chelated

(Hg-o), F4/elemental Hg (Hg-e), and F5/mercuric sulfide (Hg-s). Soil Hg-static leaching test as a function of pH (1-12) and equilibrium time (0-4weeks) before and after washing by the ss-Na_2S (Fig. 3-1).

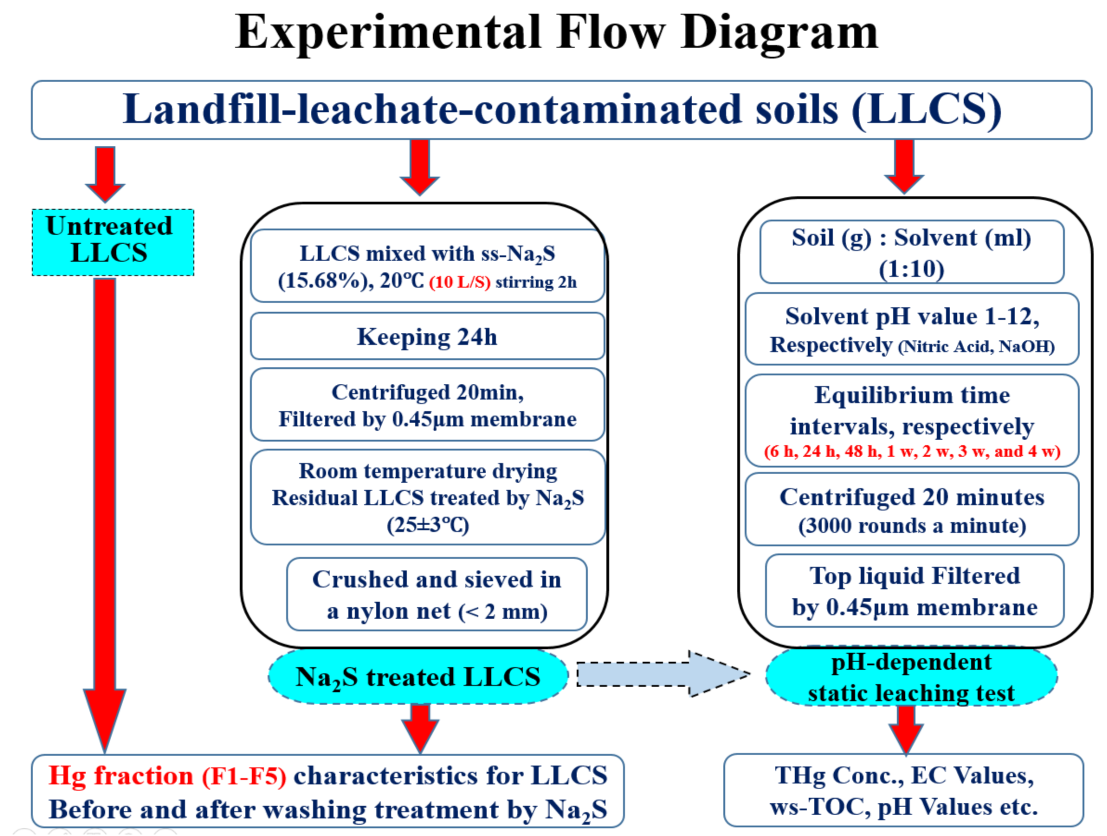


Fig. 3-1 Flow Diagram of the Landfill-leachate-contaminated soils (LLCS) before and after treated by saturated sodium sulfide (Na_2S) solution for soil Hg fractions including THg (total mercury), Hg-w (water soluble Hg), Hg-h (human stomach acid soluble Hg), Hg-o (organic-chelated Hg), Hg-e (elemental Hg), Hg-s (mercuric sulfide Hg) by a validated 5-steps sequential extraction for Hg-fractions, THg-static leaching test as a function of pH and equilibrium time.

Approximately 50 kg (wet weight) of surface LLCS (0-15 cm) of leachate-contaminated zone was obtained by composite sampling of a 15 × 15 m^2 area in way of five points sampling that was the initial sampling sites in previous study. The composite LLCS was air-dried darkly to constant dry weight (d.w.) in room temperature condition of laboratory (25±3℃, ~40 % humidity). Gently crushed by an agate mortar-pestle and sieved with a 2 mm diameter nylon. Prepared LLCS sample was manually stirred, thoroughly homogenized prior to the experiment.

In order to obtain the background information of LLCS before Na_2S washing, the general properties and geochemical components are analyzed, respectively. Including Soil pH and electrical conductivity (EC) values were measured in soil-distilled water suspensions with 1:2 v/v ratio by pH meter (Eutech instruments Cyberscan, pH 1100) and conductivity meter (Horiba B-771, Japan) (Xu et al., 2014). Soil main geochemical components were entrusted analysis using X-ray fluorescence spectrometer in accredited laboratory (XRF, Asahi Environment Analysis Center, Japan). Soil moisture was determined by weighting the sample and drying at 45℃ to a constant mass in a drying oven, the water content is calculated using the loss of mass and the mass of the dry specimen. The soil particle size distribution (PSD) was classified as sands (0.050 < % < 2 mm), Silts (0.002 < % < 0.050 mm) and Clays (% < 0.002 mm) in a 0.053 mm nylon sieve (T. A. Kettler, 2001). Soil organic matter (SOM) was analyzed by determining the content of loss on ignition (LOI) at 550℃ for 2h (ISUZU AT-S13) (Heiri et al., 2001). Soil total organic carbon (TOC) was determined in an elemental analyzer (Flash EA1112, Japan) (Tsutsumi et al., 2015). Water-soluble organic carbon (WSOC) of suspensions in soil leaching test was determined using a total organic carbon analyzer (TOC-Vcsn, Shimadzu, Japan) (Jara-Samaniego et al., 2017). All analyses were tested in triplicate at least.

2.3 Substrates preparation of ss-Na_2S treated LLCS

The substrates of ss-Na_2S liquid for was prepared using above homogenized the original dry Hg-containing LLCS, the LLCS were mixed with ss-Na_2S (15.68%) at a ratio of 10 L/S (liquid/ml versus solid/g) in a container, keep stirring at room temperature (25±3℃) with a magnetic stirrer for 24 h equilibrium incubation in the dark condition (Step 1). Then, static clarification for 2 hours, and the upper liquid was removed after 30 min of centrifugation (3000 rpm). The residual immobilized and washed LLCS by ss-Na_2S was air dried at room temperature for constant mass in the dark condition, and then fully crushed and sieved in a nylon net (< 2 mm), stored in poly ethylene terephthalate (PET) bottles in a desiccator with calcium chloride before the pH-dependent leaching test (Fig. 3-1).

2.4 Hg fractions characteristics of LLCS

The Hg chemical fractions were extracted measuring before and after Hg immobilization and wash removing of LLCS using saturated sodium sulfide (Na_2S) solution Na_2S immobilized LLCS by the reported a validated five-step sequential selective extraction procedures (Bloom et al., 2003). This method differentiates soil Hg fractions based on their biogeochemical characteristics and defined fractions as the following: F1 / water soluble Hg (Hg-w), F2 / human stomach acid soluble Hg (Hg-h), F3 / organic-chelated (Hg-o), F4 / elemental Hg (Hg-e), and F5 / mercuric sulfide (Hg-s). The extractants of method were summarized and listed in Table 3-1 and Fig. 3-1. The extraction procedures started with 2 g of dried soil samples in 150 ml PET bottle with Teflon-lined caps. Following each step of the extraction procedure, F1 - F4 steps were first added and mixed with 25ml of corresponding extractant except for F5 with aqua regia (1 versus 31.5) was conducted. The above mixed extraction was shaken in an orbital shaker at 220 rpm for 18h in F1 to F4 and 12h in F5 at room temperature, respectively. 20 min centrifugation was conducted after shaking in each step, then, the supernatant was carefully filtered by 0.45 µm filter into a new 150 ml PET bottle in the first three steps (F1 - F3), and the last two steps (F4 - F5) do not employ a filtration because the extraction is high acidity. Especially, the residues from each step (F1 - F4) were refilled with 25 ml same extractant, shaking, re-centrifuged and filtered as the first-time extracted operation. All rinse supernatant was combined and diluted to 100 ml with Milli-Q water in F1 - F4, and diluted to 65 ml in F5, respectively. The 0.2 M bromine monochloride (BrCl) solution 1.25 ml was added into F1, F2, and F4 extraction, and 10 ml 0.2 M BrCl solution was added into the F3 extraction because of high acid neutralizing capacity, which was used as an oxide to preserve THg analysis in extraction solution. All experiments were carried out in triplicate and with procedural blanks.

Table 3-1

Summary of extractants and Hg fractions used in sequential extraction procedure

Fraction of extraction step	[1]Soil Hg fractions	Extractant reagent	Solid-to-liquid (soil： Extractant)	Extraction method
[2]F1	Water soluble Hg (Hg-w)	Milli-Q water	1:100	18±2 h Shaking at room temperature
F2	Human stomach acid soluble Hg (Hg-h)	0.1 M CH3COOH + 0.01 M HCl (pH = 2)	1:100	18±2 h Shaking at room temperature
F3	Organo-chelated (Hg-o)	1 M KOH	1:100	18±2 h Shaking at room temperature
F4	Elemental Hg (Hg-e)	12 M HNO3	1:100	18±2 h Shaking at room temperature
F5	Mercuric sulfide (Hg-s)	Aqua regia	1:31.5	12h Shaking at room temperature

Note:

1. Soil Hg fractions: water soluble Hg (Hg-w), human stomach acid soluble Hg (Hg-h), organic-chelated Hg (Hg-o), elemental Hg (Hg-e), mercuric sulfide Hg (Hg-s).

2. F: Fraction.

2.5 pH-dependent leaching test procedures

Before and after the LLCS washing treated by saturated-Na_2S-solution, a developed pH-dependent leaching tests from Committee European Normalization (CEN 2006, TS14997 and TS14429) was performed to determine the Hg leaching of LLCS samples (CEN, 2006; Vítková et al., 2013; Xu et al., 2014). The pH values ranged from1 to 12 (1, 2, 3, 4, 5, 6, 6.4, 7, 8, 11, and 12) covered natural pH of the initial LLCS sample (4.2 – 5.3), and various experimental equilibrium time intervals (6 h, 24 h, 48 h, 1 w, 2 w, 3 w, and 4 w) were selected in each batch of the pH-dependent leaching tests experiments. A mass of 15 g dried pretreatment LLCS sample (< 2 mm) and the appropriate volume of demineralized Milli-Q water were placed in acid-cleaned 250 ml poly ethylene terephthalate (PET) bottle. Nine batch of the pH-static suspensions were adjusted in 0.1 M HNO_3 and 1 M NaOH (analytical grade) in the stage of corresponding pH equilibration (1, 2, 3, 4, 5, 6, 6.4, 7, 8, 11, and 12) at a ratio of 10 L/S (liquid versus solid) L/kg, Seven equilibration time intervals (6, 24, 48 h, 1, 2, 3, and 4 w) was scheduled and marked on the test bottles, respectively. All above batch pH-dependent THg leaching experiments were conducted via two parallel tests and with procedural blanks separately and simultaneously. These bottles were continuously rotated on an end-to end using a shaker at a constant (30 rpm) at room temperature for solution equilibrium time (22 ± 3℃) of laboratory condition. To avoid the pH buffering capacity of soil samples, the sample and the leaching solution were sufficient mixed by vigorous shaking for 2 min at the beginning of each selected pH-static leaching condition, and the pH was checked and adjusted when necessary. During equilibration period, each settled leaching solution pH value was kept via intermittent monitor and adjustment with 0.1 M HNO_3 and/or 1 M NaOH in manual titration. Then, samples were conducted 20 min centrifugation after finishing each corresponding equilibrium time of experiments, respectively. The extraction supernatants were obtained and immediately filtered through 0.45 µm polypropylene filters after centrifugation. The final filtered supernatant was kept at 4℃ condition and divided into subsamples for measuring the concentration of THg within 48h (Fig. 3-1).

2.6 Trial Modeling used for exploring Hg leaching behavior from LLCS

The reason to select the Visual MINTEQ as trial Hg leaching behavior modeling from LLCS is that Visual MINTEQ is a geochemical equilibrium speciation modeling software (Gustafsson, 2018). This software can be used to simulate the equilibrium mass distribution on dissolution/precipitation/complexation among their default parameters in laboratory or natural aqueous solutions. Input mass concentrations for predicting THg leaching behavior of the pH-dependent test are based on the initial concentrations using the XRF analysis results of LLCS as the reported studies, for the active geochemical Hg concentration of Visual MINTEQ modeling was assumed 50% of total initial Hg concentration in the solution (L/S = 10) of pH-dependent leaching experiments (Xu et al., 2014; Zhang et al., 2008).

Visual MINTQ was first released in 2000 as a Windows user interface to the U.S. EPA model MINTEQA2. The original version of this model (called MINTEQ) was developed in the early 1980's at Battelle Pacific Northwest Laboratory (Felmy et al., 1984) as a cooperative effort funded by the U.S. Department of Energy and the U.S. EPA. The original MINTEQ developed by (Felmy et al., 1984) was produced by combining the mathematical structure of MINEQL (Lennon et al., 1976) with the thermodynamic database of the WATEQ3 model developed by the U.S. Geological Survey (Allison and Brown, 1995). The MINTEQ model was then renamed to MINTEQA1 by the U.S. EPA Environmental Research Laboratory at Athens, Georgia (AERL) in 1985. The model was anticipated to diverge from the original as it was adapted to the special needs of the U.S. EPA, but in reality, there were very few differences between MINTEQA1 and the original MINTEQ. The distribution package, available for DOS-based PC’s or for Digital Equipment Corporation VAX machines, included a preprocessor program PRODEFA1 for the preparation of MINTEQA1 input files.

On one hand, considering the MINTEQA 2 and Visual MINTEQ are similarities and differences. Because Visual MINTEQ and MINTEQA2 have the same origins and largely the same procedures for calculations, there are many similarities between the two codes. For example, the same terminology is used in both codes (e.g., components, infinite and finite solids, etc.). Still, users of Visual MINTEQ may still find it useful to consult the manual for MINTEQA2 ver. 4.0, especially for background descriptions. However, since Visual MINTEQ started to diverge from MINTEQA2 in 2000, there are now a number of differences between the codes. In most cases this is because a number of new options and features have been added. Here are some examples of enhancements in Visual MINTEQ:

1. Visual MINTEQ can account for the effects of mixing and dilution when a titrant of a given composition is added to a solution.

2. There are six different surface complexation models to choose from; Visual MINTEQ includes the Basic Stern, the Three Plane and the Non-Electrostatic models.

3. It is now possible to use different surface complexation models on different surfaces.

4. The charge of the reference surface component is now allowed to be different from zero. For one-pK models, the charge is typically –0.5 by default – this value can be changed by the user.

5. The surface can have a cylindrical or a spherical geometry.

6. Fixed-charge sites can be defined for ion-exchange calculations to permanently charged surfaces

7. Complexation to natural organic matter can be simulated with state-of-the-art models (SHM, NICA-Donnan)

8. In surface complexation models, each surface can have a maximum of six coordination sites and include one fixed-charge site for ion-exchange reactions.

9. Visual MINTEQ can consider counter-ion accumulation in the diffuse layer of charged surfaces

10. Interfacing with Microsoft Excel to facilitate the handling of input and output data.

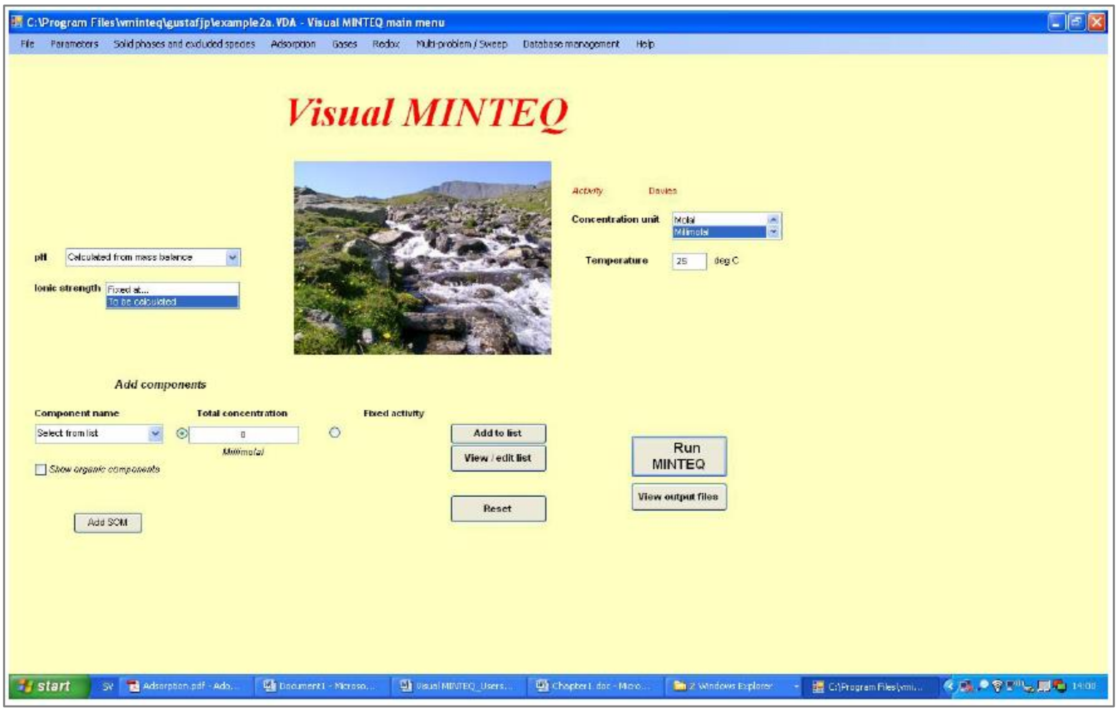
11. The thermodynamic databases are different, as Visual MINTEQ contains updated and expanded data from the NIST Critical Stability Constants database. However, the database of MINTEQA2 ver. 4.0 can be downloaded and used as well.

On the other hand, considering some technical specifications. Visual MINTEQ has been coded in Microsoft Visual Basic *.NET 2005. Microsoft *.NET Framework 1.1 or higher is required for Visual MINTEQ to run. The program will run on most Windows platforms (except ME). Other operating systems are not supported, but Visual 4 MINTEQ has been reported to run under a Windows emulator such as Wine. Microsoft Excel 2003 or higher is required for the interaction with Excel. There are a number of executable (.EXE) files associated with the software. Of these there are two that can be considered being the core of Visual MINTEQ:

- The Vminteq EXE file (vminteq13.exe in Visual MINTEQ 3.1) provides most of the interface for Visual MINTEQ. Input files are created, sent to the Mintrun EXE file (see below) and the results are read and presented on the output pages.
- The Mintrun EXE file (mintrun13.exe in Visual MINTEQ 3.1) carries out all the calculations. It appears as a black box during runtime.

These two EXE files communicate with one another using ASCII text files. More specifically, when the user presses the **Run MINTEQ** button in the interface, the text file “minin.vda” is created. This file contains all information necessary for the simulation. Mintrun reads in “minin.vda” and when it terminates the calculations, the output will be written to two other ASCII text files, “vmint.out” and “vmint.ou2”, where the former contains all detailed results for each problem, and the latter contains the information used to set up the Table **Selected sweep** results for multi-problem / sweep files. This program structure is admittedly rather old-fashioned. It is inherited from MINTEQA2 ver. 4.0, where the two cores .EXE files are “Prodefa2” and “Minteqa2”.

Therefore, if the user opens the file “minin.vda”, this represents the last simulation that was carried out by Visual MINTEQ. Also, when the user presses the **View output files** button from the main menu, Visual MINTEQ will always open the results from the last simulation, which are stored in “vmint.out” and “vmint.ou2” (Fig. 3-2).


