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Investigation of biomethylation of arsenic and tellurium during composting

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ABSTRACT

Though the process of composting features a high microbiological activity, its potential to methylate metals and metalloids has been little investigated so far in spite of the high impact of this process on metal(loid) toxicity and mobility. Here, we studied the biotransformation of arsenic, tellurium, antimony, tin and germanium during composting. Time resolved investigation revealed a highly dynamic process during self-heated composting with markedly differing time patterns for arsenic and tellurium species. Extraordinary high concentrations of up to 150 mg kg^{-1} methylated arsenic species as well as conversion rates up to 50% for arsenic and 5% for tellurium were observed. In contrast, little to no conversion was observed for antimony, tin and germanium. In addition to experiments with metal(loid) salts, composting of arsenic hyperaccumulating ferns *Pteris vittata* and *P. cretica* grown on As-amended soils was studied. Arsenic accumulated in the fronds was efficiently methylated resulting in up to 8 mg kg^{-1} methylated arsenic species. Overall, these studies indicate that metal(loid)s can undergo intensive biomethylation during composting. Due to the high mobility of methylated species this process needs to be considered in organic waste treatment of metal(loid) contaminated waste materials.

1. Introduction

Biomethylation of metals and metalloids is a widespread and relevant process in the environment, as methylated compounds show a markedly differing mobility, bioavailability and toxicity in comparison to their inorganic precursors [1]. The stepwise methylation results in both non-volatile partly methylated species as well as fully methylated metal(loid) species, which are volatile and can therefore be detected in the gas phase. In the environment, microorganisms are the most important actors in biotransforming metal(loid)s. In general, biomethylation of metal(oid)s takes place in habitats where both microbial activity and bioavailable metal(loid)s are present. Both prerequisites are found in biological waste treatment, where microbiological activity is intentionally optimized for rapid conversion of organic waste materials. Indeed, intensive biomethylation was found in waste deposits [2–5] as well as anaerobic waste treatment [6,7].

In contrast to anaerobic waste treatment, the formation of organometal(loid) compounds during the aerobic composting process has been investigated more recently. Koesters et al. identified several arsenic, tin, antimony and tellurium species in composted organic waste amended with contaminated soil [8]. In a screening study of 34 industrial composting plants, maximal concentrations of methylated arsenic compounds in the mid μg kg^{-1} range and of methylated Sn and Sb species in the low μg kg^{-1} range were reported [9]. Composting of source-separated organic household waste showed a rapid increase of methylated metal(loid)s during the hot phase of composting and a subsequent decrease during the maturation phase. Furthermore, volatile arsenic, selenium, tin, antimony, tellurium, lead and bismuth species were detected during composting duck manure and with exception of lead, composting of source-separated organic household waste [9,10].

Composting is a complex and highly dynamic process characterized by a rapid succession of microbial communities [11]. While in the initial phase the composition of the bacterial community is determined by the bacteria present in the input material, after self-heating to temperatures above 60 °C thermophilic bacterial communities dominate during the hot phase of composting. After decline of the temperatures in the subsequent maturation phase, mesophilic bacteria as well as fungi dominate [11].

The aim of this study was to investigate the formation of methylated metal(loid) species under well-defined conditions in order to gain control over the most important factors, such as the input material, aeration and agitation. In order to study the time-resolved formation of methylated metal(loid) species during self-heating, hot phase and subsequent maturation, a laboratory-