C:\Program Files\vminteq\gustafjp\example2a.VDA - Visual MINTEQ main menu

Activity Davies

Concentration unit: Molal / Millimolal

Temperature: 25 deg C

pH: Calculated from mass balance

Ionic strength: Fixed at... / To be calculated

Component name: Select from list

Total concentration: 0 Millimolal

Fig. 3-2 Visual MINTEQ 3.1 user guide

2.7 Mercury determination and quality control (QC)

Reagents of analytical grade were used for the analyses. Analytical operations (e.g., beaker, vessels, and tubes) were conducted with ultra-clean handling to avoid any possible contamination: soaked in Merck Extran detergent 5% for 24 h and immobilized with tap water 3 times, and finally soaked in 10% HNO_3 for 24 h. All items were rinsed 3 times with Milli-Q water, and then air-dried in a clean environment to avoid cross-contamination. The anhydrous sodium sulfide (Na_2S, 100% purity) for washing solution (ss-Na_2S) was purchased from the Wako Pure Chemical Industries, Ltd. (Osaka, Japan). L-Cysteine (L-Cys) were purchased from Kanto Chemical Co. (Tokyo, Japan). All reagents used for analyzing soil and earthworm in this study were analytical grade. The $HgCl_2$ standard solution ($HgCl_2$ 1,000mg/L) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Glacial acetic acid, hydrochloric acid (HCl), 5M sodium hydroxide (NaOH), nitric acid (HNO_3), hydrogen bromide.

All analytical procedure and extraction materials (beaker, vessels, centrifuge tubes, etc.) were conducted ultra-clean handling to avoid cross-contamination and then soaking and washing with a detergent (Extran MA02, Merck, Ltd.) and Milli-Q water rinsing 3 times. And then following with soaking into 20% HNO_3 24h and Milli-Q water rinsing 3 times. Finally, all materials were air-dried in clean environment.

The accuracy and precision of analyses were validated by measuring the Hg content in duplicate of the subsamples, blanks, and verified recovery of certified reference materials (CRMs). All CRMs were purchased from the National Metrology Institute of Japan (NMIJ). The swordfish tissue (7403-a No.165) and codfish tissue (7402-a No.250) were used for the extraction analysis of T-Hg and MeHg. The marine sediments (7302-a No. A-0210) and estuarine sediments (7303-a No. E-0190) were used as the CRMs for the sequential extraction of soil T-Hg (Bloom et al., 2003; Zagury et al., 2006). Three replicates ($n = 3$) of the samples, and random intervals of CRMs and blanks were determined in each series of the sample analyses. Blanks were used to confirm the purity of the reagents and to correct the results of the analysis. Average values were used in statistical and modeling processing. In addition, the largest value of deviations was excluded in the calculation of mean values if the coefficient of variation (CV) of the replicates ($n = 5$) was more than 0.3. The analytical calibration curves were set up with different volumes of standard $HgCl_2$ solutions (0.1 mg L^{-1} and 1 mg L^{-1}), prepared using 1000 mg L^{-1} $HgCl_2$ standard solution. The range of calibration curve was from 0 to 200 ng (0, 2, 5, 10, 20, 50, 100, and 200 ng).

The expected recovery was within 95% confidence limits of the recommended standard T-Hg/MeHg values for the above certified materials ($n = 6$). The extraction recovery of T-Hg ranged between 90.2% and 105.4% (mean: 95.4%), and that of MeHg ranged between 93.9% and 113.8% (mean: 102.3%). Therefore, we used 102.3% as the correction factor for the calculation of MeHg concentration extracted from the earthworm samples (Nakano et al., 2010; Yoshimoto et al., 2016). The recovery of sequential extractions of Hg had a range of 96.7 – 103.3% in the sediment CRMs, and no statistically significant differences ($p > 0.05$) were found between the total Hg in the extracted fractions and the total Hg in the digest sediment CRMs of the sequential extraction. Hg concentrations in the reagents and blanks were always below the detection limit for the analysis. The accuracy and precision of the methods were larger than 95%.

The limit of detection (LOD) of instrument for T-Hg and MeHg were defined as 3 times the standard deviation (SD) signal-to-noise ratio and limit of quantitation (LOQ) as 10 times signal-to-noise ratio of the lowest detectable concentration of mixture of standards. The LOD was 0.2 ng/g d.w. (dry weight) and the LOQ was 0.6 ng/g d.w.. By reducing-vaporization atomic absorption measurements of MA3000, the LOD for T-Hg was 1 ng/L, and the LOQ was 3 ng/L in this study (Watanuki et al., 2015).

The THg concentrations in LLCS samples (d.w.) were directly determined by a Thermal Decomposition Hg Analyzer MA-3000 (Nippon Instruments Corporation, Tokyo, Japan). The clear filtrates of the leaching tests were acidized with H_2SO_4 (1:1), reduced by 10% $SnCl_2$ solutions, and analyzed by reducing-vaporization atomic absorption measurements of MA3000 (Nippon Instruments Corporation, Tokyo, Japan). All the analysis reagents used were an analytical grade or higher. All the containers of analytical procedures were soaked in 10% HNO_3 solution for 24h and rinsed 3 times in deionized water, and then dried before use. The reported Hg detection limit was 0.2 ng/g d.w. (d.w.), and 1 ng/L for Hg in the liquid phase (Watanuki et al., 2015). The QC of analytical procedures including applying reference material, duplicate of subsamples, and blank intervals. The precision and accuracy of the Hg analytical methods were confirmed against standard reference materials (CRM) be recommended by the National Metrology Institute of Japan (NMIJ) standard reference material (CRM 7302-a, THg value: 0.52 ± 0.03 mg kg-1) and (CRM 7303-a THg value: 0.067 ± 0.006 mg kg-1), which purchased from National Institute of Advanced Industrial Science and Technology (Tsukuba, Japan). The results of mean concentrations (n=6) were 0.522 ± 0.021 mg kg^{-1} of CRM 7302-a and 0.066 ± 0.002 mg kg-1 of CRM 7303-a, within in 95% confidence limits of the recommended Hg values for these CRM in a random experiment, respectively. The recovery ranged from 96.7% to 102.3% of the certified value (n=5) for checking the validated five-step soil Hg sequential extraction procedures. Mercury concentrations in all reagents and blanks were always below detection limit of each relevant analytical procedures.

2.8 Data statistical analysis

All data are presented as means ± standard deviation (SD). The Statistical Product and Service Solutions (SPSS) version 21.0 for Windows and Excel 2013 were used for data statistical treatment. The geochemical modeling of Visual MINTEQ 3.1 software was used to simulate the leaching THg behavior of LLCS in pH-dependent leaching test. At last, Figures are made use of the plotting function of the Graph-pad Prim version 5.01. Less than 0.05 was the significant difference levels in all statistical treatment of data. The reducing efficiency ratios (%) of leaching THg concentration of the treated LLCS by saturated Na_2S solution were calculated as the following formula: Reducing efficiency (%) = (1 - leaching THg concentrations of Na_2S treated LLCS divided the leaching THg of untreated LLCS at the corresponding pH value and equilibrium time during in pH-independent test) × 100%. The ratio of leaching THg (‰) from Hg containing untreated LLCS = the leaching THg concentration (ppb/µg L^{-1}) of untreated LLCS divided the THg concentration (ppm/mg kg^{-1}) * 1000 * ‰, or the ratio of leaching THg (‰) from Hg containing treated LLCS = the leaching THg concentration (ppb/µg L^{-1}) of washing treated LLCS by saturated Na_2S solution divided the THg concentration (ppm/mg kg^{-1}) of washing treated LLCS by saturated Na_2S solution * 1000 * ‰ in corresponding leaching condition of the static dependent leaching test as a function of pH and equilibrium time.

3. Results and discussion

3.1 Mineralogical characterization of the LLCS

The data in Table 3-2 showed the general soil characteristics of the study LLCS. Soil with the pH values range 4.2 ~ 5.3, indicating that soil is in high acidity in study zone. The soil measured SOM, moisture and TOC ranged from 11.9% to 13.1%, 59.6 to 63.0% and 4.5% to 5.5%, respectively. The average EC was 84.4 ± 2.9 mS m^{-1} and DOC contents of 54.8 ± 1.4 mg L^{-1} in initial LLCS. The PSD was found that clay 32.3 ± 0.6%, slit 16.7 ± 1.5%, sand 51.0 ± 2.0%. The average of THg concentrations was 1.827 ± 0.119 mg kg^{-1}. Most studies on soil THg have been conducted in polluted sites, and have found a wide range in concentration of THg, from a few milligrams to several thousand milligrams per kilogram of soil (Fernández-Martínez et al., 2015; Reis et al., 2009). According to a survey of environmental Hg levels conducted in 18 prefectures of Japan, the residual soil THg concentrations varied between 0.002 mg kg^{-1} and 78.6 mg kg^{-1} (Nakagawa, 2008). the general consensus of previous studies is that atmospheric Hg deposition is the major source of Hg in forest soils (Buch et al., 2017; Rieder et al., 2011). Besides, it is recommended that the probable effect concentration (PEC) of Hg should be 1.06 mg kg^{-1}; Hg pollution exceeding this limit is very harmful to organisms (MacDonald et al., 2000). The USA Environmental Protection Agency (USEPA 2013) recommended that inorganic Hg (IHg) limit [guidelines for Soil Screening Levels (SSL)] is 23 mg kg^{-1} for residential soils and 310 mg kg^{-1} for industrial soils (United States Environmental Protection Agency (USEPA), 2013). The SSL limits were also established for MeHg and elemental Hg in industrial soils (MeHg:100 mg kg^{-1}; elemental Hg: 43 mg kg^{-1}) and in residential soils (MeHg: 7.8 mg kg^{-1}; elemental Hg:

10 mg kg^{-1}) (Rimondi et al., 2012; United States Environmental Protection Agency (USEPA), 2013). However, it must be noticed that the PEC of Hg is established to access sediments and is not directly applicable to soils in vicinities of MSW's landfill. The soil T-Hg levels in the landfill of this study met the USEPA industrial SSL. The present soil T-Hg levels were very high in comparison to the concentrations of the natural background soils 0.087 mg kg^{-1} found in our previous study, recently (He et al., 2018).

The mineralogical compositions of the LLCS are given in Table 3-3. The main mineralogical compositions of the LLCS were constituted by SiO_2 (63.19%), Al_2O_3 (21.93%), Fe_2O_3 (6.03%) and K_2O (3.5%), and a certain amount of MgO, Na_2O, TiO_4, CaO, MnO etc. were also observed from XRF analysis results (Fig. 3-3). It has been reported that soil-Hg are associated with Fe and Mn oxides in soils (Liu et al., 2006). The composition of MnO was 0.35% in pretreated LLCS for this experimental study. Moreover, the was found that a significant correlation between the total mercury concentrations, aluminum (Al_2O_3) and iron (Fe_2O_3) in soils (Fadini and Jardim, 2001).

Table 3-2

Initial characteristics of the landfill-leachate-contaminated soil

Soil properties	Unit	Value (n=6)
pH	-	4.2 ~ 5.3
Soil organic matter (SOM)	%	12.5 ± 0.6
Electrical conductivity (EC)	mS m^{-1}	84.4 ± 2.9
Moisture (n=6)	%	61.3 ± 1.7
Soil total organic carbon (TOC) (n=6)	%	5 ± 0.5
Dissolved organic carbon (DOC)	mg L^{-1}	54.8 ± 1.4
Soil particle size (PSD)		
Clays	%	32.3 ± 0.6
Silts	%	16.7 ± 1.5
Sands	%	51 ± 2
THg concentration (n=3)	mg kg^{-1}	1.827 ± 0.119

Table 3-3

General mineralogical composition contents of landfill-leachate-contaminated soil (LLCS) using XRF analysis

Chemical component	Content (*d.w. %)	Chemical component	Content (d.w. %)
Na_2O	0.96	MnO	0.35
MgO	1.47	Fe_2O_3	6.03
Al_2O_3	21.93	Co_2O_3	< 0.02
SiO_2	63.19	NiO	< 0.02
P_2O_5	0.34	CuO	< 0.02
SO_3	0.42	ZnO	0.01
Cl	< 0.04	Rb_2O	< 0.01
K_2O	3.5	SrO	< 0.01
CaO	0.71	ZrO_2	< 0.05
TiO_2	0.92	BaO	< 0.1
Cr_2O_3	< 0.03	Ga_2O_3	< 0.02

Note: * d.w.: dry weight.

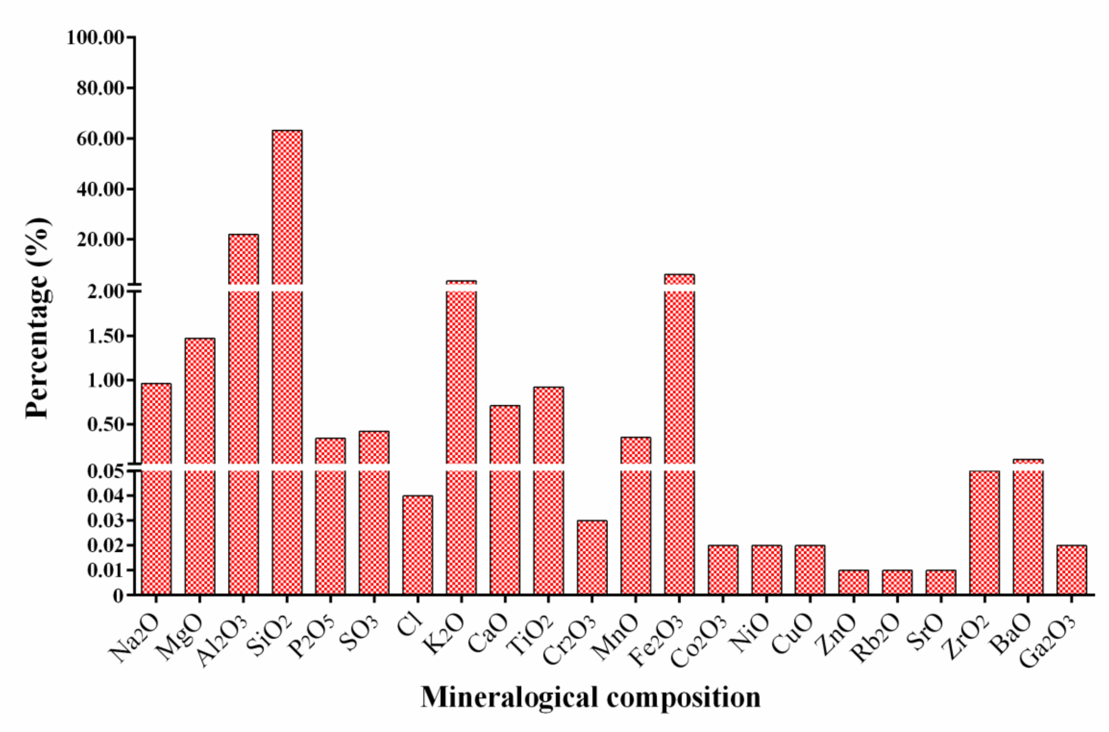


Fig. 3-3 The mineralogical composition analysis of the landfill-leachate-contaminated soils (LLCS) using XRF analysis.

3.2 Hg fractions in LLCS

The respective average concentrations of soil-Hg fractions of F1-F5 are shown in Table 3-4, 3-5. F1/Hg-w ranged between 0.0008 and 0.0061 mg kg^{-1} (median: 0.0052 mg kg^{-1} dw), F2/Hg-h ranged between 0.0093 and 0.0985 mg kg^{-1} (median: 0.0904 mg kg^{-1} dw), F3/Hg-o ranged between 0.1530 and 1.2203 mg kg^{-1} (median: 1.0680 mg kg^{-1} dw), F4/Hg-e ranged between 0.0762 and 0.5746 mg kg^{-1} (median: 0.4936 mg kg^{-1} dw), and F5/Hg-s ranged between 0.0066 and 0.0681 mg kg^{-1} (median: 0.0639 mg kg^{-1} dw), and the main concentrations of Hg fractions are the F3/Hg-o, F4/Hg-e and F2/Hg-h observed in control LLCS (CS), respectively. The percentages of the 5 Hg fractions were in the order of F3/Hg-o (58.68%–67.92%, mean: 62.05%) > F4/Hg-e (23.56%–31.78%, mean: 28.68%) > F2/Hg-h (4.47%–6.32%, mean: 5.25%) > F5/Hg-s (3.61%–3.79%, mean: 3.71%) > F1/Hg-w (0.26%–0.39%, mean: 0.30%) in the control LLCS (Figs. 3-4a and 3-4b, Fig. 3-5). The F3/Hg-o and F4/Hg-e were the dominant components in control LLCS, occupying 62.05% and 28.68% of the total Hg fractions in control LLCS, respectively (Fig. 3-4, and Fig. 3-5). In washing removal treatment of

LLCS by ss-Na_2S, the respective average concentrations of soil-Hg fractions of F1-F5 are shown in Table 3-4, 3-5. F1/Hg-w ranged between 0.0001 and 0.0002 mg kg^{-1} (median: 0.0002mg kg^{-1} dw), F2/Hg-h ranged between 0.0031 and 0.0058 mg kg^{-1} (median: 0.0040 mg kg^{-1} dw), F3/Hg-o ranged between 0.7260 and 0.9130 mg kg^{-1} (median: 0.8133 mg kg^{-1} dw), F4/Hg-e ranged between 0.3956 and 0.5327 mg kg^{-1} (median: 0.4644 mg kg^{-1} dw), and F5/Hg-s ranged between 0.0002 and 0.0011 mg kg^{-1} (median: 0.0005 mg kg^{-1} dw), also the main concentrations of Hg fractions are the F3/Hg-o, F4/Hg-e and F2/Hg-h observed in washing treated LLCS (TS), respectively. The composition of Hg fractions already has been changed under washing treatment of ss-Na_2S in laboratory study. The percentages of the 5 Hg fractions were in the order of F3/Hg-o (60.64%–66.71%, mean: 63.43%) > F4/Hg-e (32.94%–38.84%, mean: 36.22%) > F2/Hg-h (0.22%–0.48%, mean: 0.31%) > F5/Hg-s (0.01%–0.09%, mean: 0.04%) > F1/Hg-w (0.01%–0.02%, mean: 0.01%) in the washing treated LLCS by ss-Na_2S (Figs. 3-4a and 3-4b, Fig. 3-5). The F3/Hg-o and F4/Hg-e were still the dominant components in treated LLCS by ss-Na_2S, occupying 63.43% and 36.22% of the total Hg fractions in treated LLCS, respectively (Fig. 3-4, and Fig. 3-5).

The results of Hg chemical fractions (F1-F5) extracted from the untreated and Na_2S treated LLCS of the Hg contaminated traditional landfill area are shown in Table 3-4. The total amount of Hg fractions in five-steps sequential extraction has a good consistency with the LLCS THg, and the recovery ratios were accepted for analyzing, which ranged from 92.13 to 95.80% (mean: 94.15%), 84.96 to 94.63% (mean: 89.90%) in untreated and Na_2S treated LLCS, respectively. Although the averaged THg concentration of LLCS reduced by 22.02% only (from 1.8266 μg L^{-1} down to 1.4244 mg kg^{-1}). But even so, the concentration composition of some Hg fractions in LLCS have changed before and after treatment significantly, including the average concentration of F1/Hg-w was reduced by 96.15% (from 0.0052 mg kg^{-1} down to 0.0002 mg kg^{-1}) , F2/Hg-h was reduced by 95.58% (from 0.0904 mg kg^{-1} down to 0.0040 mg kg^{-1}), F3/Hg-o was reduced by 23.85% (from 1.0680 mg kg^{-1} down to 0.8133 mg kg^{-1}), F4/Hg-e was reduced by 5.92% (from 0.4936 mg kg^{-1} down to 0.4644 mg kg^{-1}), F5/Hg-s was reduced by 99.22% (from 1.7211 mg kg^{-1} down to 1.2824 mg kg^{-1}), respectively (Fig. 3-4a, 4b and Fig. 3-5). Our study shows that the saturated Na_2S solution as a Hg immobilization application is an effective method to minimize the amount of leaching THg from Hg-containing LLCS. The main minimizing mechanism of the amount of leaching THg is to reduce the concentration of exchangeable and stable non-mobile Hg (i.e. Hg-s) of LLCS, that more than 95% of exchangeable Hg (Hg-w, Hg-h) and Hg-s to be immobilized and removed by the saturated Na_2S solution in LLCS proved via a validated Hg five-steps sequential extraction procedure.

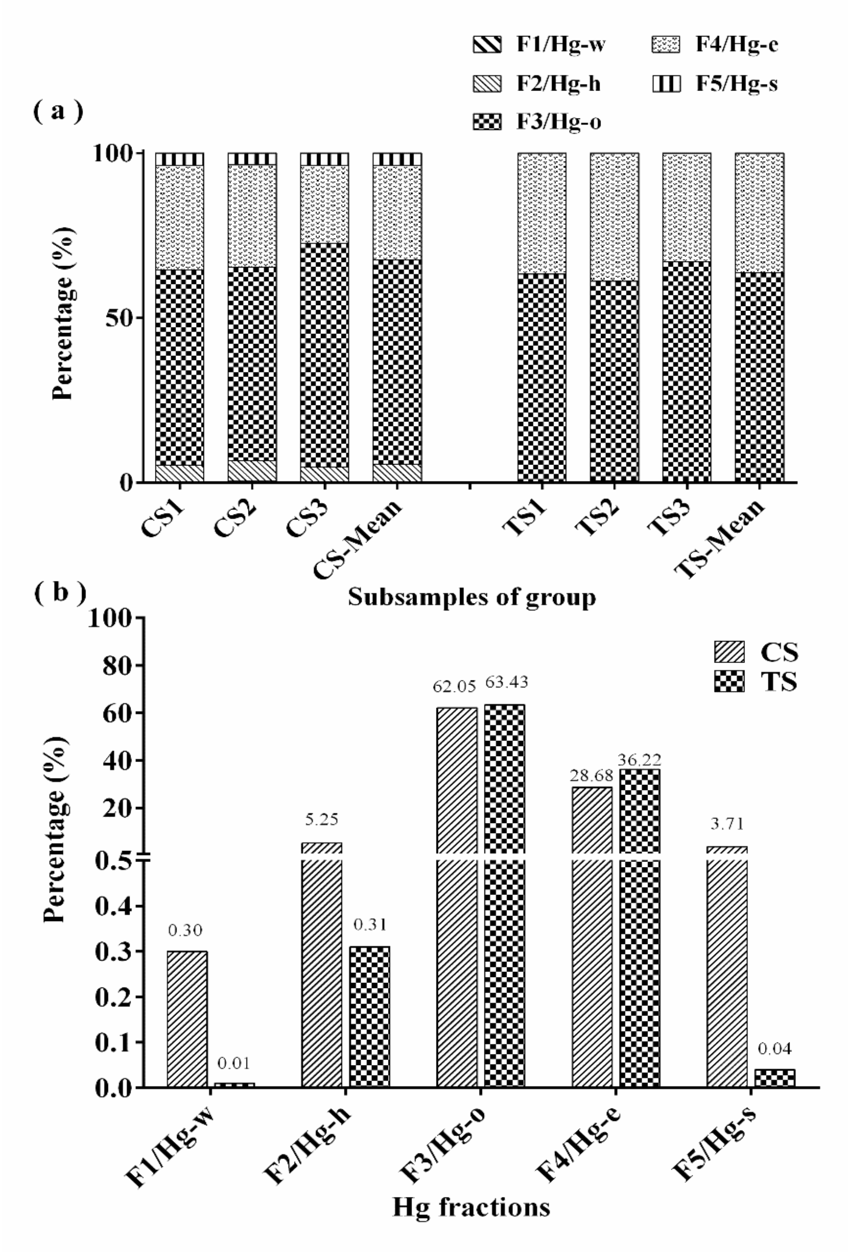


Fig. 3-**4 (a, b)** Hg fraction composition of landfill-leachate-contaminated soil (LLCS) in 5-steps sequence extraction procedures before and after Na_2S treating. (a) Percentages of Hg fractions in each subsample. (b) Mean percentages of the Hg fractions in each subsample of groups. (Soil Hg fractions: water soluble Hg (Hg-w), human stomach acid soluble Hg (Hg-h), organic-chelated Hg (Hg-o), elemental Hg (Hg-e), mercuric sulfide Hg (Hg-s)).

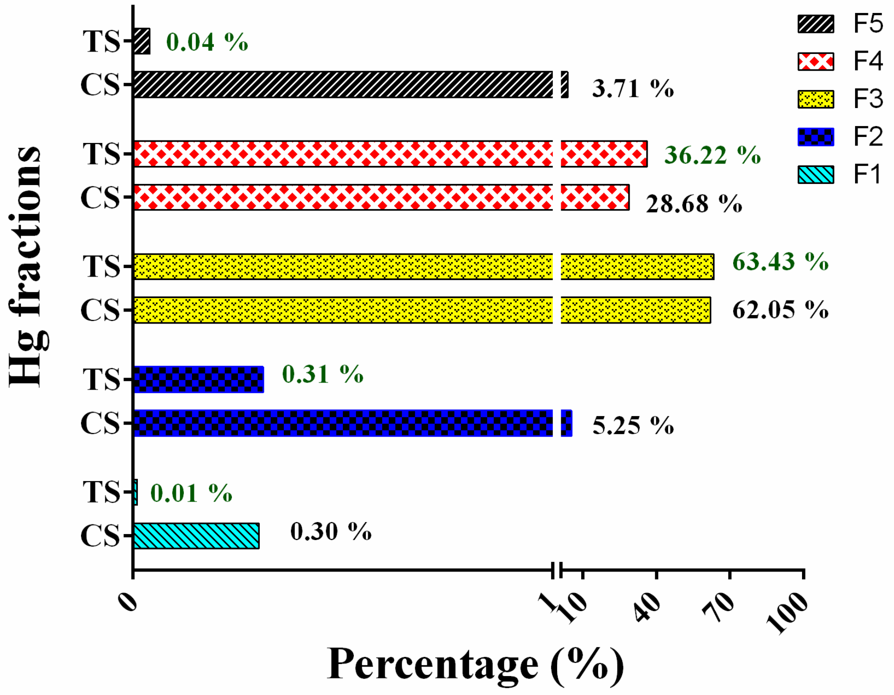


Fig. 3-5 Mean percentages of Hg fraction compositions of landfill-leachate-contaminated forest soil (LLCS) in 5-steps sequence extraction procedures before and after Na_2S treating. (CS: Control LLCS sample; TS: Na_2S treated LLCS; Soil Hg fractions: water soluble Hg (Hg-w), human stomach acid soluble Hg (Hg-h), organic-chelated Hg (Hg-o), elemental Hg (Hg-e), mercuric sulfide Hg (Hg-s)).

The analysis results of sequential extraction indicated that the F3/Hg-o and F4/Hg-e keeps as the dominant components, and more than 90% of the total five Hg fractions. Contrary, the total of F1/Hg-w, F2/Hg-h and F5/Hg-s is not enough 10% Hg of THg in LLCS. The content of Hg fractions has the order of the F3/Hg-o > F4/Hg-e > F5/Hg-s > F2/Hg-w > F1/Hg-h before and after Na_2S treated LLCS (Figs. 3-4a and 3-4b). For the THg of LLCS, amounts of 22.02% decreasing rate (from 1.8266 ± 0.1189 mg kg^{-1} to 1.4244 ± 0.1019 mg kg^{-1}) was observed in Na_2S treated LLCS only. However, to further studies found that a part of the Hg fractions composition has already significantly decline among the five extraction fractions, including the fractions of exchangeable Hg (Hg-w, Hg-g) and stable Hg (Hg-s), the concentrations of the F1/Hg-h, F2/Hg-w and F5/Hg-s were removed over 95% after treating LLCS by Na_2S, the corresponding of theirs mean percentage compositions decreased from 0.30 to 0.01

(96.15%), 5.25 to 0.31 (95.58%) and 3.71 to 0.04 (99.22%), respectively (Figs. 3-4b, Table 3-4 and Table 3-5). While no significant variation was observed for other fractions (Hg-o and Hg-e), because of the average THg concentration of the LLCS reduced from 1.8266 mg kg^{-1} to 1.4244 mg kg^{-1}, therefore, the amounts of Hg-o and Hg-e were increased changing from 62.05 to 63.43% and 28.68 to 36.22%, respectively (Fig. 3-4a and 3-4b).

This technique mechanism of Hg washing removal is based on the following well-known chemical reactions. The Hg-fraction compositions of the LLCS has changed under the condition of the washing treatment by the saturated Na_2S solution. This is most likely reason related with the characteristics of the Hg-w and Hg-h, which representing the most important fractions of high mobility and potential bioavailability in view of environmental concerns. Hg-w is weakly absorbed by soils, and Hg-h is a susceptible reactive Hg fraction to pH change of environmental conditions, those forms associated with different soil phases are including more easily transported Hg fraction (e.g., $HgCl_2$) (Bloom et al., 2003). $HgCl_2$ (Hg^{2+}) can easily reacted with Na_2S (S^{2-}), and Mercury (II) sulfide (HgS) was then formed, but another reaction is going to happen when the excessed ss-Na_2S in whole process. Since the chemical reaction will happen to change the precipitate of Hg_2S just formed as follows (Fig. 3-6):

e.g., $HgCl_2$:

① Na_2S (aq) + $HgCl_2$ (aq) ➡ ② HgS (s) + 2 NaCl (aq) ➡ ③

③ HgS (s) + ↑ss-Na_2S (aq) ➡ ④ $Na_2[HgS2]$ ▪ $5H_2O$ (aq) + ↓ss-Na_2S (aq) ➡ ⑤ HgS (s)
(Note: aq=aqueous phase, s=solid phase)

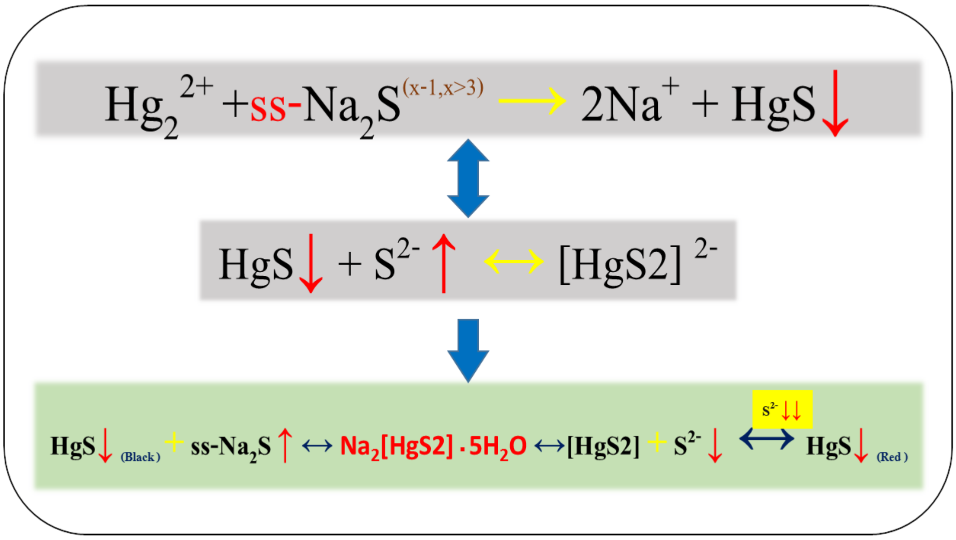


Fig. 3-6 Speculation of the chemical complexing reactions for the landfill-leachate-contaminated soils (LLCS) treated with excessing saturated Na_2S solution in this laboratory study from our results (ss-Na_2S = Saturated Na_2S solution).

The reaction balances to a 1:1 ratio of reactant molar, so if more moles of ss-Na_2S are present in the mixture than $HgCl_2$. Therefore, all the Hg (II) included in the LLCS will be first immobilized into Hg_2S, and to be washing removed from LLCS together with the residual Hg_2S in the LLCS itself. The reason speculated as follows.

In case of amount of saturated Na_2S solution were excessed for neutralizing the anion of Hg (II) in washing solution, Formed HgS can react with sodium sulfide to produce sodium dithio-mercurate (II). Excessed saturated Na_2S solution will improve the solubility of cinnabar (HgS). Because of Hg-w and Hg-h were the first easily available fractions, were more can be bio-accumulated than other fractions and very important in environment concerns even in low contents in view of high mobility and potential bioavailability. Hg-w fraction can be weakly adsorbed on soil surface, with most mobile and available characteristics in interstitial soil. It's usually called as “labile Hg species”, including more readily bio-accumulated Hg species than other Hg species, and able to diffuse into deeper soil and groundwater (e.g. Hg chloride ($HgCl_2$) is more easily to be transported and methylated than other inorganic Hg species (Fernández-Martínez and Rucandio, 2013; Han et al., 2003). Hg-s including sulfide Hg and other stable forms associated with silicates (Bloom et al., 2003).

These observations form our results suggest and supports that exchangeable Hg (Hg-w and Hg-h) and stable Hg (Hg-s) were the main soil Hg fractions of LLCS to be immobilized firstly and then to be dissolved and washed separation from LLCS by excessed Na_2S solution treatment in laboratory experiment of pretreatment. Therefore, this speculation of the chemical reactions was proved that the related fraction was reduced by the excessed saturated Na_2S solution pretreatment through the chemical complexing reactions for Hg-w, Hg-h, and Hg-s in the mixing suspension of landfill-leachate-contaminated soils (LLCS) and saturated Na_2S solution pretreatment under the certain liquid-solid ratio of liquid (ml) versus solid (g) (10 L /S) (Fig. 3-4, Fig. 3-5, Fig. 3-6, Table 3-4 and Table 3-5). It has been reported that many environmental hazardous pollutants can be released into the soil through leachate from landfill. The Hg specific compounds (speciation or fraction) in bonding with their interaction with the soil native substrates will determine the Hg mobility and bioavailability together under different native environmental conditions (Bloom et al., 2003). Particularly, the alterations of Hg physical or chemical compounds in complex environmental conditions of traditional MSW landfill was hard to predict and control (Lee et al., 2016). Soil-Hg fractions were performed by the sequential extractions to better understand the different characteristics of Hg geological solid phase (e.g., possible mobility, eco-toxicological) in soils (Bloom et al., 2003). For getting and assessing the changes of specific “make sense” Hg biogeochemical fraction will better understand the application effect of soil washing technologies for remediating to Hg contaminated soil.

In the previous reports, various sulfur compounds have been used for remediating Hg-contaminated soils (Stepan et al., 1993; Wang et al., 2012). Sulfur has the high affinity capacity for bonding Hg, and the Hg sulfide compounds with low solubility will precipitates in water solution. However, the leachability of Hg sulfide compound precipitations will be increased with the time changes of incubation or hydration (Conner, 1990; Meng et al., 1998). Because the solubility of formed Hg sulfide compounds can be dissolved in the condition of excess amount of sulfide solution (e.g. saturated Na_2S) (Meng et al., 1998; Wang and Driscoll, 1995; Zhu et al., 2013).

Table 3-4 Comparison of soil Hg fractions (mg kg^{-1}) in the landfill-leachate-contaminated soil (LLCS) between the untreated and washing treated by Na_2S solution (0.1 M, S/L = 1 : 10) using sequential selective extraction procedures

[2]Soil THg / fractions	[1]LLCS subsamples of group										[3]Removal efficiency of Hg fractions (%)
	Control LLCS (CS)					Na_2S treated LLCS (TS)					
	CS-1	CS-2	CS-3	SD	Average	TS-1	TS-2	TS-3	SD	Average	
THg	1.9132	1.6910	1.8755	0.1189	1.8266	1.5314	1.3285	1.4134	0.1019	1.4244	22.02
F1 / Hg-w	0.0047	0.0061	0.0048	0.0008	0.0052	0.0001	0.0002	-	0.0001	0.0002	96.15
F2 / Hg-h	0.0923	0.0985	0.0803	0.0093	0.0904	0.0032	0.0058	0.0031	0.0015	0.0040	95.58
F3 / Hg-o	1.0693	0.9143	1.2203	0.1530	1.0680	0.9130	0.7260	0.8010	0.0941	0.8133	23.85
F4 / Hg-e	0.5746	0.4828	0.4233	0.0762	0.4936	0.5327	0.4650	0.3956	0.0686	0.4644	5.92
F5 / Hg-s	0.0672	0.0563	0.0681	0.0066	0.0639	0.0002	0.0003	0.0011	0.0005	0.0005	99.22
[4]Sum Fractions	1.8081	1.5580	1.7968	0.1412	1.7211	1.4492	1.1973	1.2008	0.1444	1.2824	25.49
[5]Recovery%	94.51	92.13	95.80	1.8618	94.15	94.63	90.12	84.96	4.84	89.90	

Note:

1. LLCS: Landfill-Leachate-contaminated soil
2. Soil THg / fractions: total mercury (THg), water soluble Hg (Hg-w), human stomach acid soluble Hg (Hg-h), organic-chelated Hg (Hg-o), elemental Hg (Hg-e), mercuric sulfide Hg (Hg-s)
3. Removing efficiency of Hg fractions (%) = Control average / Na_2S washing average
4. Sum fractions = Hg-w + Hg-h + Hg-o + Hg-e + Hg-s
5. Recovery % = Sum Fractions / THg of LLCS

Table 3-5 Percentage comparison of Hg fractions (mg kg^{-1}) in the landfill-leachate-contaminated soil (LLCS) between the untreated and washing treated by Na_2S solution (0.1 M, S/L = 1:10) using sequential selective extraction procedures

Groups	Subsamples	[1]Hg fractions (%)					
		F1 / Hg-w	F2 / Hg-h	F3 / Hg-o	F4 / Hg-e	F5 / Hg-s	Sum
Control [2]LLCS (CS)	CS-1	0.26	5.1	59.14	31.78	3.72	100
	CS-2	0.39	6.32	58.68	30.99	3.61	100
	CS-3	0.27	4.47	67.92	23.56	3.79	100
	Mean	0.3	5.25	62.05	28.68	3.71	100
Na2S treated LLCS (TS)	TS-1	0.01	0.22	63.00	36.76	0.01	100
	TS-2	0.02	0.48	60.64	38.84	0.03	100
	TS-3	[3]ND	0.26	66.71	32.94	0.09	100
	Mean	0.01	0.31	63.43	36.22	0.04	100

1. Hg fractions Including water soluble Hg (F1/Hg-w), human stomach acid soluble Hg (F2/Hg-h), organo-chelated Hg (F3/ Hg-o), elemental Hg (F4/Hg-e), sulfide Hg (F5/Hg-s).

2. LLCS: Landfill-Leachate-contaminated soil.

3. ND: Below the limit of detection. Not determined in LOD (limit of detection) of MA 3000 mercury analysis instrument.

3.3 Batch pH-static dependent THg leaching behavior of LLCS

3.3.1 Characteristics of the untreated LLCS in the batch THg leaching test

From our results in batch pH-static dependent THg leaching test as a function of differential pH value and equilibrium time. The average leaching THg (Table S3-2, three replicates, n=3) from untreated LLCS was a wide ranged from 0.101 to 1.019 µg L^{-1} (mean: 0.275 µg L^{-1}). Most of the average leaching THg concentrations from untreated LLCS are below the Japanese THg leaching limited standard (less than 0.5 µg L^{-1}) of soil. In general, the high hydroxyl ion (OH-) condition of pH-static leaching solutions will increase THg releasing for Hg-containing LLCS during long-term equilibrium time of leaching in this experiment. From the results of the static leaching test as a function of pH and time, only 3 leaching condition of pH11 at 1w equilibrium time, pH12 at 1w and 2w equilibrium time are exceeded the Japanese THg leaching limited standard of soil, the corresponding average leaching THg concentration are 0.538 µg L^{-1}, 1.019 µg L^{-1}, 0.566 µg L^{-1} during the period of the 4w limited equilibrium time, respectively. the max (pH12) THg leaching value appears in 1 weeks of equilibrium time in leaching test. We also found that the leaching THg from the Hg-containing LLCS under the natural pH 4.2-5.3 were not exceeding the Japanese THg leaching limited standard (less than 0.5 µg L^{-1}) of soil. This THg concentration standard is also the Japanese groundwater standard. The leaching THg concentrations in untreated LLCS are presented in Fig. 3-7 and Fig. 3-8. The results suggest that the leaching THg from untreated LLCS is safety relatively under natural pH values referencing the Japanese THg leaching limited standard (less than 0.5 µg L^{-1}) of soil, and the ratio of leaching THg from LLCS is lower under the relatively wide range of the pH value (1-12) and long-term equilibrium time (6h-4w) (Fig. 3-7, Fig. 3-8 and Table S3-4.1).