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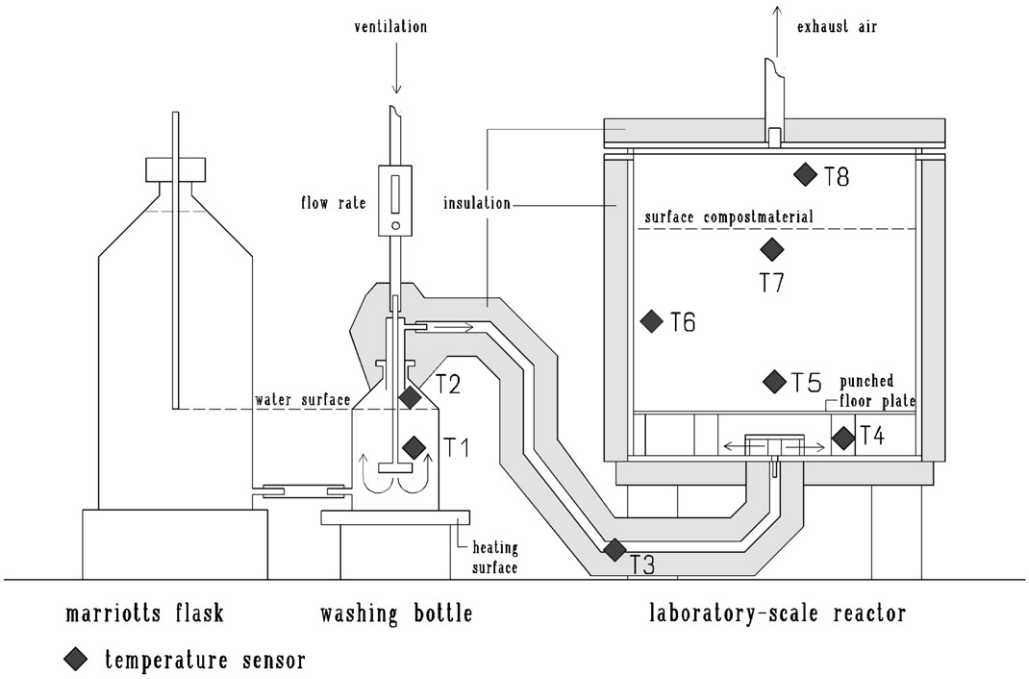

Fig. 1. Setup of the laboratory-scale reactor (LSR) system used for time-resolved investigation of biomethylation during composting.

scale reactor (LSR) was developed. Furthermore, we investigated the influence of metal(loid) concentration on the formation of methylated metal(loid) species during composting as well as the cross-influence between different metal(loid)s. In addition to experiments with metal(loid) salts, we studied the biomethylation of arsenic during composting of arsenic hyperaccumulating *Pteris vittata* (Chinese brake fern/Ladder brake fern) and *P. cretica* (Cretan brake fern/Table fern). Both ferns are cosmopolitan and found in tropic and subtropic areas worldwide and have been reported to reach arsenic concentrations in their aboveground biomass up to the g kg^{-1} range [12,13]. As arsenic accumulating ferns are endemic in arsenic-contaminated sites and their use for remediation of As-contaminated sites has been proposed [14], the availability of arsenic accumulated in fern material needed to be studied.

2. Experimental

2.1. Reagents and standards

All reagents were of analytical grade or better. Sodium tetrahydroborate (purity > 99%) was obtained by Acros Organics (Geel, Belgium). Trace analysis grade hydrochloric acid was used from Fisher Scientific (Schwerte, Germany). Water of 18.2 MΩ quality was prepared using a Seralpur Pro 90 CN system (Elga Berkefeld GmbH, Celle, Germany). Trisodium citrate dihydrate from Merck (Darmstadt, Germany), citric acid from Fluka (St. Gallen, Switzerland) and sodium hydroxide from Carl Roth GmbH (Karlsruhe, Germany) were used.

Organometal(loid) standards used for speciation analysis were reported in Ref. [15]. ICP-MS standards for As, Rh, Sb, Sn, Sc and Y were obtained by Merck (Darmstadt, Germany), others by Bernd Kraft GmbH (Duisburg, Germany). Nitric acid (p.a., JT Baker, Deventer, Netherlands) was subboiled before use. Argon of 99.996% purity and Helium of 99.999% purity (both Air Liquide, France) were used for ICP-MS and GC, respectively.

For metal(loid) amendment of composting experiments the following compounds were used: sodium hydrogen arsenate(V) (98.5+%); Na-(meta) arsenite(III) (99%); telluric(VI) acid (99+%): Fluka (St. Gallen, Switzerland); germanium(IV) oxide (99.999%): ABCR (Karlsruhe, Germany); tin(II) oxalate (98%): Lancaster Synthesis (Mühlheim am Main, Germany); potassium antimony(V) oxide tartrate (p.a.): Merck (Darmstadt, Germany).

2.2. Time-resolved analysis of metal(loid) methylation during composting

In order to allow multiple sampling for time-resolved analysis of metal(loid) methylation, a laboratory-scale reactor (LSR) was developed (Fig. 1). Height and diameter of the acrylic glass cylinder were 50 cm with an inner volume of 98 L. For minimization of heterogeneity within the compost heap, the LSR was pressure aerated bottom-up using humidified air. An aeration rate of 1.28 L min^{-1} resp. 0.14 L min^{-1} kg_{DW}^{-1} (dry weight) was chosen, which is in the lower range of the aeration rates applied in full-scale composting plants (0.06 and 2.3 L min^{-1} kg_{DW}^{-1}), as intensive heat losses by ventilation and through the walls has been reported for composting systems at laboratory-scale and pilot-scale [16]. The walls of the LSR were insulated by 5 cm mineral fibrous material laminated with aluminum. Top and bottom were insulated by 5 cm foamed polystyrene boards. Eight temperature sensors were arranged inside the composting system.