In order to explore the relationships of variation trend between average total mercury (THg), total organic carbon (TOC) and electrical conductivity (EC) in the static dependent leaching test as a function of pH (1-12) and time (6h-4w). The average value of THg, TOC, and EC was detected in elution solution of the leaching test, respectively, their concentrations of THg, EC, and TOC from untreated LLCS in static dependent leaching test are presented in Fig. 3-9 (a) 6h, 3-9 (b) 24h, 3-9 (c) 48h, 3-9 (d) 1W, 3-9 (e) 2W, 3-9 (f) 3W, 3-9 (g) 4W for leaching solution with different leaching condition of pH values and equilibrium time.

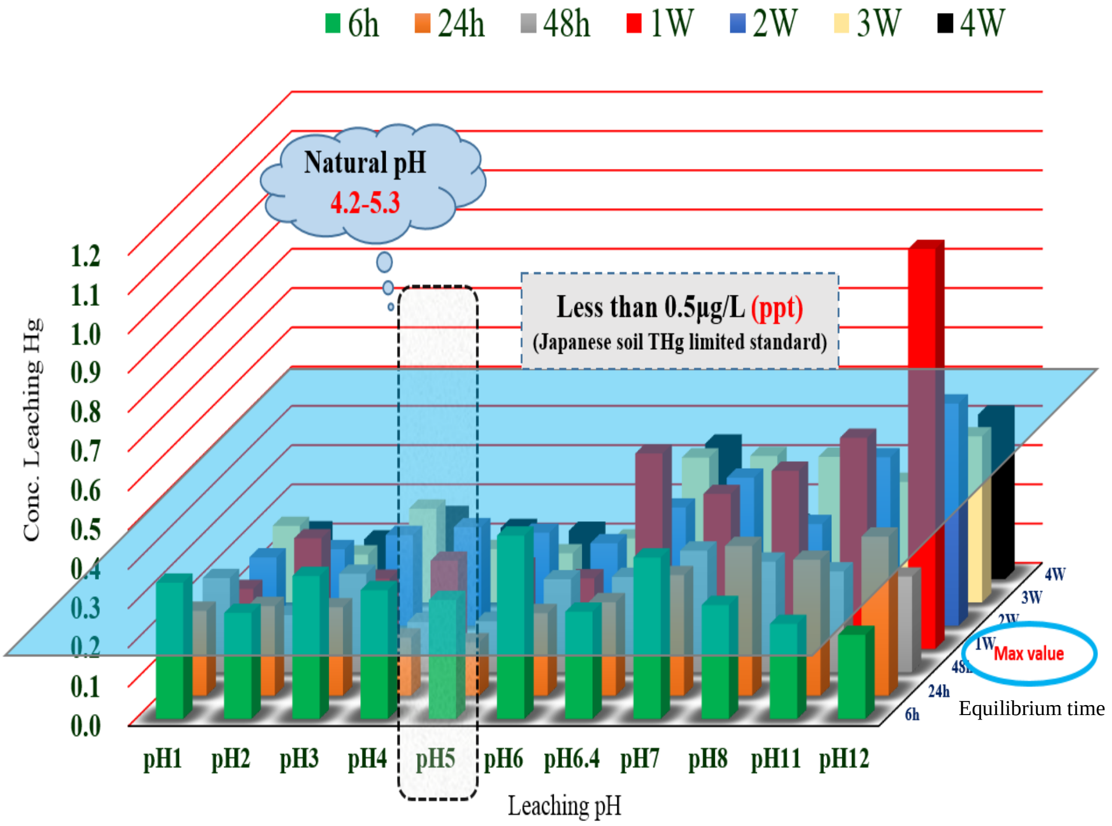


Fig. 3-7 Characteristics of total mercury (THg) in the untreated landfill-leachate-contaminated soil (LLCS) at the batch static dependent leaching test as a function of pH and time. (Natural pH 4.2-5.3 was indicated in a vertical oblong circular dashed line, and a horizonal virtual plane is the Japanese soil limit of the leaching THg concentration from soil).

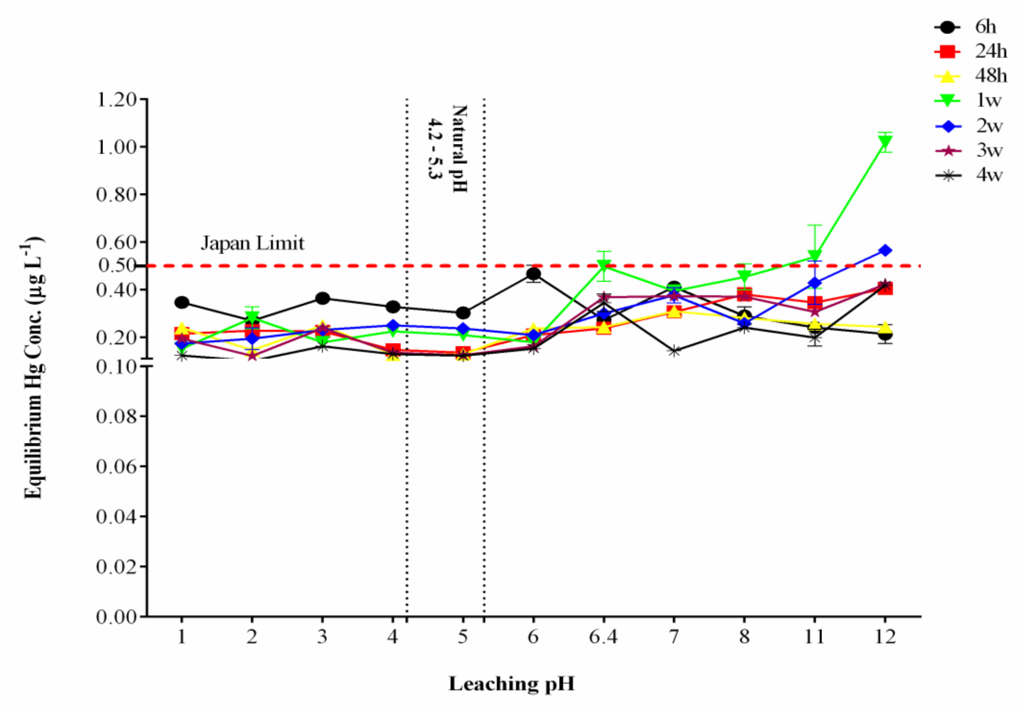


Fig. 3-8 The variation trends of leaching THg form untreated LLCS in pH-dependent static leaching test. (Natural pH 4.2-5.3 was indicated in two vertical dashed line, and a horizonal dashed line is the Japanese soil limit of the leaching THg concentration from soil).

A poor correlation relationship was found between THg, TOC, and EC from this pH static dependent leaching test for untreated LLCS, only the leaching THg concentration has happened significantly concentration changes during the period of the equilibrium time under the wide range of pH values (1-12). The leachability of THg was increased significantly when the high pH values of leaching condition. The possibility reason maybe is that the stable Hg (HgS) can be dissolved through the chemical formation soluble mercuric polysulfide [HgS_2^{2-}] complexes and / or hydrosulfide [$Hg(HS)_2$] (Baker, 1994; Nriagu, 1979; Wang and Driscoll, 1995).

when the pH increased to above the neutral conditions and elevated concentrations of OH^- in batch static dependent leaching test as a function of pH values and equilibrium time.

When compared with other studies, this poor correction is similar with study focusing water in the river both total and reactive Hg and TOC concentrations (Fadini and Jardim, 2001). However, it is important to bear in mind that this results differs from the ones reported for the lake water, the values of TOC are very close THg and active Hg of water (Driscoll et al., 1995). Otherwise, no significant changes of the concentration of EC and TOC were found during the period of the whole equilibrium time. Previous studies have reported that there was a good positive correlation between the concentration of metal ions and the EC in groundwater (Liu et al., 2003). High inorganic Hg contents coexist with high EC in soils (Rodríguez Álvarez et al., 2014). The results suggest that the leaching THg from untreated LLCS is not relate with the leaching concentration of EC and TOC. The change of the leaching THg concentration of LLCS may be mainly influenced by the pH value. The leaching EC and TOC concentrations from untreated LLCS under different pH condition may not to be influenced by change of equilibrium time for 4 weeks.

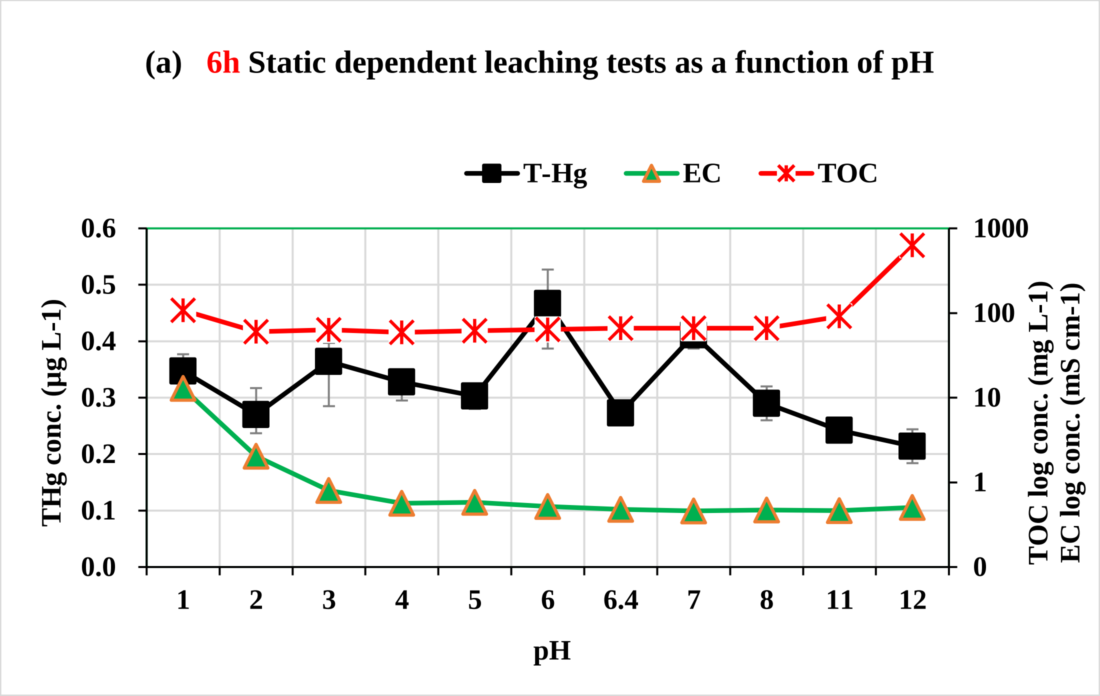


***Fig. 3-9 (a)**

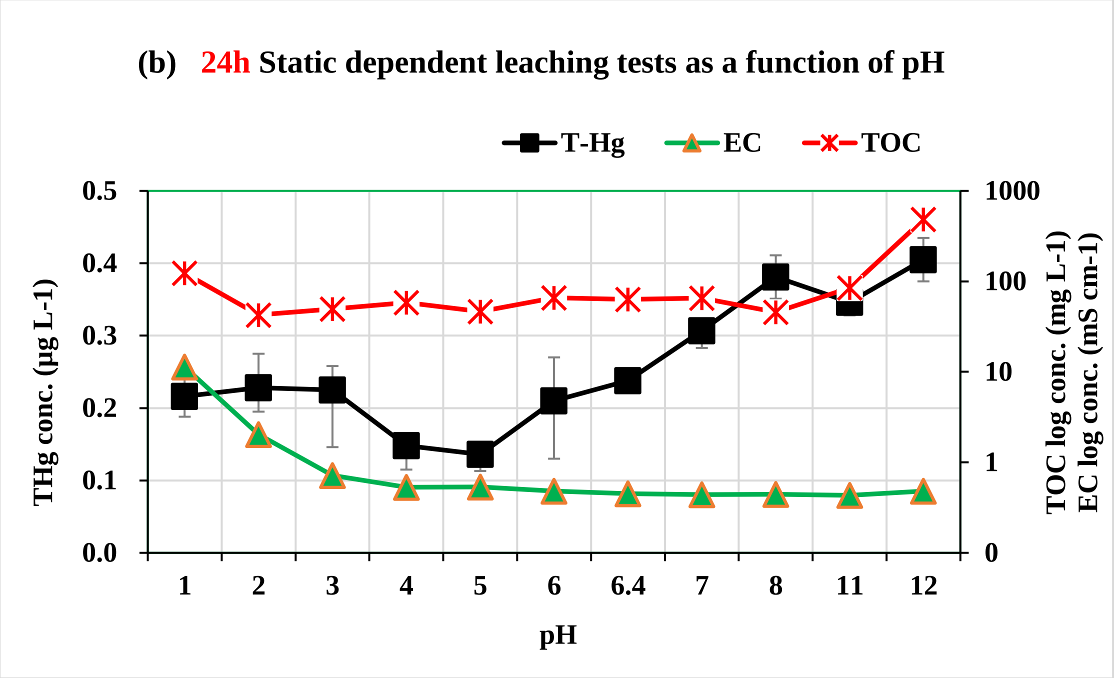


***Fig. 3-9 (b)**

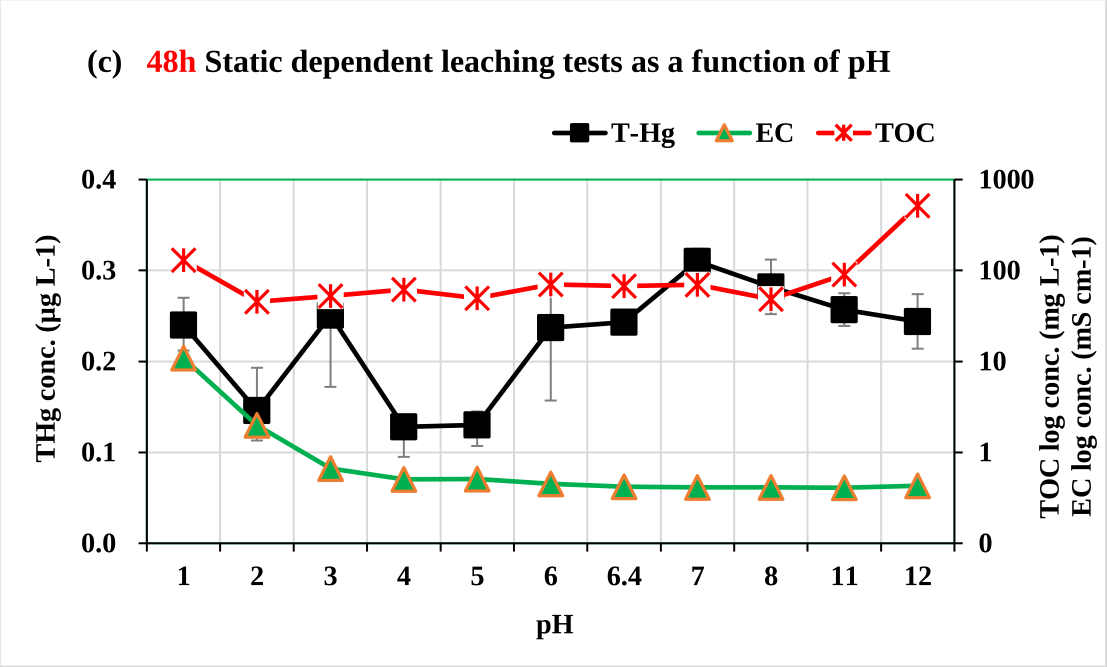


***Fig. 3-9 (c)**

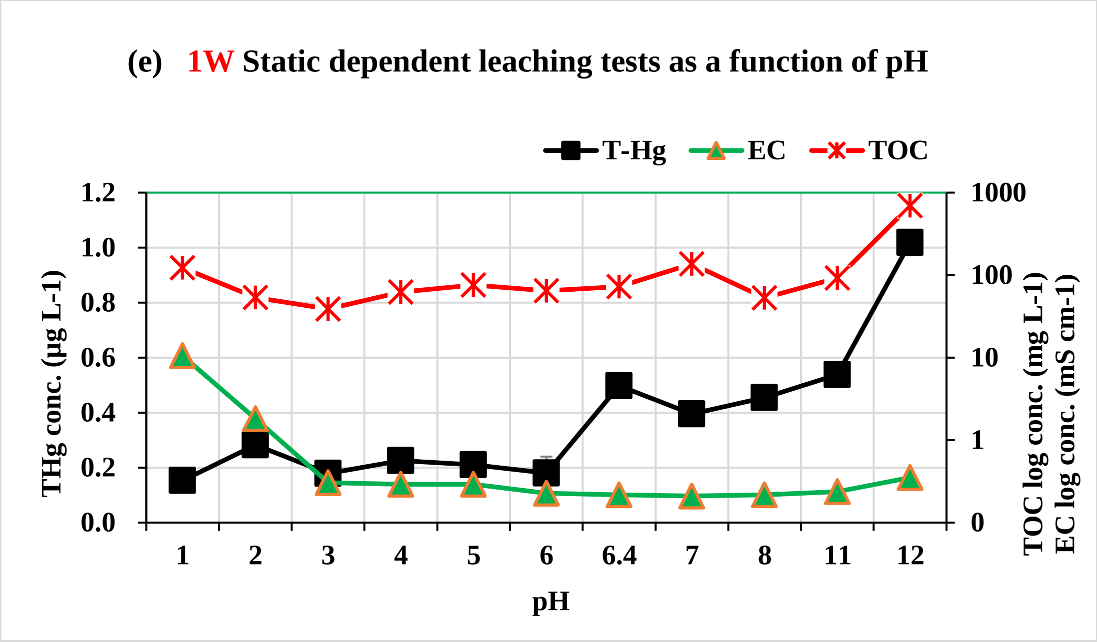


***Fig. 3-9 (e)**

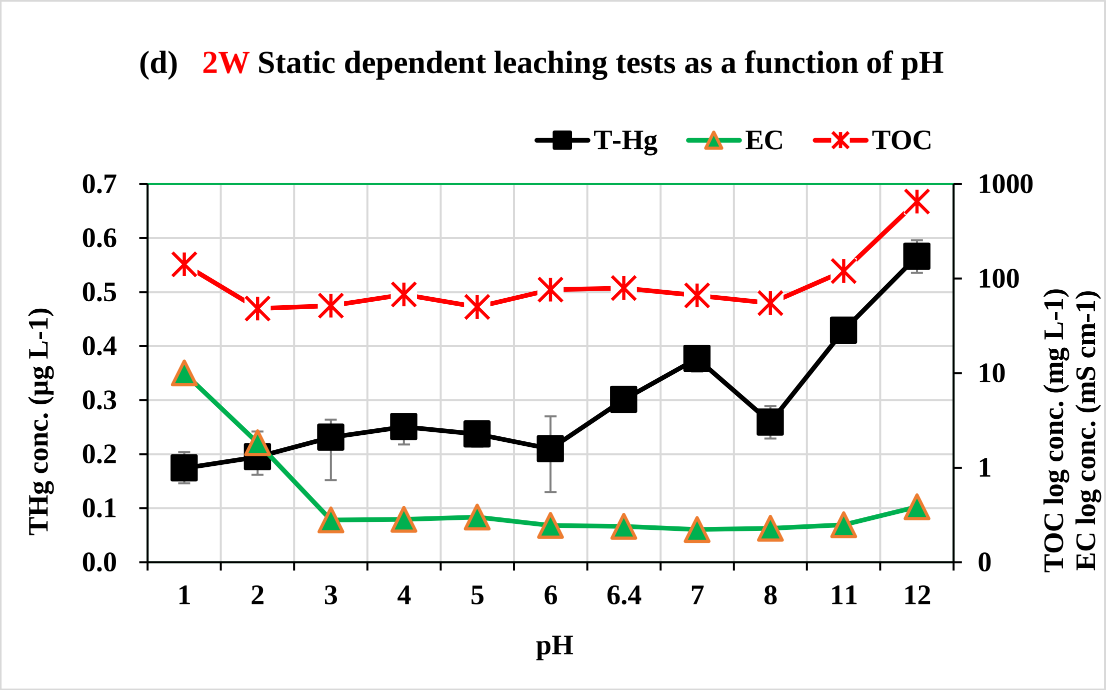


***Fig. 3-9 (d)**

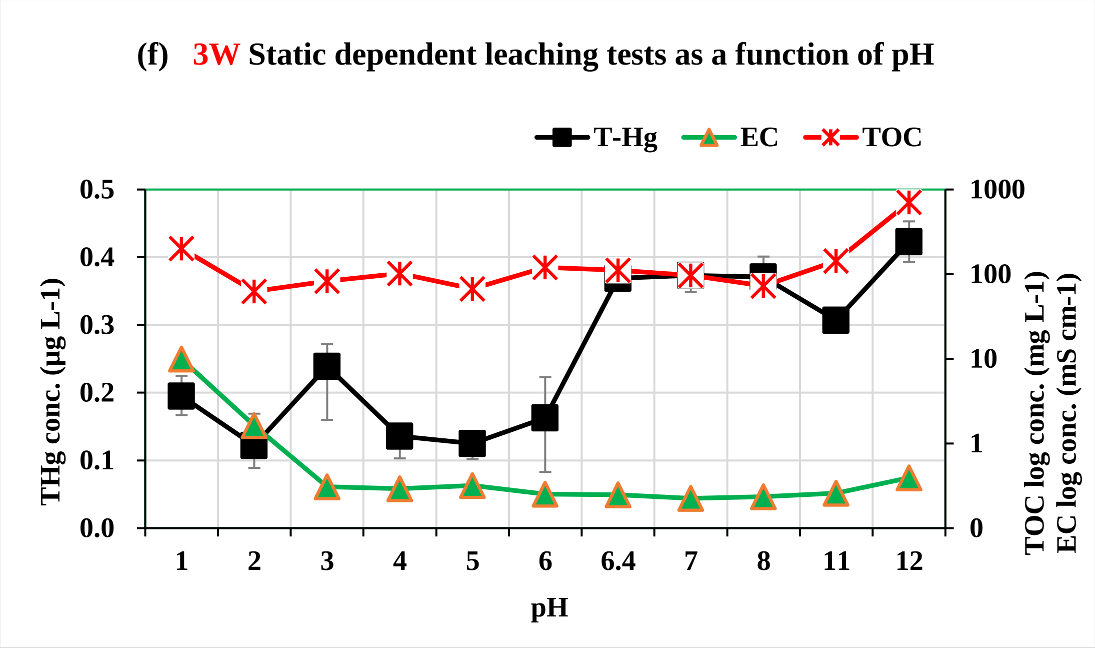


***Fig. 3-9 (f)**

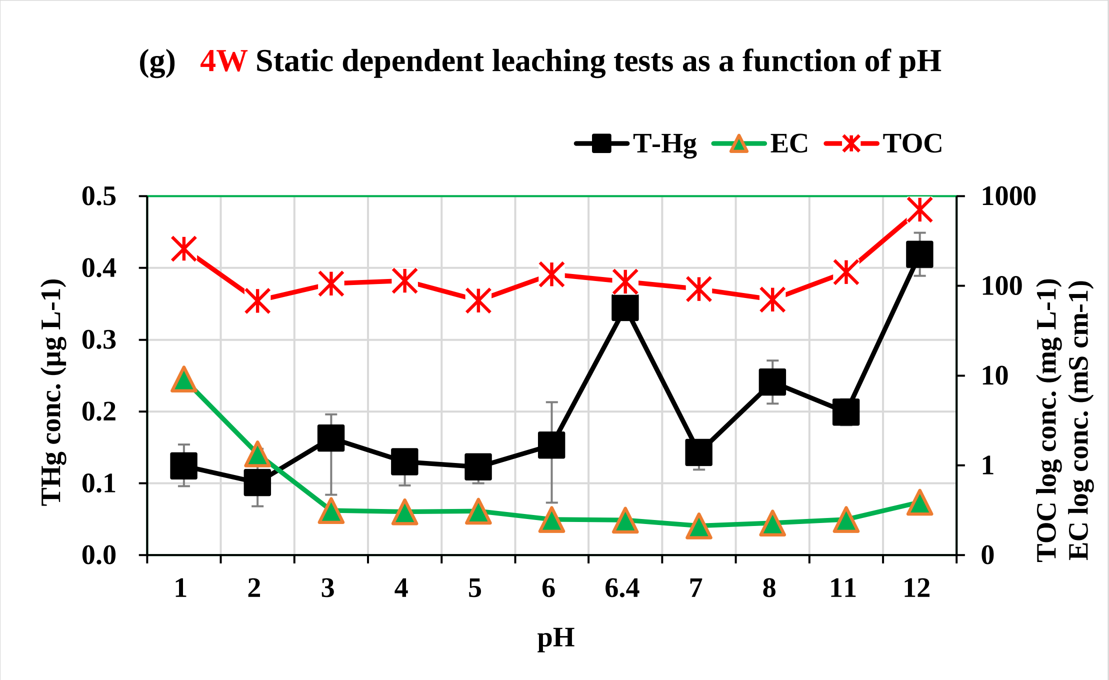


***Fig. 3-9 (g)**

Fig. 9 (a, b, c, d, e, f, g). Relationships of variation trend between average total mercury (THg), total organic carbon (TOC) and electrical conductivity (EC) in the static dependent leaching test as a function of pH (1-12) and time (6h-4w), the average value of THg, TOC, and EC was detected in (a) 6h, (b) 24h, (c) 48h, (d) 1W, (e) 2W, (f) 3W, (g) 4W, respectively.

3.3.2 Characteristics of the Na_2S treated LLCS in the batch THg leaching test

From our results of the treated LLCS by ss-Na_2S in batch pH-static dependent THg leaching test as a function of differential pH value and equilibrium time. The average leaching THg (Table S3-2, three replicates, n=3) from untreated LLCS was a wide ranged from 0.021 to 0.077 µg L^{-1} (mean: 0.043 µg L^{-1}) in the batch THg leaching test during the period of limited 4w equilibrium time. all the average leaching THg concentrations from treated LLCS are below the Japanese THg leaching limited standard (less than 0.5 µg L^{-1}) of soil. Also, the high hydroxyl ion (OH-) condition of pH-static leaching solutions will increase THg releasing for Hg-containing LLCS washing treated by ss-Na_2S during long-term equilibrium time (4w) in this experiment. Similarly, the max average THg leaching value (0.077 µg L^{-1}) appears at the pH12 leaching condition of in 1 week of equilibrium time in leaching test, the next 2 largest average leaching THg value are 0.052 µg L^{-1} and 0.051 µg L^{-1} appearing in pH 3 and pH 6 under the 6h equilibrium time, respectively. Also, we found that the leaching THg from the Hg-containing LLCS under the natural pH 4.2-5.3 were not exceeding the Japanese THg leaching limited standard (less than 0.5 µg L^{-1}) of soil as untreated LLCS, Moreover, the whole leaching soil THg concentrations from treated LLCS are significantly lower than the untreated LLCS under setting leaching condition as a function of pH values (1-12) and equilibrium time (6h-4w) (Fig. 3-10 and Fig. 3-11). The results suggest that the leaching THg from washing treated LLCS were reduced significantly by the treatment of the saturated Na_2S solution comparing with the untreated LLCS.

Various sulfur compounds have been used for remediating Hg-contaminated soils (Stepan et al., 1993; Wang et al., 2012). Sulfur has the high affinity capacity for bonding Hg, and the Hg sulfide compounds with low solubility will precipitates in water solution. However, the leachability of Hg sulfide compound precipitations will be increased with the time changes of incubation or hydration (Conner, 1990; Meng et al., 1998). Because the solubility of formed Hg sulfide compounds can be dissolved in the condition of excess amount of sulfide solution (e.g. ss-Na_2S) (Meng et al., 1998; Wang and Driscoll, 1995; Zhu et al., 2013). Through comparing the leaching THg from LLS before and after soil washing treated by ss-Na_2S for Hg containing LLCS. the changes of leaching THg in LLCS was better controlled by washing with ss-Na_2S treatment, the stable ability of treated LLCS for leaching THg were validated and examined under the controlled condition of the different pH values and equilibrium times in leaching test.

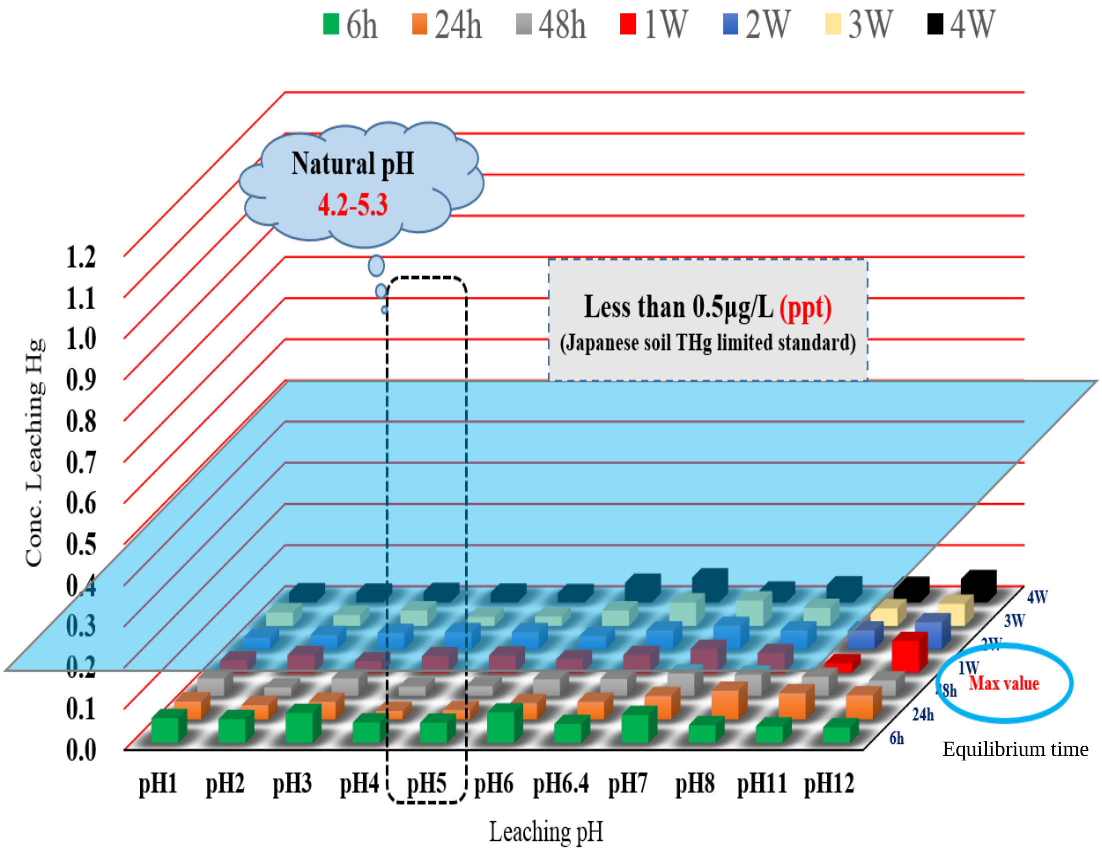


Fig. 3-10 Characteristics of total mercury (THg) in the washing treated landfill-leachate-contaminated soil (LLCS) by the ss-Na_2S at the batch static dependent leaching test as a function of pH and time. (Natural pH 4.2-5.3 was indicated in a vertical oblong circular dashed line, and a horizonal virtual plane is the Japanese soil limit of the leaching THg concentration from soil).

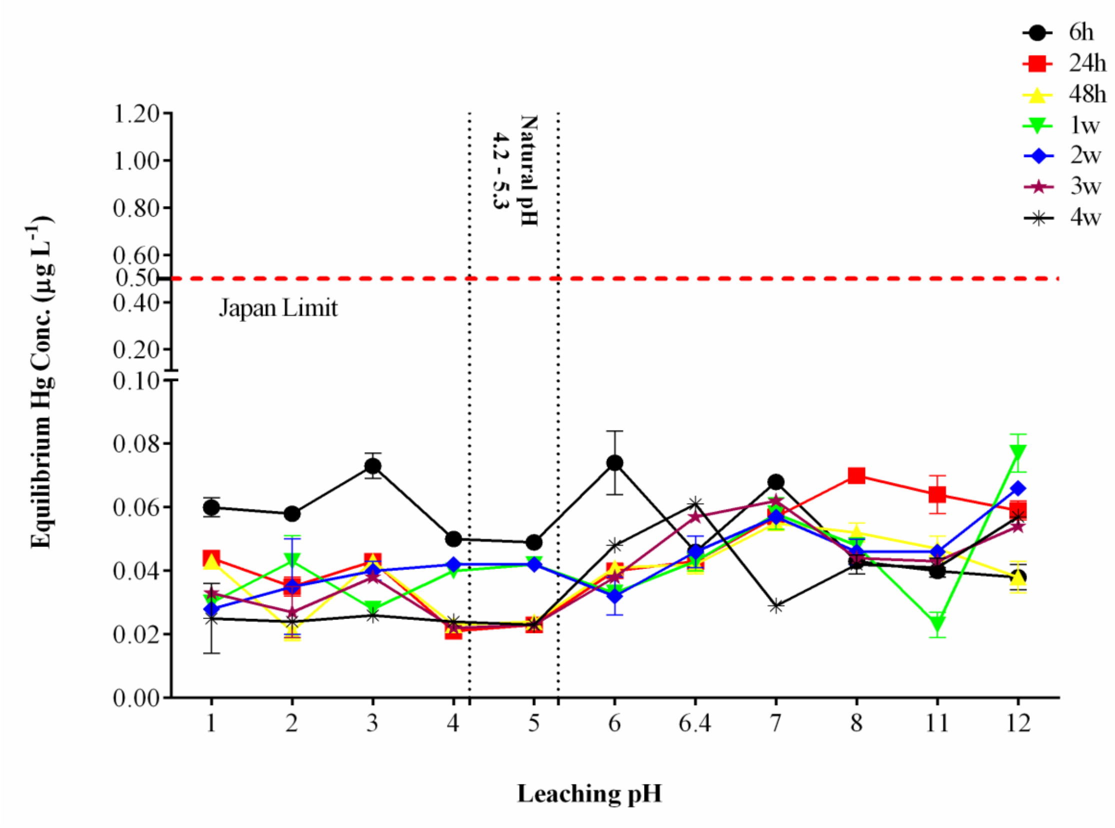


Fig. 3-11 The variation trends of leaching THg form washing treated LLCS by ss-Na_2S in pH-dependent static leaching test. (Natural pH 4.2-5.3 was indicated in two vertical dashed line, and a horizonal dashed line is the Japanese soil limit of the leaching THg concentration from soil).

3.3.3 Relationships and comparison of THg leaching between the untreated and treated LLCS in batch leaching test

The simulated THg leaching behavior were conducted in Visual TINTEQ. The related elements are depicted in Table 3-6. Main initial element concentrations were calculated basing on the XRF analysis (Table 3-3) as total amount of the element, and the initial various element concentration (mg L^{-1}) of LLCS in pH-static dependent leaching solution are listed in Table 3-6.

Table 3-6 Initial concentration of exchangeable element (mg L^{-1}) according to the amount of total element of by LLCS XRF analysis.

Element	Concentration (mg L^{-1})	Element	Concentration (mg L^{-1})
Al^{3+}	11610	Mn^{2+}	271.13
Fe^{3+}	4502.4	$PO4^{3-}$	454.93
K^{1+}	2904.26	Zn^{2+}	8.02
Mg^{2+}	882	$SO4^{2+}$	504
Na^{1+}	712.26	Hg^{2+}	0.09
Ca^{2+}	507.14		

The results of modeling using Visual MINTEQ showed that the variation trends of leaching THg from untreated and treated LLCS by the washing treatment of saturated Na_2S solution could not be simulated via the Visual MINTEQ modeling in pH-dependent static leaching test (Fig. 3-12). The THg leaching behavior of LLCS before and after washing treated by saturated Na_2S solution was investigated via Visual MINTEQ simulation and the pH-dependent leaching tests as a function of pH values (1-12) and equilibrium time (6h-4w), their simulation and experiment results are shown in Fig. 3-12.

According to the MINTEQ modeling, it can be observed from Fig. 3-12 that dissolved THg concentration of untreated LLCS was decreased to minimum from pH value 1 to 8; while the dissolved THg was gradually raised when pH value increased to more than 8. In addition, the leachability of dissolved THg was relatively lower than pH values less than 8. According to above leaching modeling, it can be found that pH value performed an important influence factor to THg leaching from LLCS. Moreover, the simulated variation trends of dissolved THg concentrations are higher than the Japanese guideline limit for soil Hg leaching (less than 0.5 µg L^{-1}) at a given pH, LLCS natural pH and equilibrium time (4.2-5.2) in Fig. 3-12.

However, the actual experiment results of the static dependent leaching test of soil THg as a function of pH values and equilibrium time before and after washing treated LLCS by ss-Na_2S procedures. The simulated and actual results in pH-dependent leaching tests were shown in Fig. 3-12. From the variation trends of leaching soil THg from untreated and washing treated by ss-Na_2S, the dissolved THg concentrations at the corresponding leaching condition as a function of pH and equilibrium time are not in concordance with the leaching behavior of MINTEQ simulation of main initial element concentrations using XRF analysis. First, at whole of pH 1-12 during 4w equilibrium time, the aqueous phase of THg concentrations are much lower than the dissolved THg concentration simulated by MINTEQ modeling, whether in untreated or Na_2S washing treated LLCS. From the observation of the actual trends of leaching THg concentrations, most leaching THg concentrations from pH value 1 to 8 were below the Japanese guideline limit for soil Hg leaching (less than 0.5 μg L^{-1}). while the leaching THg was gradually raised and part of them exceed guideline limit when pH value increased to more than 8 to 12. For untreated LLCS, the leaching THg reached maximum concentration (1.019 ± 0.042 μg L^{-1}) at pH 12 in first equilibrium week of this investigation in Fig. 3-8. Similarly, the leaching THg reached maximum concentration (0.077 ± 0.006 μg L^{-1}) at pH 12 in first equilibrium week from the treated LLCS, (Fig. 3-11, Table S3-3 and Fig. 3-13). All the results of THg leaching from untreated and treated LLCS are completely different with the simulation results of MINTEQ modeling. It can be speculated that the MINTEQ modeling is not suitable for simulating the leaching trends of THg concentrations from Hg-containing landfill-leachate-contaminated soils (LLCS) around the traditional MSW landfill.

In a word, although the Visual MINTEQ has been commonly developed currently in the simulation research on heavy metals leaching behaviors based on geochemical exchangeable element in soil. However, it was found that the trial Visual MINTEQ modeling according to mineralogical composition analysis is not suitable to predict the variation trends of leaching Hg in pH-dependent leaching test both in control and ss-Na_2S-washing treated Hg-containing LLCS. Therefore, considering the composition of LLCS is very complex under contaminated by leachate from traditional MSW landfill. It is needed to further explore the simulation research of Hg leaching behavior and find a more scientific method for evaluation leaching Hg from LLCS.