Pressed alfalfa hay (*Medicago sativa*) was used as composting starting material, which is commercially available as horse food with a particle size ranging from 1 mm × 2 mm to 2 mm × 7 mm (Scheiper's Mühle, Dortmund). Dry weight, ignition loss and C/N ratio (determined by Elementar Vario Max V 4.5) of this material were determined as 91.1%, 93.1% and 29, respectively.

For time-resolved analysis of metal(loid) methylation during composting, 10 mg L^{-1} Ge, Sb, Sn and Te as well as 20 mg L^{-1} sodium arsenate were added to 10 L of tap water. Then, 20 g of each starch and glucose were added for improvement of self-heating and the water was thoroughly mixed with 10 kg of dry alfalfa hay. The amended alfalfa hay was filled into the LSR. The LSR was sampled after 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 19, 21, 23, 25, 28, 32, 35, 41, 48 and 56 days. In order to avert stratifications and allow homogenous sampling, the entire compost material was removed,

mixed and refilled. Loss of compost material during refilling was carefully avoided.

2.3. Influence of metal(loid) concentration on biomethylation during composting

For investigation of the influence of metal(loid) concentrations on biomethylation during composting, 250 g dried alfalfa hay cobs were mixed with 250 mL tap water amended with different combinations of metal(loid) salts (see Table 1). In brief, three different composting experiment series were conducted. At first, experiments with As^{III}, Te, Ge, Sn, Sb in concentrations of 0–1000 $\mu g\,L^{-1}$ were conducted. Second, arsenite was amended in concentrations of 5000–100,000 $\mu g\,L^{-1}$. Finally, different combinations of As^{III}, As^{V}, Te, Ge, Sn and Sb were studied for investigation of inter-element cross-influence. Starch and glucose were not used in this experiment series. The damp material was then filled into 2 L temperature isolated vessels (TIV) with an inner diameter of 10 cm and a height of 29 cm. A 3 cm bottom layer of beech shavings was used to separate the seepage water from the substrate. The temperature was monitored online with a sensor placed in the centre of the vessel. The compost material was sampled after the hot phase at day 14 (multielement and arsenic concentration row) resp. day 17 (interelement cross-influence). In case of the multielement and arsenic concentration rows, the dry top layer of the compost material was removed and the underlying damp compost material was mixed and subsequently sampled. In case of the experiments studying the interelement cross-influence, the entire material was sampled.

2.4. Biomethylation of As during composting of P. vittata and P. cretica

Forty *P. cretica* and six *P. vittata* plants with an age of approximately 2.5 years were replanted to each 1 kg of potting soil amended with concentrations of arsenate (*P. cretica* 0, 250, 500, 1000 and 1500 mg kg_{DW}^{-1}, each eight plants, *P. vittata* 1000 mg kg_{DW}^{-1} six plants). The ferns were grown under optimal green house conditions (8–12 °C by night, 21 °C at day, 80% relative humidity, partially shaded). After 4, 8 resp. 12 weeks each 2, 3 resp. 3 *P. cretica* plants were sampled. All 6 *P. vittata* plants were sampled after 12 weeks. After removal of the soil material, roots, shoots and fronds were analyzed separately for total arsenic concentration.

For composting experiments, 50 g (wet weight) frond material of *P. cretica* and *P. vittata* collected after 12 weeks growth on soil amended with 1000 mg As kg^{-1} as well as 50 g of frond material of *P. cretica* grown without As were used. The frond material was mixed with 200 g alfalfa hay, humidified with 250 mL tap water and composted in TIV as described above. Total arsenic concentrations of the *P. vittata* resp. *P. cretica* material used for composting can be consulted in Table 2. After 14 and 21 days composting, the entire material was removed from the TIV, mixed, sampled and refilled again to the TIV.

2.5. Total metal analysis

All samples were first cryomilled using the Freezer Mill 6850 (Spex CertiPrep, Metuchen, USA) and stored at −20 °C until analysis. For the analysis of *aqua regia* extractable metal content, 0.2–0.5 g of the sample material was digested with 6 mL HNO_3 and 2 mL HCl at a temperature of 180 °C for 20 min in a Mars 5 microwave digester (CEM Corporation). The extracts were diluted with deionised water to 100 mL and 10 $\mu g\,L^{-1}$ Ga, Y, and Tl were added for internal standardization. Arsenic analysis by ICP-MS was performed using He collision cell mode (ICP-MS 7500ce, Agilent Technologies).