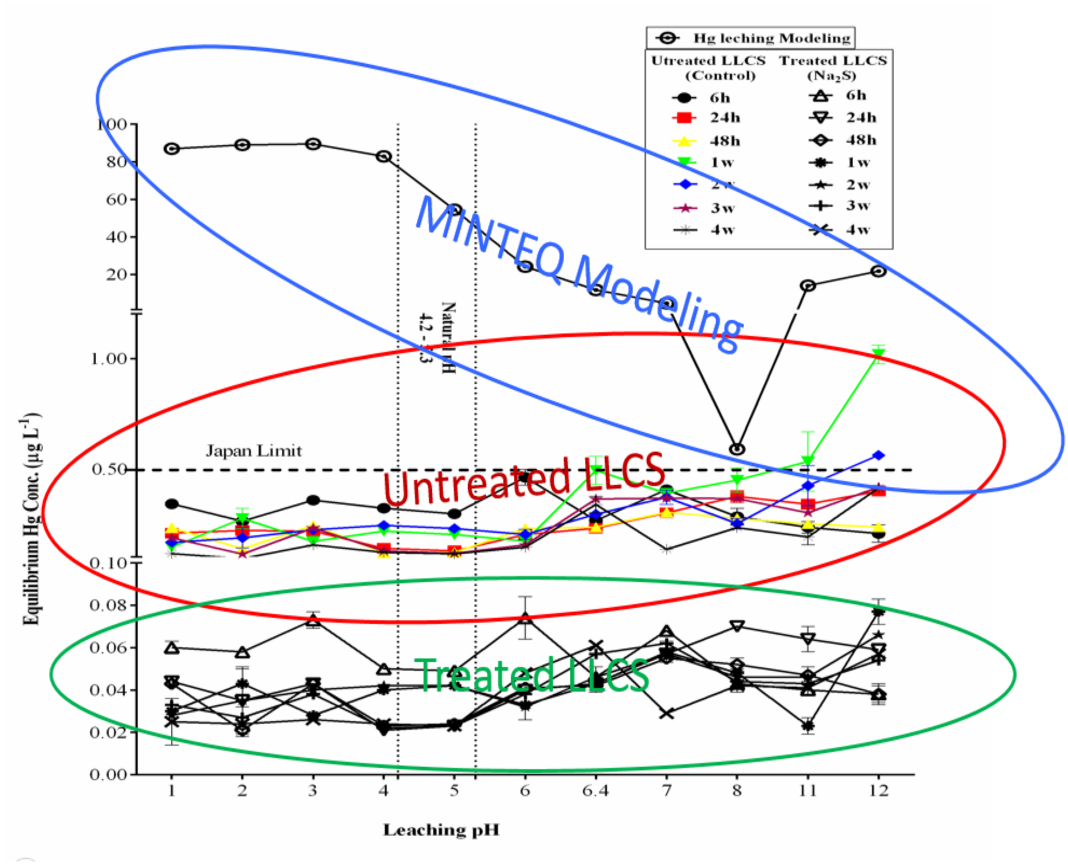


Fig. 3-12 The variation trends of leaching THg from untreated landfill-leachate-contaminated soil (LLCS) circling region with the red oval, washing treated by saturated Na_2S solution (ss-Na_2S) circling region with the green oval, and simulated by the Visual MINTEQ modeling circling region with the blue oval in pH-dependent static leaching test. (Natural pH 4.2-5.3 was indicated in two vertical dashed line, and a horizonal dashed line is the Japanese soil limit of the leaching THg concentration from contaminated soil).

The stability of residual Hg in treated LLCS by the process of saturated Na_2S solution were investigated via pH-dependent THg leaching test as a function of pH and equilibrium time intervals. The results showed that the leaching THg always maintains at a very low concentration level during 4w equilibrium phases under set pH values ranged from 1 to 12. Significant differences of the leaching THg concentration were observed before and after washing treated LLCS by ss-Na_2S. Generally, the whole variation trends of leaching THg has a relative high concentration level from pH value 1-7 during first 6h and from pH value from 8-11 during 24h equilibrium time, respectively. However, the maximum of leaching THg concentration was only (0.077 ± 0.006 µg L^{-1}) appeared at pH 12 in first equilibrium week of this laboratory study, this value of leaching THg from treated LLCS are significantly less than the maximum of leaching THg concentration from untreated LLCS (1.019 ± 0.042 µg L^{-1}) same appeared in first equilibrium week. The amount of leaching THg from treated LLCS were significantly immobilized and washed removal from the LLCS by saturated Na_2S solutions. Meanwhile, all leaching THg of pH-dependent test from saturated Na_2S solutions treated LLCS are less than the Japanese guideline limit for Hg leaching from soil during the whole equilibrium time of four weeks (Table S3-3, Fig. 3-12 and Fig. 3-13). Also, this standard of less than 0.5 µg L^{-1} is the Japanese guideline of dissolved Hg in groundwater. Therefore, referencing the Japanese guideline limit for Hg leaching from soil, it is unlikely to cause groundwater contamination by Hg leaching from LLCS at the natural pH (4.2-5.2) of LLCS, even so under a given pH, and equilibrium time in this laboratory study.

By comparing the concentration variation of leaching THg before and after saturated Na_2S solution washing treated LLCS at a given pH (1-12) for four weeks equilibrium time. The results show that the ss-Na_2S (L/S = 10) for removing the leaching THg (exchangeable Hg) is high efficiency from Hg-containing LLCS revealed in Table 3-4.1 and Table 3-4.2, the total dissolved THg (exchangeable Hg) was reduced significantly and ranged between 68.63% and 95.72% at a given pH (1-12) for four weeks equilibrium time in our laboratory study, and the optimal maximum efficiency of 95.72% was observed at pH 11 in first equilibrium week (Fig. 3-13, Fig. S3-1).

In general, the reasons for low leaching THg concentrations of Na_2S washing treated LLCS can probably be attributed to reduce the composition of exchangeable Hg, because the significant decline of Hg fractions (Hg-w and Hg-h) were proved through the process of a validated five-step sequential extractions (Fig. 3-4 and Fig. 3-5). Above results indicated that F1/Hg-w and F2/Hg-h were constituted 0.3% and 5.25% of THg in untreated LLCS only, respectively. Contrast, after washing treated LLCS with saturated Na_2S precipitation process, this percentage of F1/Hg-w and F2/Hg-h were decreased to 0.01% and 0.31% of THg in treated LLCS, the corresponding reducing efficiency reached 96.15% and 95.58%, respectively. The treated LLCS maintained the lowest level of leaching THg than untreated LLCS whose removal efficiency of leaching THg reached between 68.63% and 95.72%, which have been observed from the series experimental results in THg leaching test of batch pH-static dependent as a function of differential pH value and equilibrium time. Second, the exchangeable Hg was reduced by F1/Hg-w and F2/Hg-h with high mobility can be easily bio-accumulated than other fractions (F3/Hg-o, F4/Hg-e, and F5/Hg-s) and very important in environment concerns and public health, even in low contents in view of high mobility and potential bioavailability. Hg-w fraction can be weakly adsorbed on soil surface, with most mobile and available characteristics in interstitial soil. It's usually called as"labile Hg species", including more readily bio-accumulated Hg species than other Hg species, and able to diffuse into deeper soil and groundwater (e.g. Hg chloride ($HgCl_2$) is more easily to be transported and methylated than other inorganic Hg species (Fernández-Martínez and Rucandio, 2013; Han et al., 2003). Hg-s including sulfide Hg and other stable forms associated with silicates (Bloom et al., 2003).

Nevertheless, the heavy metal fractionation results do not always perfectly explain metal removal efficiency in remediation practice of heavy metal contaminated soil, because the removal efficiency of heavy metal also depends on the other practical factors such as heavy metal concentration and soil geochemistry (Dermont et al., 2008; Sun et al., 2001). It is a well-known reaction that stable compound of HgS will dissolve in exceeded volume of saturated Na_2S solutions (Baker, 1994; Nriagu, 1979; Wang and Driscoll, 1995). Meanwhile, considering some uncertain situations that the HgS dissolved in saturated Na_2S solutions may be influenced by many of complex pollutants in LLCS, but there was nothing factors changing this reaction to be observed in this laboratory study, the stable Hg-S fraction from the Hg fractionation for Hg-containing LLCS before and after washing treated by Na_2S to be confirmed in a validated five-step sequential extraction. Generally, it is significant that the stable Hg fraction Hg-s and the exchangeable fraction (F1/Hg-w and F2/Hg-h) can be better washing removed from LLCS (Fig. 3-4 and Fig. 3-5). From the experimental results indicated that the percentage of F5/Hg-S, F1/Hg-w and F2/Hg-h of THg in LLCS from 3.71% decrease to 0.04 % (removal efficiency 99.22%), 0.3% decrease to 0.01% (removal efficiency 96.15%), and 5.25% decrease to 0.31% (removal efficiency 95.58%) before and after washing treated LLCS saturated Na_2S solution, respectively (Fig. 3-5). Therefore, the saturated Na_2S solution has high immobilization capacity into stable Hg (Hg-S) for exchangeable Hg of LLCS, and then dissolved the stable Hg (Hg-S) to achieved washing removal the exchangeable Hg (F1/Hg-w and F2/Hg-h) and stable Hg (F5/Hg-s) from Hg-containing LLCS. Batch the results of static dependent as a function of differential pH value and equilibrium time demonstrate that the saturated Na_2S solution can be used to effectively immobilize exchangeable (Hg^{2+}) and dissolve stable Hg (HgS) for washing removal from Hg-containing LLCS, to reduce the concentration of soil-Hg fractions with high mobility and potential bioavailability in environment, lower the potential risks of secondary Hg poisoning from Hg-containing LLCS around traditional MSW landfill for public health on environment concerns.

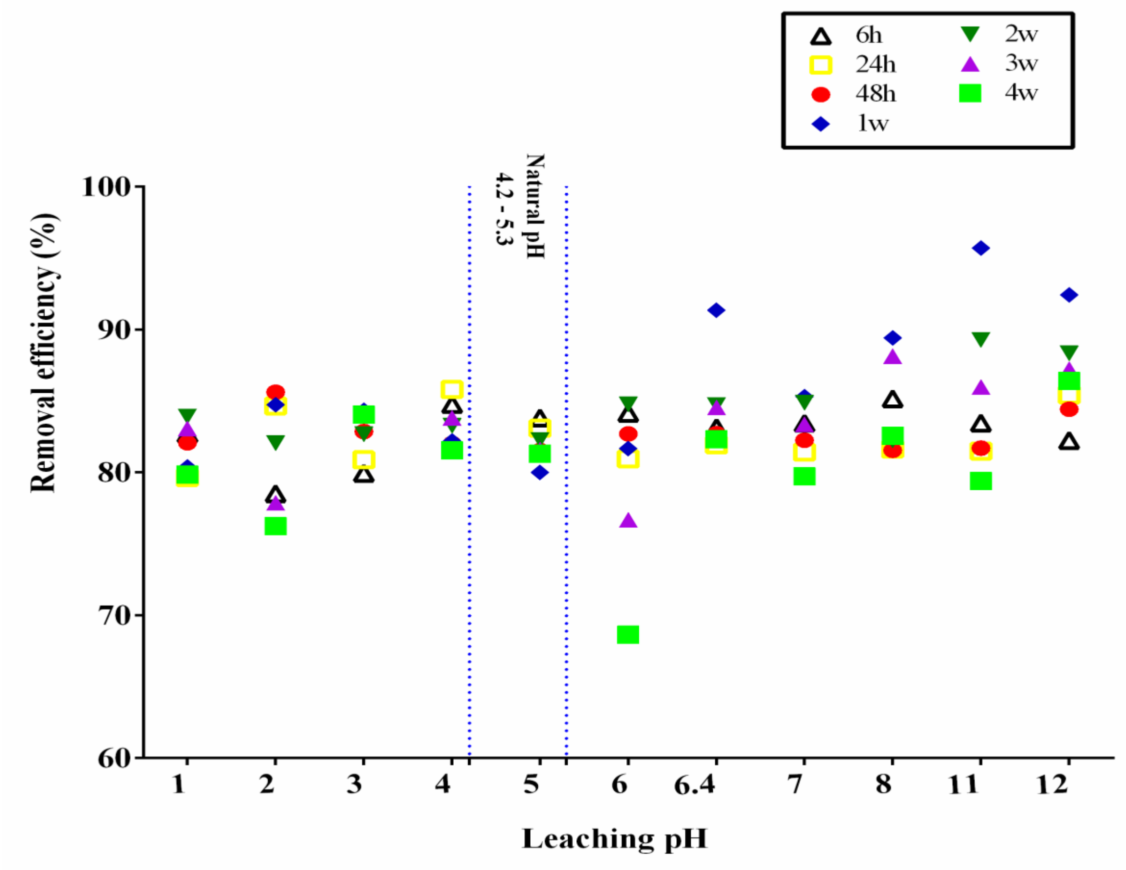


Fig. 3-13 The removal efficiency of leaching THg from treated landfill-leachate-contaminated soil (LLCS) by saturated Na_2S solution (ss-Na_2S) comparing with the leaching THg from untreated LLCS in the batch pH-static dependent leaching test as a function of pH (1-12) and equilibrium time (6h-4W).

However, because of relative narrow Hg concentration range of LLCS in this study and limitations of the extraction procedures of soil Hg fractions and pH-dependent-static-leaching tests, the applicability of our findings might be limited under field conditions (i.e., waste tape and composition, seasonal weather variation, dumping site age, precipitation by rainwater) in LLCS. The large-scale application of washing treated by ss-Na_2S processes presents some disadvantages: (1) this ss-Na_2S ex situ treatment system requires a large stirring equipment and large spaces for soil treatment; (2) the treatment of washing water may be required, thus will increase additional cost on water recycling and treatment (Agency, 1995; Dermont et al., 2008); (3) the microbiological and physico-chemical properties have been influenced and may be inappropriate directly revegetation and on-site disposal for safety; (4) if a part of the residual washing agents involved in the washing treated soils may be problematic discharge, or may complicate to cause other environmental issues in environment; (5) the residual sludge in discharged washing water is difficult to manage because it may be rich in other heavy metals, wastewater treatment may produce new toxic sludges. Therefore, more laboratory experiments are necessary to quantitatively examine the potential influencing factors for Hg washing removal, and the removed soil must be carefully managed, such as long-term stability, mobility, transformation of different soil-Hg fractions, and natural factors.

4. Conclusions

The objective of this laboratory study is to investigate the washing remediation effect of saturated sodium sulfide (ss-Na_2S) solution for mercury (Hg)-containing LLCS through considering the changes of soil total mercury (THg) concentration, a validated 5-steps sequential extraction for Hg-fractions, THg-static leaching test as a function of pH and equilibrium time, and the varied trends of leaching THg concentration were simulated by Visual MINTEQ modeling according to the mineralogical composition analysis. The effectiveness of used ss-Na_2S for immobilizing and washing treatment to Hg compounds in Hg-containing LLCS, before and after treatment in much more detailed insights into release controlling processes using THg-static leaching test as a function of pH and equilibrium time, a validated 5-steps sequential extraction for Hg-fractions, THg of LLCS.

The leaching behavior of mercury (Hg) using the pH-dependent leaching test, as a function of pH (1-12) and various experimental equilibrium time intervals (6 h, 24 h, 48 h, 1 w, 2 w, 3 w, and 4 w) were investigated in the Hg-containing LLCS from an unlined and Hg contaminated traditional landfill, also five fractions of Hg named water soluble Hg (F1/Hg-w), human stomach acid soluble Hg (F2/Hg-h), organic-chelated (F3/Hg-o), elemental Hg (F4/Hg-e), and mercuric sulfide (F5/Hg-s) by a validated sequential extraction procedure were carried out for assessing the effect, before and after Hg immobilization and wash removing of LLCS using ss-Na_2S, simultaneously.

To test the trends of Hg concentration-time in Hg static-leaching test, as a function of different equilibrium time and pH values. For better understanding the Hg long-term leaching behavior and potential environmental risks on LLCS before and after treating by saturated Na_2S solution. The variation trends of leaching dissolved total mercury (THg) in the pH range (1-12) from the LLCS and Na_2S-immobilized LLCS were combined with the Visual MINTEQ geochemical framework modeling according to the mineralogical composition analysis using the X-ray fluorescence (XRF).

From an overall perspective, the exchangeable Hg (F1/Hg-w, F2/Hg-h) and F5/Hg-s to be removed by the treatment of ss-Na_2S in Hg-containing LLCS are significantly. Although the averaged THg concentration of LLCS reduced by 22.02% only (from 1.8266 mg kg^{-1} down to mg kg^{-1}). But even so, the concentration composition of some Hg fractions in LLCS have changed before and after treatment significantly, including the average concentration of F1/Hg-w was reduced by 96.15% (from 0.0052 mg kg^{-1} down to 0.0002 mg kg^{-1}) , F2/Hg-h was reduced by 95.58% (from 0.0904 mg kg^{-1} down to 0.0040 mg kg^{-1}), F3/Hg-o was reduced by 23.85% (from 1.0680 mg kg^{-1} down to 0.8133 mg kg^{-1}), F4/Hg-e was reduced by 5.92% (from 0.4936 mg kg^{-1} down to 0.4644 mg kg^{-1}), F5/Hg-s was reduced by 99.22% (from 1.7211 mg kg^{-1} down to 1.2824 mg kg^{-1}), respectively.

The experimental results indicate that the LLCS immobilized and washed pretreatment using the saturated Na_2S solution can significantly reduce the soluble THg within the pH-dependent leaching test at various equilibrium interval periods. It was found that the highest concentration of leaching THg achieved at the 1st equilibrium week of pH12 both in the control LLCS and Na_2S-treated LLCS, while the lowest concentration of leaching THg occurred in pH 2 at the 4th equilibrium week in control LLCS, and at the 48h equilibrium time in Na_2S-treated LLCS in pH-dependent leaching test, respectively.

The optimal reducing efficiency of leaching THg in Na_2S-treated LLCS was achieved at pH 11, reaching up maximum 95.72% in the 1st week comparing with the corresponding equilibrium interval of the control LLCS. However, according to the XRF mineralogical composition analysis, it was not in agreement with Visual MINTEQ modeling that the leaching THg results during whole pH-dependent leaching test of control and Na_2S-treated LLCS. Also, the variation trends of leaching THg could not be simulated via the Visual MINTEQ modeling according to the speculating the elemental concentration from the mineralogical composition by XRF analysis.

Our study shows that the saturated Na_2S solution as a Hg immobilization application is an effective method to minimize the amount of leaching THg from Hg-containing LLCS. The main minimizing mechanism of the amount of leaching THg is to reduce the concentration of exchangeable and stable non-mobile Hg (i.e. Hg-s) of LLCS, that more than 95% of exchangeable Hg (Hg-w, Hg-h) and Hg-s to be immobilized and removed by the saturated Na_2S solution in LLCS proved via a validated Hg five-steps sequential extraction procedure. Meanwhile, its effectiveness is relatively stable with a wide range of equilibrium time (6h - 4w) and pH value (1 - 12) in this laboratory study.

However, Visual MINTEQ modeling simulated by mineralogical composition analysis is not suitable to predict the variation trends of leaching THg in pH-dependent leaching test both in control and Na_2S-immobilized Hg-contaminated LLCS. The exchangeable Hg (F1/Hg-w, F2/Hg-h) and stable F5/Hg-s can be removed by the washing treatment of saturated Na_2S solution from Hg-containing LLCS, also the stability and efficiency of the treated LLCS have been validated and compared through a sequential extractions and static dependent leaching test as a function of pH and equilibrium time.

The ss-Na_2S washing treatment can significantly reduce the Hg leaching from LLCS. Considering the exchangeable Hg fraction (Hg-w, Hg-h) were the high mobility and potential bioavailability than other Hg fraction in soil, the ss-Na_2S ex situ treatment may be developed a cost-effective Hg immobilization and washing process for removing or reducing exchangeable Hg fraction (Hg-w, Hg-h) and non-mobile Hg fraction (Hg-s) from Hg-containing LLCS simultaneously for reducing or eliminating the Hg potential environmental risks of LLCS referencing the Hg safety guide of Japanese in soil.

5. Summary

The ss-Na_2S washing treatment can significantly reduce the Hg leaching from LLCS. Considering the exchangeable Hg fraction (Hg-w, Hg-h) were the high mobility and potential bioavailability than other Hg fraction in soil, the ss-Na_2S ex situ treatment may be developed a cost-effective Hg immobilization and washing process for removing or reducing exchangeable Hg fraction (Hg-w, Hg-h) and non-mobile Hg fraction (Hg-s) from Hg-containing LLCS simultaneously for reducing or eliminating the Hg potential environmental risks of Hg-containing LLCS referencing the Hg safety guide of Japanese in soil.