Table 1
Influence of metal(loid) concentrations on biomethylation of arsenic and tellurium during composting. 250 mL water amended with different combinations of metal(loid) salts was added to 250 g alfalfa hay. Composting was performed in temperature isolated vessels. Values are means of triplicates ± SD. <LOD: below limit of detection.

Metal(loid)s amended	Composting time (day)	Dry mass (%)	Ignition loss (%)	As total content ($\mu g\,kg_{DW}^{-1}$)	MMAs ($\mu g\,kg_{DW}^{-1}$)	DMAs ($\mu g\,kg_{DW}^{-1}$)	TMAs ($\mu g\,kg_{DW}^{-1}$)	Relative fraction methylated species	Te total content ($\mu g\,kg_{DW}^{-1}$)	DMTe ($\mu g\,kg_{DW}^{-1}$)	Relative fraction methylated species
Multielement concentration row 0–1000 $\mu g\,L^{-1}$											
As^{III}, Te, Ge, Sn, Sb 0 $\mu g\,L^{-1}$	14	49%	88%	302 ± 19	0.5 ± 0.04	5 ± 0.0	<LOD	1.7%	18 ± 13	0.004 ± 0.001	0.02%
As^{III}, Te, Ge, Sn, Sb 10 $\mu g\,L^{-1}$	14	40%	88%	437 ± 38	0.6 ± 0.2	12 ± 0.2	7 ± 5	4.5%	77 ± 34	0.14 ± 0.02	0.18%
As^{III}, Te, Ge, Sn, Sb 100 $\mu g\,L^{-1}$	14	41%	88%	809 ± 29	0.9 ± 0.04	48 ± 0.0	38 ± 1	10.8%	307 ± 37	0.58 ± 0.01	0.19%
As^{III}, Te, Ge, Sn, Sb 500 $\mu g\,L^{-1}$	14	34%	88%	2042 ± 44	8.0 ± 3.2	554 ± 3.2	80 ± 20	31.4%	1674 ± 148	8.30 ± 1.12	0.50%
As^{III}, Te, Ge, Sn, Sb 1,000 $\mu g\,L^{-1}$	14	26%	89%	4691 ± 114	18.1 ± 0.5	1872 ± 0.5	658 ± 74	54.3%	4044 ± 251	25.93 ± 3.28	0.64%
Arsenic concentration row 5000–100,000 $\mu g\,L^{-1}$											
As^{III} 5000 $\mu g\,L^{-1}$	14	18%	90%	35,105 ± 393	71.4 ± 65.5	914 ± 65.5	14,130 ± 11,071	43.1%	39 ± 33	0.11 ± 0.17	0.28%
As^{III} 7500 $\mu g\,L^{-1}$	14	20%	88%	46,147 ± 498	21.7 ± 13.2	234 ± 13.2	9357 ± 2395	20.8%	17 ± 8	0.15 ± 0.25	0.87%
As^{III} 25,000 $\mu g\,L^{-1}$	14	18%	86%	133,210 ± 1452	132.5 ± 22.6	2986 ± 22.6	52,254 ± 2932	41.6%	14 ± 2	0.06 ± 0.06	0.39%
As^{III} 50,000 $\mu g\,L^{-1}$	14	21%	89%	216,910 ± 2950	71.2 ± 22.0	1794 ± 22.0	78,347 ± 11,047	37.0%	23 ± 17	0.05 ± 0.05	0.22%
As^{III} 75,000 $\mu g\,L^{-1}$	14	21%	89%	442,426 ± 7433	165.9 ± 88.6	6490 ± 88.6	61,590 ± 24,434	15.4%	35 ± 28	0.05 ± 0.05	0.14%
As^{III} 100,000 $\mu g\,L^{-1}$	14	18%	89%	556,716 ± 4231	720.7 ± 30.6	17,873 ± 30.6	137,717 ± 6179	28.1%	28 ± 17	0.11 ± 0.00	0.40%
Interelement cross-influence											
As^{III} 50,000 $\mu g\,L^{-1}$, Te, Ge, Sn, Sb 5,000 $\mu g\,L^{-1}$	17	66%	93%	33,947 ± 160	13.5 ± 0.2	203 ± 0.2	18,920 ± 2007	56.4%	3371 ± 61	124.24 ± 4.33	3.69%
As^{V} 50,000 $\mu g\,L^{-1}$, Te, Ge, Sn, Sb 5000 $\mu g\,L^{-1}$	17	63%	92%	47,926 ± 326	6.7 ± 1.0	60 ± 1.0	20,661 ± 886	43.2%	4237 ± 59	120.99 ± 10.19	2.86%
Te, Ge, Sn, Sb 5000 $\mu g\,L^{-1}$	17	60%	90%	265 ± 38	0.16 ± 0.01	1.1 ± 0.006	2.8 ± 2.6	1.6%	5634 ± 181	13.68 ± 4.69	0.24%
Te 5000 $\mu g\,L^{-1}$	17	61%	92%	205 ± 11	0.19 ± 0.01	1.7 ± 0.010	3.4 ± 1.5	2.6%	4981 ± 118	7.14 ± 0.67	0.14%
Sb 5000 $\mu g\,L^{-1}$	17	66%	93%	272 ± 28	0.23 ± 0.00	0.8 ± 0.004	1.5 ± 0.2	0.9%	12 ± 18	1.10 ± 0.88	9.00%