Supplementary data of " Mercury immobilization and washing removal effect in landfill-leachate-contaminated soils (LLCS) using saturated sodium sulfide solution (ss-Na_2S): A laboratory study "

Table S3-1.1

Average concentrations of the THg, EC, and TOC in the static leaching test of untreated LLCS under the different pH value condition.

Equilibrium time	Items	Average concentrations of the THg, EC, and TOC in untreated [1]LLCS										
		1	2	3	4	5	6	6.4	7	8	11	12
6h	[2]THg	0.347	0.270	0.364	0.328	0.303	0.467	0.273	0.411	0.290	0.242	0.214
	[3]EC	12.883	2.037	0.800	0.568	0.582	0.520	0.478	0.460	0.470	0.465	0.505
	[4]TOC	95.004	57.996	62.352	58.536	61.056	63.180	65.628	65.448	65.484	91.008	628.560
24h	THg	0.216	0.228	0.225	0.148	0.136	0.210	0.238	0.307	0.381	0.346	0.405
	EC	11.325	2.028	0.715	0.529	0.534	0.481	0.449	0.441	0.442	0.432	0.483
	TOC	111.461	40.562	48.677	57.550	46.016	65.402	62.756	65.101	45.131	84.132	483.448
48h	THg	0.240	0.146	0.251	0.128	0.130	0.237	0.243	0.310	0.282	0.257	0.244
	EC	10.864	1.988	0.663	0.506	0.510	0.450	0.419	0.411	0.412	0.408	0.428
	TOC	118.892	43.266	51.922	61.387	49.083	69.762	66.939	69.441	48.140	89.741	515.678
1W	THg	0.153	0.282	0.179	0.225	0.210	0.180	0.498	0.395	0.454	0.538	1.019
	EC	10.517	1.817	0.305	0.292	0.290	0.227	0.217	0.210	0.217	0.238	0.350
	TOC	112.680	51.480	38.520	61.752	75.678	64.296	72.162	136.170	53.046	92.142	691.596
2W	THg	0.174	0.195	0.231	0.251	0.237	0.210	0.301	0.377	0.259	0.429	0.566
	EC	10.033	1.838	0.280	0.285	0.300	0.245	0.240	0.222	0.228	0.248	0.385
	TOC	131.796	46.440	51.372	67.560	49.940	75.852	78.894	65.916	54.828	120.038	651.807
3W	THg	0.195	0.122	0.239	0.136	0.125	0.163	0.369	0.373	0.371	0.307	0.423
	EC	9.933	1.625	0.308	0.293	0.320	0.252	0.248	0.225	0.235	0.258	0.393
	TOC	190.944	61.056	82.476	101.268	66.864	119.952	111.384	96.156	72.324	143.352	705.699
4W	THg	0.124	0.101	0.163	0.130	0.123	0.153	0.345	0.143	0.241	0.199	0.419
	EC	9.150	1.345	0.315	0.305	0.310	0.250	0.245	0.212	0.229	0.250	0.390
	TOC	249.804	66.960	106.020	113.736	68.572	134.532	111.222	92.016	70.236	142.385	706.473

Note:

1. LLCS: Landfill-Leachate-contaminated soil (d.w.=dry weight); 2. THg (total mercury, ppb/μg L^{-1}); 3. EC: Electrical conductivity (mS/m);
4. TOC: Total organic carbon (mg L^{-1}).

Table S3-1.2

Standard deviation (SD) of the THg, EC, and TOC concentrations in the static leaching test of untreated LLCS.

Equilibrium time	Items	Standard deviation (SD) of the concentrations in untreated [1]LLCS										
		1	2	3	4	5	6	6.4	7	8	11	12
6h	[2]THg	0.028	0.033	0.079	0.033	0.023	0.080	0.011	0.024	0.030	0.018	0.030
	[3]EC	0.895	0.147	0.110	0.020	0.004	0.078	0.108	0.088	0.099	0.093	0.093
	[4]TOC	2.880	1.080	1.080	1.260	0.972	0.360	0.756	2.304	0.396	1.476	5.184
24h	THg	0.021	0.024	0.015	0.007	0.017	0.011	0.015	0.025	0.028	0.031	0.030
	EC	1.404	0.189	0.057	0.001	0.021	0.065	0.102	0.092	0.083	0.085	0.106
	TOC	1.728	0.720	0.360	1.260	0.684	7.560	1.872	0.684	1.908	1.764	7.416
48h	THg	0.012	0.023	0.008	0.009	0.010	0.012	0.016	0.018	0.019	0.022	0.024
	EC	1.336	0.200	0.010	0.023	0.044	0.044	0.088	0.076	0.070	0.079	0.068
	TOC	1.836	1.310	1.550	1.450	1.880	5.960	7.320	2.320	1.220	0.400	3.420
1W	THg	0.007	0.034	0.015	0.011	0.015	0.025	0.139	0.059	0.099	0.236	0.309
	EC	1.224	0.136	0.028	0.013	0.000	0.015	0.005	0.000	0.005	0.004	0.033
	TOC	2.160	2.690	0.920	2.050	5.920	0.760	-0.050	0.420	0.460	0.300	0.400
2W	THg	0.022	0.094	0.042	0.012	0.008	0.101	0.025	0.076	0.023	0.103	0.095
	EC	1.242	0.044	0.034	0.016	0.000	0.016	0.013	0.013	0.010	0.010	0.038
	TOC	1.800	0.030	0.030	0.023	0.078	0.110	0.000	0.010	0.010	0.020	0.050
3W	THg	0.033	0.075	0.039	0.006	0.025	0.029	0.019	0.021	0.060	0.029	0.055
	EC	1.169	0.018	0.053	0.015	0.000	0.031	0.020	0.005	0.027	0.020	0.026
	TOC	2.880	0.030	0.030	0.021	0.035	0.060	0.030	0.010	0.060	0.030	0.070
4W	THg	0.017	0.021	0.053	0.008	0.010	0.037	0.026	0.087	0.013	0.035	0.041
	EC	1.041	0.030	0.049	0.016	0.000	0.033	0.027	0.004	0.034	0.022	0.033
	TOC	2.160	0.010	0.030	0.021	0.024	0.030	0.060	0.030	0.100	0.090	0.160

Note:

1. LLCS: Landfill-Leachate-contaminated soil (d.w.=dry weight); 2. THg (total mercury, ppb/µg L^{-1}); 3. EC: Electrical conductivity (mS/m);
4. TOC: Total organic carbon (mg L^{-1}).

Table S3-2

Initial data of the leaching THg concentrations (μg L^{-1}) of pH-dependent test from **untreated LLCS** and washing **treated LLCS** by saturated Na_2S solutions (ss-Na_2S) as different pH values (1-12) and equilibrium time intervals.

pH leaching conditions	Leaching replicates	Intervals of the ^{1}equilibrium time in the pH-static dependent leaching test													
		Untreated ^{2}LLCS							Treated LLCS						
		6h	24h	48h	1W	2W	3W	4W	6h	24h	48h	1W	2W	3W	4W
pH1	1	0.355	0.218	0.227	0.128	0.163	0.188	0.127	0.058	0.046	0.044	0.030	0.029	0.034	0.038
	2	0.357	0.203	0.247	0.165	0.185	0.198	0.130	0.063	0.043	0.043	0.031	0.030	0.034	0.018
	3	0.328	0.227	0.245	0.167	0.175	0.200	0.115	0.058	0.043	0.041	0.029	0.026	0.031	0.021
pH2	1	0.266	0.235	0.150	0.330	0.243	0.127	0.124	0.059	0.035	0.021	0.052	0.051	0.035	0.022
	2	0.278	0.240	0.157	0.238	0.152	0.120	0.093	0.058	0.032	0.019	0.041	0.029	0.022	0.025
	3	0.265	0.208	0.130	0.278	0.190	0.120	0.086	0.058	0.038	0.024	0.036	0.023	0.022	0.026
pH3	1	0.340	0.215	0.248	0.178	0.213	0.228	0.153	0.070	0.045	0.044	0.028	0.037	0.037	0.026
	2	0.370	0.223	0.252	0.190	0.232	0.235	0.183	0.078	0.042	0.043	0.029	0.042	0.039	0.026
	3	0.382	0.237	0.253	0.170	0.247	0.253	0.152	0.072	0.041	0.042	0.028	0.041	0.039	0.027
pH4	1	0.340	0.142	0.130	0.215	0.248	0.157	0.132	0.051	0.022	0.024	0.041	0.043	0.023	0.025
	2	0.322	0.170	0.127	0.223	0.252	0.128	0.127	0.050	0.021	0.023	0.041	0.042	0.022	0.025
	3	0.323	0.132	0.127	0.237	0.253	0.123	0.130	0.049	0.021	0.023	0.038	0.040	0.022	0.023
pH5	1	0.288	0.157	0.132	0.200	0.230	0.118	0.133	0.048	0.023	0.024	0.044	0.043	0.023	0.024
	2	0.307	0.128	0.127	0.212	0.240	0.135	0.123	0.051	0.023	0.024	0.042	0.042	0.022	0.022
	3	0.315	0.123	0.130	0.218	0.242	0.123	0.112	0.048	0.023	0.023	0.041	0.042	0.023	0.023

Table S3-2 (*Continued*)

PH leaching conditions	Leaching replicates	Intervals of the [1]equilibrium time in the pH-static dependent leaching test													
		Untreated [2]LLCS							Treated LLCS						
		6h	24h	48h	1W	2W	3W	4W	6h	24h	48h	1W	2W	3W	4W
pH6	1	0.468	0.200	0.230	0.188	0.233	0.183	0.142	0.066	0.041	0.042	0.033	0.026	0.038	0.047
	2	0.502	0.212	0.240	0.178	0.185	0.147	0.163	0.071	0.041	0.041	0.033	0.037	0.038	0.048
	3	0.430	0.218	0.242	0.173	0.212	0.160	0.155	0.085	0.037	0.040	0.035	0.035	0.038	0.049
pH6.4	1	0.273	0.242	0.240	0.437	0.282	0.355	0.302	0.045	0.045	0.046	0.040	0.042	0.055	0.061
	2	0.285	0.242	0.232	0.495	0.300	0.368	0.370	0.044	0.041	0.042	0.044	0.051	0.057	0.061
	3	0.260	0.230	0.257	0.562	0.322	0.383	0.362	0.048	0.043	0.039	0.044	0.046	0.059	0.060
pH7	1	0.418	0.308	0.318	0.385	0.365	0.373	0.140	0.067	0.058	0.055	0.053	0.055	0.062	0.032
	2	0.413	0.310	0.315	0.408	0.413	0.383	0.143	0.067	0.058	0.055	0.061	0.059	0.062	0.029
	3	0.402	0.302	0.297	0.392	0.353	0.362	0.147	0.070	0.055	0.054	0.060	0.057	0.063	0.027
pH8	1	0.332	0.412	0.288	0.510	0.268	0.373	0.243	0.044	0.071	0.050	0.047	0.042	0.042	0.045
	2	0.282	0.373	0.282	0.400	0.277	0.375	0.248	0.044	0.070	0.050	0.047	0.050	0.046	0.041
	3	0.257	0.358	0.275	0.453	0.232	0.365	0.233	0.042	0.069	0.055	0.049	0.047	0.044	0.040
pH11	1	0.260	0.353	0.253	0.413	0.348	0.317	0.202	0.040	0.059	0.049	0.018	0.044	0.044	0.044
	2	0.255	0.352	0.242	0.522	0.412	0.305	0.163	0.041	0.061	0.048	0.025	0.047	0.043	0.038
	3	0.212	0.333	0.275	0.678	0.527	0.300	0.233	0.038	0.071	0.042	0.025	0.046	0.042	0.041
pH12	1	0.260	0.410	0.262	1.067	0.590	0.427	0.418	0.042	0.058	0.044	0.071	0.068	0.052	0.058
	2	0.190	0.402	0.227	1.003	0.552	0.420	0.427	0.038	0.063	0.036	0.076	0.066	0.055	0.058
	3	0.193	0.402	0.242	0.987	0.555	0.422	0.413	0.034	0.057	0.035	0.082	0.064	0.055	0.056

Note:

1. Equilibrium time intervals including 6 hours (6 h), 24 hours (24 h), 48 hours (48 h), 1 week (1 w), 2 weeks (2 w), 3 weeks (3 w), and 4 weeks (4 w).

2. LLCS: Landfill-Leachate-contaminated soil.

Table S3-3

Leaching THg concentrations (μg L^{-1}) of pH-dependent test from **untreated LLCS** and **washing treated LLCS** by saturated Na_2S solutions (ss-Na_2S) as different pH values and equilibrium time intervals (Mean ± SD).

[1]LLCS	pH values	[2]Equilibrium time intervals						
		6h	24h	48h	1W	2W	3W	4W
Control LLCS (CS)	1	0.347 ± 0.016	0.216 ± 0.012	0.240 ± 0.011	0.153 ± 0.022	0.174 ± 0.011	0.195 ± 0.006	0.124 ± 0.008
	2	0.270 ± 0.007	0.228 ± 0.017	0.146 ± 0.014	0.282 ± 0.046	0.195 ± 0.046	0.122 ± 0.004	0.101 ± 0.020
	3	0.364 ± 0.022	0.225 ± 0.011	0.251 ± 0.003	0.179 ± 0.010	0.231 ± 0.017	0.239 ± 0.013	0.163 ± 0.018
	4	0.328 ± 0.010	0.148 ± 0.020	0.128 ± 0.002	0.225 ± 0.011	0.251 ± 0.003	0.136 ± 0.018	0.130 ± 0.003
	5	0.303 ± 0.014	0.136 ± 0.018	0.130 ± 0.003	0.210 ± 0.009	0.237 ± 0.006	0.125 ± 0.009	0.123 ± 0.011
	6	0.467 ± 0.036	0.210 ± 0.009	0.237 ± 0.006	0.180 ± 0.008	0.210 ± 0.024	0.163 ± 0.018	0.153 ± 0.011
	6.4	0.273 ± 0.013	0.238 ± 0.007	0.243 ± 0.013	0.498 ± 0.063	0.301 ± 0.020	0.369 ± 0.014	0.345 ± 0.037
	7	0.411 ± 0.008	0.307 ± 0.004	0.310 ± 0.011	0.395 ± 0.012	0.377 ± 0.032	0.373 ± 0.011	0.143 ± 0.004
	8	0.290 ± 0.038	0.381 ± 0.028	0.282 ± 0.007	0.454 ± 0.055	0.259 ± 0.024	0.371 ± 0.005	0.241 ± 0.008
	11	0.242 ± 0.026	0.346 ± 0.011	0.257 ± 0.017	0.538 ± 0.133	0.429 ± 0.091	0.307 ± 0.009	0.199 ± 0.035
	12	0.214 ± 0.040	0.405 ± 0.005	0.244 ± 0.018	1.019 ± 0.042	0.566 ± 0.021	0.423 ± 0.004	0.419 ± 0.007
Na_2S treated LLCS (TS)	1	0.060 ± 0.003	0.044 ± 0.001	0.043 ± 0.002	0.030 ± 0.001	0.028 ± 0.002	0.033 ± 0.002	0.025 ± 0.011
	2	0.058 ± 0.000	0.035 ± 0.003	0.021 ± 0.003	0.043 ± 0.008	0.035 ± 0.015	0.027 ± 0.008	0.024 ± 0.002
	3	0.073 ± 0.004	0.043 ± 0.002	0.043 ± 0.001	0.028 ± 0.001	0.040 ± 0.003	0.038 ± 0.002	0.026 ± 0.001
	4	0.050 ± 0.001	0.021 ± 0.001	0.023 ± 0.001	0.040 ± 0.002	0.042 ± 0.001	0.022 ± 0.000	0.024 ± 0.001
	5	0.049 ± 0.001	0.023 ± 0.000	0.024 ± 0.000	0.042 ± 0.002	0.042 ± 0.001	0.023 ± 0.001	0.023 ± 0.001
	6	0.074 ± 0.010	0.040 ± 0.002	0.041 ± 0.001	0.033 ± 0.001	0.032 ± 0.006	0.038 ± 0.000	0.048 ± 0.001
	6.4	0.046 ± 0.002	0.043 ± 0.002	0.042 ± 0.003	0.043 ± 0.003	0.046 ± 0.005	0.057 ± 0.002	0.061 ± 0.001
	7	0.068 ± 0.001	0.057 ± 0.002	0.055 ± 0.001	0.058 ± 0.005	0.057 ± 0.002	0.062 ± 0.001	0.029 ± 0.002
	8	0.043 ± 0.001	0.070 ± 0.001	0.052 ± 0.003	0.048 ± 0.001	0.046 ± 0.004	0.044 ± 0.002	0.042 ± 0.003
	11	0.040 ± 0.001	0.064 ± 0.006	0.047 ± 0.004	0.023 ± 0.004	0.046 ± 0.002	0.043 ± 0.001	0.041 ± 0.003
	12	0.038 ± 0.004	0.059 ± 0.003	0.038 ± 0.005	0.077 ± 0.006	0.066 ± 0.002	0.054 ± 0.002	0.057 ± 0.001

1. LLCS: Landfill-Leachate-contaminated soil
2. Equilibrium time intervals including 6 hours (6 h), 24 hours (24 h), 48 hours (48 h), 1 week (1 w), 2 weeks (2 w), 3 weeks (3 w), and 4 weeks (4 w).

Table S3-4.1

The ratio of leaching ^{1}THg (‰) from untreated LLCS and washing treated by ss-Na_2S in static dependent leaching test as a function of pH and equilibrium time.

PH leaching condition	^{2}Equilibrium Time of pH-static dependent leaching test													
	Untreated ^{3}LLCS **(‰)**							Treated LLCS **(‰)**						
	6h	24h	48h	1W	2W	3W	4W	6h	24h	48h	1W	2W	3W	4W
pH1	0.190	0.118	0.131	0.084	0.095	0.107	0.068	0.042	0.031	0.030	0.021	0.020	0.023	0.018
pH2	0.148	0.125	0.080	0.154	0.107	0.067	***0.055**	0.041	0.025	0.015	0.030	0.025	0.019	0.017
pH3	0.199	0.123	0.137	0.098	0.126	0.131	0.089	0.051	0.030	0.030	0.020	0.028	0.027	0.018
pH4	0.180	0.081	0.070	0.123	0.137	0.074	0.071	0.035	0.015	0.016	0.028	0.029	***0.015**	0.017
pH5	0.166	0.074	0.071	0.115	0.130	0.068	0.067	0.034	0.016	0.017	0.029	0.029	0.016	0.016
pH6	0.256	0.115	0.130	0.099	0.115	0.089	0.084	0.052	0.028	0.029	0.023	0.022	0.027	0.034
pH6.4	0.149	0.130	0.133	0.273	0.165	0.202	0.189	0.032	0.030	0.029	0.030	0.032	0.040	0.043
pH7	0.225	0.168	0.170	0.216	0.206	0.204	0.078	0.048	0.040	0.039	0.041	0.040	0.044	0.020
pH8	0.159	0.209	0.154	0.249	0.142	0.203	0.132	0.030	0.049	0.037	0.034	0.032	0.031	0.029
pH11	0.132	0.189	0.141	0.295	0.235	0.168	0.109	0.028	0.045	0.033	0.016	0.032	0.030	0.029
pH12	0.117	0.222	0.134	***0.558**	0.310	0.232	0.229	0.027	0.041	0.027	***0.054**	0.046	0.038	0.040

1. The ratio of leaching THg (‰) from Hg containing untreated LLCS = leaching THg concentration (ppb/µg L^{-1}) of untreated LLCS divided THg concentration (ppm/mg kg^{-1}) ⁎ 1000 ⁎ ‰, or the ratio of leaching THg (‰) from Hg containing treated LLCS = leaching THg concentration (ppb/µg L^{-1}) of washing treated LLCS by saturated Na_2S solution divided the THg concentration (ppm/mg kg^{-1}) of washing treated LLCS by saturated Na_2S solution ⁎ 1000 ⁎ ‰.

2. Equilibrium time intervals including 6 hours (6 h), 24 hours (24 h), 48 hours (48 h), 1 week (1 w), 2 weeks (2 w), 3 weeks (3 w), and 4 weeks (4 w).

3. LLCS: Landfill-Leachate-contaminated soil.

Note: * The maximum and minimum values are shown in text red and bold.

Table S3-4.2

Average reducing [1]efficiency (%) of leaching THg concentrations from the ss-Na_2S washing treated LLCS basing on the untreated LLCS in the corresponding pH-dependent as different pH values and equilibrium time intervals.

Leaching pH	Intervals of [3]equilibrium time						
	6h	24h	48h	1W	2W	3W	4W
1	82.71	79.63	82.08	80.39	83.91	83.08	79.84
2	78.52	84.65	85.62	84.75	82.05	77.87	76.24
3	79.95	80.89	82.87	84.36	82.68	84.10	84.05
4	84.76	85.81	82.03	82.22	83.27	83.82	81.54
5	83.83	83.09	81.54	80.00	82.28	81.60	81.30
6	84.15	80.95	82.70	81.67	84.76	76.69	***68.63**
6.4	83.15	81.93	82.72	91.37	84.72	84.55	82.32
7	83.45	81.43	82.26	85.32	84.88	83.38	79.72
8	85.17	81.63	81.56	89.43	82.24	88.14	82.57
11	83.47	81.50	81.71	***95.72**	89.28	85.99	79.40
12	82.24	85.43	84.43	92.44	88.34	87.23	86.40

1. Reducing efficiency (%) = (1 - leaching THg concentrations of Na_2S treated LLCS divided the leaching THg of untreated LLCS at the corresponding pH value and equilibrium time during in pH-independent test) × 100%. in corresponding leaching condition of the static dependent leaching test as a function of pH and equilibrium time.

2. LLCS: Landfill-Leachate-contaminated soil.

3. Equilibrium time intervals including 6 hours (6 h), 24 hours (24 h), 48 hours (48 h), 1 week (1 w), 2 weeks (2 w), 3 weeks (3 w), and 4 weeks (4 w).

Note: * The maximum and minimum values are shown in text red and bold.

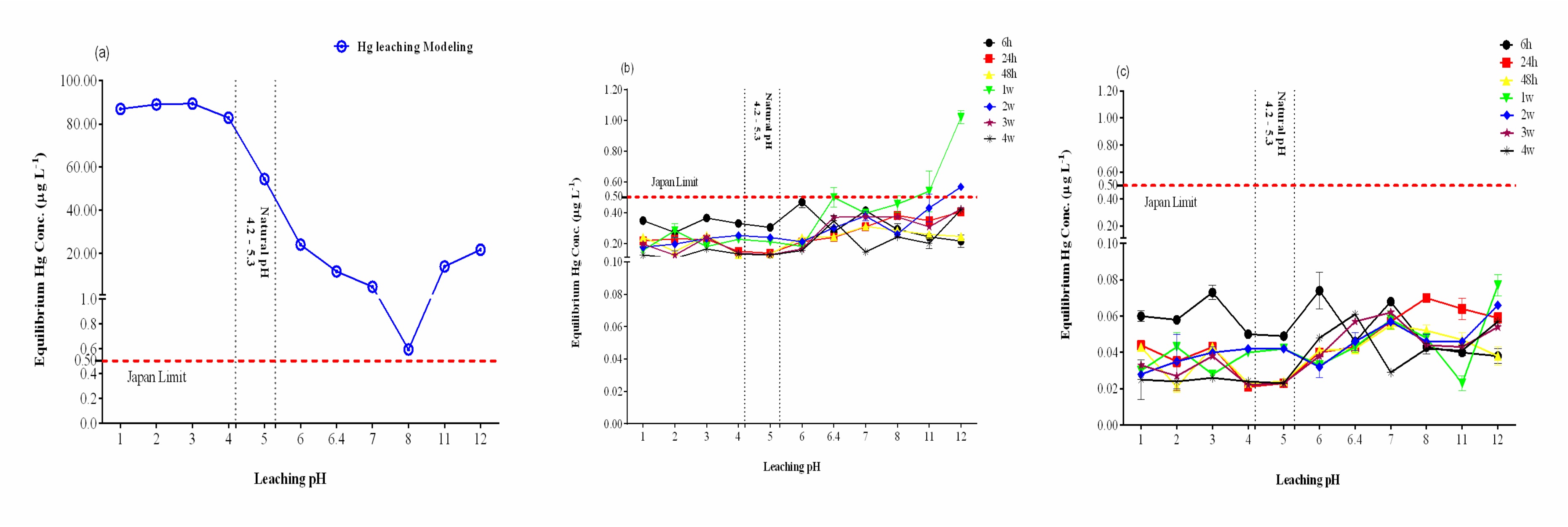


Fig. S3-1

The variation trends of the leaching THg concentrations (μg L^{-1}) of pH-dependent test as different pH values and equilibrium time intervals, (a) leaching THg simulated by the Visual MINTEQ modeling, (b) leaching THg from the untreated LLCS and (c) leaching THg from the washing treated by saturated Na_2S solutions (ss-Na_2S).

CHAPTTR 4

Conclusions and perspectives

Hg-containing Municipal solid waste (MSW) are unavoidably deposited in soil of the traditional landfills, although direct discharge of Hg-containing MSW into the environment has been declined substantially worldwide. Considering Hg with the potential high risks of public health can be accumulated and amplified in predatory animals and humans through the food chain directly and indirectly (e.g., neurotoxicity, mortality, and reproductive toxicity). Earthworms are considered useful bio-indicators for assessing soil quality and biotic effects for heavy metal pollution. It is meaningful to study the native earthworm relationships with mercuric biogeochemical fractions, spatial distribution, and compare THg and MeHg bioaccumulation in native *B. parvus* earthworm in the Hg-containing LLCS by field investigation, to explore the effects of washing remediation using saturated Na_2S solution (ss-Na_2S) for Hg-containing LLCS through contrasting the changes in soil mercuric fractions and leaching THg of Hg-containing LLCS before and after washing treatment of ss-Na_2S.

In this study, spatial distributions on fractionation of soil-Hg and Hg speciation (T-Hg and MeHg) of native *B. parvus* earthworms were investigated in the leachate-contaminated zone around a Hg polluted traditional MSW landfill. Soil T-Hg concentration ranged between 0.227 and 2.919 mg kg^{-1} dry weight (dw). The T-Hg and MeHg concentrations of *B. parvus* species ranged from 1.242 to 6.775 mg kg^{-1} dw and from 0.031 to 0.218 mg kg^{-1} dw, respectively. Percentages of soil-Hg fractions were in the order of F3/Hg-o > F4/ Hg-e > F5/ Hg-s > F1/Hg-w > F2 / Hg-h, and the fractions of Hg-o and Hg-e were 55.50% and 35.31%, respectively. The results indicated that easily bioavailable and soluble Hg fractions (Hg-w, Hg-h) of the soil were not appropriate to illustrate the distribution of Hg in native *B. parvus*. Instead, the stable soil-Hg fractions (Hg-o, Hg-e, and Hg-s) demonstrated good relationships of spatial distribution with *B. parvus* Hg in leachate-contaminated soil. It is advisable to preclude the evaluation of Hg biological distribution using soluble Hg fractions only. Stable Hg fractions in leachate-contaminated soil should also be included for assessing the biological distribution of Hg in leachate-contaminated soils.

THg and MeHg bioaccumulation were explored in the *B. parvus* earthworm of the Hg-containing LLCS. It was found that the average THg concentrations in *B. parvus* and in LLCS were 10.21 and 14.90 times higher than those in the reference sites, respectively, whereas the MeHg levels were similar in LLCS (< 0.01 mg kg^{-1}) and in *B.*

parvus (0.100–0.114 mg kg^{-1}) between contaminated and reference sites. In addition, the average BAF$_{THg}$ of *B. parvus* were similar between the LLCS and the reference sites. *B. parvus* showed a high ability to accumulate THg and MeHg in both leachate-contaminated and reference forest soils. It was proposed that Hg exposure to food chains is possible through *B. parvus*. However, the role of *B. parvus* in MeHg production is not clear, and it is possible that the MeHg in *B. parvus* was firstly formed within forest soils and then accumulated in their tissues.

The effects of Hg washing removal using ss-Na$_2$S for Hg-containing LLCS was to be explore through contrasting the change of soil mercuric fractions and leaching THg from Hg-containing LLCS before and after washing treatment of saturated Na$_2$S solution. From an overall perspective, the exchangeable Hg (F1/Hg-w, F2/Hg-h) and F5/Hg-s to be removed by the treatment of ss-Na$_2$S in Hg-containing LLCS are significantly. Although the averaged THg concentration of LLCS reduced by 22.02% only (from 1.8266 mg kg^{-1} down to 1.4244 mg kg^{-1}). But even so, the concentration composition of some Hg fractions in LLCS have changed before and after ss-Na$_2$S treatment significantly, including the average concentration of F1/Hg-w was reduced by 96.15%, F2/Hg-h was reduced by 95.58%, F3/Hg-o was reduced by 23.85%, F4/Hg-e was reduced by 5.92%, F5/Hg-s was reduced by 99.22%, respectively. Considering the exchangeable Hg fraction (Hg-w, Hg-h) were the high mobility and potential bioavailability than other Hg fraction in soil, for reducing or eliminating the Hg potential environmental risks of leaching Hg from LLCS according to the soil safety guide of environmental Hg reference standard. The ss-Na$_2$S ex situ treatment may be developed a cost-effective Hg immobilization and washing process for removing or reducing exchangeable Hg fraction (Hg-w, Hg-h) and non-mobile Hg fraction (Hg-s) from Hg-containing LLCS, simultaneously.

The data obtained in this field investigation combined laboratory study give more information on mercuric fractionation, spatial distribution, bioaccumulation for understanding the roles of native *B. parvus* earthworm species in both soil Hg and tissues Hg in LLCS, besides the washing removal effects of soil Hg fractions using ss-Na$_2$S for Hg-containing LLCS were conducted in experiments. However, considering the relative narrow concentration range of soil T-Hg, Hg contaminated matrices, washing duration time in selected LLCS and limitations of the extraction procedures of soil Hg. Moreover, the possibility of an associated bacterial community for Hg methylation in *B. parvus* and LLCS. The uncertainties might exist and thus any definite conclusions may be premature or/and not to be widely used in different tapes of contaminated sites at this stage. Therefore, the applicability of our findings might be limited under field conditions (i.e., waste tape and composition, seasonal weather variation, age, precipitation) and different numerous coexisting pollutants (e.g., heavy metal, organic and inorganic matters) may interact with physics and chemistry each other in leachate-contaminated soils because of the traditional landfill included the complex MSW dumping. There are multiple domains of research deserving further studies.

More LLCS samples representing typical natural environments and ecosystems and quantitative data on the soil Hg-fraction or species, spatial distribution, bioaccumulation and sources of Hg-containing MSW are still necessary to sum up or corroborate the hypotheses and rigorously test the early findings hereinbefore. Multidisciplinary study approaches including laboratory simulated experiments (e.g., soil physics and chemistry, microbiology, contents of Hg contaminated matrices and their species identification) are required to examine the potential influencing factors and simulate native Hg bioaccumulation modelling quantitatively, for better understanding the potential risks or the secondary Hg poisoning of soil Hg to organisms in terrestrial ecosystems, and associated bacterial community for Hg methylation synthetically, under considering a great variety of Hg environmental chemistry, such as long-term stability, mobility, transformation of different soil-Hg fractions, and natural factors in LLCS.

The transport of Hg via leachates has the potential biogeochemical transformations with the age of the landfill (e.g., production and emissions of Hg^0, Hg chelation and mineral precipitation/dissolution, Hg methylation and demethylation). In order to accurately assess and develop the washing process using saturated Na_2S ex situ treatment to remove or reduce leaching Hg from Hg-containing LLCS. More fundamental work and laboratory experiments are needed to elucidate on the effects of washing remediation using saturated Na_2S solution for Hg-containing LLCS through considering the biogeochemical transformation of soil Hg and their species or/and fraction concentrations within the traditional landfill at the different age/stages of maturation during its different lifetime. In addition, the information on the optimum ratio of solid/liquid used saturated Na_2S for washing removal the exchangeable Hg (Hg-w, Hg-h) must be adjusted and controlled based on soil-Hg fraction of the different tapes of LLCS. These knowledges would help better understanding what affects and avoiding the additional contaminated source by the residual Na_2S in Hg washing remediated soil.

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APPENDIX

1. Abbreviations

ANOVA	Analysis of variance
aq	Aqueous phase
BAF	Bioaccumulation factors
BrCl	Bromine monochloride
C/N	Total organic carbon / Total nitrogen
CRM	Certified reference materials
CV	Coefficient of variation
EC	Electrical conductivity
F1/Hg-w	Fraction of water soluble mercury
F2/Hg-h	Fraction of human stomach acid soluble mercury
F3/ Hg-o	Fraction of organo-chelated mercury
F4/Hg-e	Fraction of elemental mercury
F5/Hg-s	Fraction of sulfide mercury
FAO	Food and agriculture organization
GC-ECD	Electron capture detector gas chromatography
$HgCl_2$	Mercury chloride
HgS	Mercury sulfide
HNO_3	Nitric acid
L-Cys	L-Cysteines
LLCS	Landfill leachate contaminated soil
LOD	Limit of detection
LOI	Loss on ignition
LOQ	Limit of quantitation
MeHg	Methylmercury
MIBK	Methyl isobutyl ketone

MSW	Municipal solid waste
NaOAc	Sodium acetate trihydrate, $CH_3COONa \cdot 3H_2O$
NaOH	Sodium hydroxide
NMIJ	National metrology institute of Japan
p	Significance probability
PEC	Probable effect concentration
PET	Polyethylene terephthalate
PP	Polypropylene
PSD	Particle size distribution
QA	Quality assurance
QC	Quality control
r	Correlation coefficients
s	Solid phase
SD	Standard deviation
SOM	soil organic materials
SPSS	Statistical product and service solutions
SRB	Sulfur-reducing bacteria
SSL	Soil screening levels
ss-Na_2S	Saturated sodium sulfide
THg	Total mercury
TN	Total nitrogen
TOC	Total organic carbon
UNEP	United nations environment program
USEPA	United states environmental protection soils
WHO	World health organization
XRD	X-ray diffraction

2. Curriculum Vitae

HE Changhua

● Education

▲ 09/2001–07/2006 Hainan Medical College of China, **Bachelor** of Public Health

▲ 09/2009–07/2012 Huazhong University of Science & Technology of China, Tongji Medical College. **Master** of Public Health

▲ 10/2015–10/2018 Prefectural University of Kumamoto of Japan. Environmental & Symbiotic Sciences, **Ph.D.** Course of Environmental & Symbiotic Sciences.

● Further Study

▲07-09/2013 Visiting scholar at Prefectural University of Kumamoto of Japan, as an assistant Researcher.

(Major in DNA Microarray technology)

▲02-03/2012 Department of Pathogen Biology, Second Military Medical University, Shanghai 200433, China.

(Major in Molecular Biology)

▲08-10/2011 Microbiology and Epidemiology of Military Medical Institute, Beijing, China.

(Major in Biological Vector Control)

▲07-10/2010 Medical English Training Program of Hainan medical college, China.

● Career Experiences

▲07-08/2006 Center for disease control and prevention, Dongfang city, Hainan province, China. Work for infection control.

▲09/2006-2018 Hainan Provincial Center for disease control and prevention, China. Work for Tropical Disease and Biological Vector control.

● **Research Experiences**

▲2012 One of the authors the monograph "The questions and answers to biological vector control in Hainan Province".

▲2011 One of the inventors of the utility model patent "a box type mousetrap".

▲2009-2010 Hainan Provincial Scientific Research Grant (Grant no. 309074).

Title: Analysis of the malaria related knowledge, attitude and practice (KAP), the serological status and the evaluation of intervention effect of population working in mountains of the malaria endemic areas, Hainan, China.

▲2006-2009 The 5th round the Global Fund for Malaria in Hainan Province.

The project assistant, officials. Work for project related applications, management, and data analysis evaluation and so on.

▲2008 Design and conduct the G6PD screening plan for the malaria patients, this study supported by the ministry of health, China.

▲ One of the research members for Hainan Provincial Scientific Research Grant (No. 813251, 30873 and 808226).

▲2006 Analysis on factors influencing of the concentrations Cadmium in Tree Vegetable of Wuzhishan. To survey the Cd concentration in tree vegetable of Wuzhishan and environmental influencing factors (soils and water).

● **Publication of original articles during Ph.D. course (Since Oct. 2015)**

[1] Changhua He, Koji Arizono, Hezhe Ji, Yuka Yakushiji, Daizhou Zhang, Kuangwei Huang, Yasuhiro Ishibashi (2018). Comparison of mercury and methylmercury bioaccumulation in earthworms (*Bimastus parvus*) native to landfill-leachate-contaminated forest soil. ***The Joural of Toxicological Sciences, vol.43, No. 7，459-471.***

[2] Changhua He, Koji Arizono, Hezhe Ji, Yuka Yakushiji, Daizhou Zhang, Kuangwei Huang, Yasuhiro Ishibashi (2018). Spatial distribution characteristics of mercury in the soils and native earthworms (Bimastos parvus) of the leachate-contaminated zone around a traditional landfill. ***Science of The Total Environment, 636, 1565-1576.***

● **Previous publication of original articles (Before Oct. 2015)**

[1] Changhua He, WANG Shanqin, HU Ximin, et al. (2014). Eliminating Plasmodium falciparum in Hainan, China: a study on the use of behavioral change communication intervention to promote malaria prevention in mountain worker populations. ***Malaria journal, 13, 273.***

[2] Changhua He, WANG Shanqing, HU Ximin, et al. (2014). Knowledge, attitudes, and practices toward malaria antibody-positive risk among population working on the mountains: A case-control study [J]. ***Chinese Journal of Public Health, Vol 30, No 1.***

[3] Changhua He, ZHAO Wei, WANG Shanqing, et al. (2014). Analysis of mosquito density and its seasonal fluctuation in urban areas of China Hainan province in 2012 [J]. ***Chin J Vector Biol & Control, Vol 25, No 1:12-14***

[4] Changhua He, LI Haishan, WANG Shanqing, et al. (2013). Malaria-related condition among township medical worker in Ledong County, Hainan province [J]. ***Journal of Public Health and Preventive Medicine, 24(5): 71-72.***

[5] LI Yong, HE Xufang, ZHAO Wei, **Changhua He (**2013). Epidemiological characteristics of 368 cases of paederus dermatitis in Hainan Province [J]. ***China Tropical Medicine, 13(07): 912-913.***

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[13] YANG Bin, HUANG Yan, CHENG Xiuyu, **HE Changhua (**2006). Evaluation of the dietary structure and nutritional status in kindergartens of Haikou City [J]. ***China Tropical Medicine, 6(10): 1802-1805.***

● Workshop and Conference Presentations During Ph.D. coure (Since Oct. 2015)

[1] The 27th Symposium on Environmental Chemistry, **Oral + Poster** (Okinawa; May 21-25, 2018).

[2] The 45th Annual Meeting of the Japanese Society of Toxicology, **Oral + Poster** (Osaka; July 18-20, 2018).

[3] 2017 Taiwan-Japan International Symposium on Environmental Science, Technology and Management (TJIS), Hg characterizations of mobilization and distribution in soil from leachate infiltration zone of an old MSW landfill for native earthworms (Bimastos parvus), **Oral,** Sep. 10-13, 2017.

[4] Shimabara Joint Training for National Universities in Kyushu Area, Mercury Elution Characteristics and Influence of Soil from MSW Landfill Vicinity, **Poster,** Nov. 26-27, 2016.

[5] 2016 Taiwan-Japan International Symposium on Environmental Science, Mercury

Elution Characteristics and Influence of Soil from MSW Landfill Vicinity, **Oral,** Technology and Management (TJIS) , Sep. 25-29, 2016.

[6] Nagasaki Sankyo YuKi Co.,Ltd. Sanpozan landfill. Nagasaki, **Oral**, December, 2017.