Table 2
Methylation of arsenic accumulated by *P. vittata* and *P. cretica* grown on arsenic-amended soil during composting. 50 g (wet weight) of fronds of the ferns grown on arsenic-amended soil were mixed with 200 g of alfalfa hay and composted in temperature isolated vessels. Values are means of triplicates ± SD. na: not analyzed.

	Composting time (day)	Dry mass (%)	As total content ($\mu g\,kg_{DW}^{-1}$)	MMAs ($\mu g\,kg_{DW}^{-1}$)	DMAs ($\mu g\,kg_{DW}^{-1}$)	TMAs ($\mu g\,kg_{DW}^{-1}$)	Relative fraction methylated species
Frond material used for composting							
P. cretica 0 $mg_{As}\,kg_{soil}^{-1}$	–	28%	2315 ± 658	na	na	na	
P. cretica 1000 $mg_{As}\,kg_{soil}^{-1}$	–	28%	3,147,721 ± 424,182	30.3 ± 4.2	9.3 ± 2.5	14.4 ± 2.7	0.002%
P. vittata 1000 $mg_{As}\,kg_{soil}^{-1}$	–	43%	3,500,100 ± 19,250	na	na	na	
Compost amended with							
P. cretica 0 $mg_{As}\,kg_{soil}^{-1}$	14	58%	626 ± 343	1.0 ± 0.2	5.6 ± 2.2	4.1 ± 2.9	1.7%
P. cretica 0 $mg_{As}\,kg_{soil}^{-1}$	21	66%	717 ± 212	0.8 ± 0.3	4.6 ± 1.2	2.2 ± 0.7	1.1%
P. cretica 1000 $mg_{As}\,kg_{soil}^{-1}$	14	54%	145,351 ± 7108	33.7 ± 6.4	165.5 ± 37.2	6961.8 ± 1701.3	4.9%
P. cretica 1000 $mg_{As}\,kg_{soil}^{-1}$	21	57%	218,511 ± 7298	93.3 ± 11.9	284.9 ± 40.0	8341.2 ± 1084.8	4.0%
P. vittata 1000 $mg_{As}\,kg_{soil}^{-1}$	14	58%	276,599 ± 13,166	29.8 ± 5.0	145.8 ± 34.6	6078.5 ± 1579.8	2.3%
P. vittata 1000 $mg_{As}\,kg_{soil}^{-1}$	21	57%	399,443 ± 4913	1122.0 ± 69.5	251.2 ± 21.9	268.3 ± 16.8	0.4%

Blank levels as well as sensitivity were controlled every ten analyses in order to correct for drifts in instrument background and sensitivity. Recovery of arsenic standard addition to the microwave digest of five different *P. cretica* samples ranged between 97.6 and 99.4%.

2.6. Speciation analysis of methylated metal(loid) compounds by pH-gradient-HG-GC–ICP-MS

For analysis of non-volatile methylated metal(loid)s, a recently developed GC–ICP-MS method was applied using pH-gradient hydride generation [15]. In brief, derivatisation was conducted in 100 mL four-neck round-bottomed flasks sealed with screw caps with PTFE lids (Bohlender GmbH, Grünsfeld, Germany). 0.2–1 g (wet weight) of cyromilled compost sample was added to 40 mL citric acid/citrate buffer and purged by 300 ml He min^{-1} for 120 s for oxygen removal. Then, 10 mL 1 M $NaBH_4$ and 10 mL 2 M HCl were continuously added via automated piston pumps with ceramic head (REGLO-CPF Ismatec, Wertheim, Germany) within 420 s. The volatile derivatives were continuously purged out of the reaction solution by 300 ml He min^{-1} and cryofocussed on a column (ID: 4 mm, length: 40 cm) packed with 1.15 g of 10% SP-2100 on 80/100 mesh Supelcoport (Sigma–Aldrich) immersed in liquid nitrogen. After derivatisation, the purge flow is maintained for additional 2 min. Then, the column was gradually heated to 180 °C within 600 s for separation of the volatile hydrides according to their boiling point.

For multielement detection of organometal(loid) species both 7500a and 7500ce ICP-MS (Agilent, Yokohama, Japan) were used. Operating parameters for the ICP-MS can be consulted in Ref. [15]. The transfer line to ICP-MS consisted of 80 cm of 1/16 in. fluorinated ethylene propylene (FEP) tubing heated to 120 °C using a resistance wire. The GC-efflux was mixed to the efflux of the spray chamber by using a T-piece inserted between spray chamber and torch. The wet aerosol from the liquid sample introduction system is used for introduction of a continuous internal standard solution (^{71}Ga and ^{205}Tl, 10 $\mu g\,L^{-1}$ each and ^{113}In, 100 $\mu g\,L^{-1}$) and for post-column quantification using interaggregate calibration (IAC) as described by Feldmann [17]. In short, this method uses the mass flow of inorganic aqueous standards introduced via the nebulizer for species-independent post-column quantification of volatile element species. Peak integration was carried out using Microcal Origin® 5.0 with manual correction of the baseline setting. For further data processing Microsoft Excel® was used.

Blank concentrations were determined threefold at the beginning of each day. Detection limits for methylated species were between 1 and 40 pg depending on the blank level in the derivatisation reagent [15]. Analysis was conducted as triplicates. Recoveries of methylated organometal(loid) standards were determined for the compost sample amended with 50 mg kg^{-1} arsenite (see Table 1) and were found in the range of 96–102% [15]. Species identification was validated using GC–MS [8].

The hydride generation method applied allows only the differentiation between different degrees of methylation, but not different redox states or oxo- and thioforms of arsenic. Therefore, the species are referred as mono-, di- resp. trimethylarsenic (MMAs, DMAs resp. TMAs) throughout this manuscript.

3. Results and discussion

3.1. Time-resolved analysis of metal(loid) methylation during composting

By use of a self-developed laboratory-scale reactor (LSR), the time-resolved formation of methylated metal(loid) species during composting was studied (Fig. 2). For these experiments alfalfa hay was used as composting material in order to ensure reproducible composting conditions and to improve homogeneity of the compost material. Furthermore, metal(loid) levels in compost from non-amended alfalfa hay was relatively low (Table 1), thereby allowing the study of low-level metal(loid) amendment.

The LSR showed good self-heating within three days with maximal temperatures up to 76 °C. After 17 days hot composting phase, temperatures declined and remained constant at a level of 30 °C until the end of the experiments. The pH curve showed a typical initial acidification due to the formation of fatty acids and subsequent shift to slightly basic conditions. Ignition loss as well as C/N ratio decreased due to respiration of organic carbon, which indicates a satisfying maturation of the organic material. Likewise, total metal(loid) contents increased by a factor of 2–2.5 (see supplemental information).

Analysis of methylated species revealed a complex time-pattern of metal(loid) methylation during composting (Fig. 2a). Until the beginning of the hot phase, concentrations of methylated species remained on a low level. During the hot phase, which is dominated by thermophilic bacteria, first MMAs, then DMAs levels rose from low $\mu g\,kg_{DW}^{-1}$ to maximum concentrations of 150 resp. 3800 $\mu g\,kg_{DW}^{-1}$. Towards the end of the hot phase, both MMAs and DMAs decreased while TMAs concentrations increased up to 5100 $\mu g\,kg_{DW}^{-1}$. In contrast to arsenic, concentrations of methylated tellurium species remained on a very low level throughout the hot composting phase. Towards the end of the experiment DMTe levels rose up to 210 $\mu g\,kg_{DW}^{-1}$. Accordingly, a garlic odor typical for DMTe was noticeable towards the end of the experiment.

The observation of consecutive maxima of methylated arsenicals indicates towards one the one hand a stepwise methylation of arsenic and on the other hand an involvement of different microbial groups in methylation of arsenic. In case of tellurium, an involve-

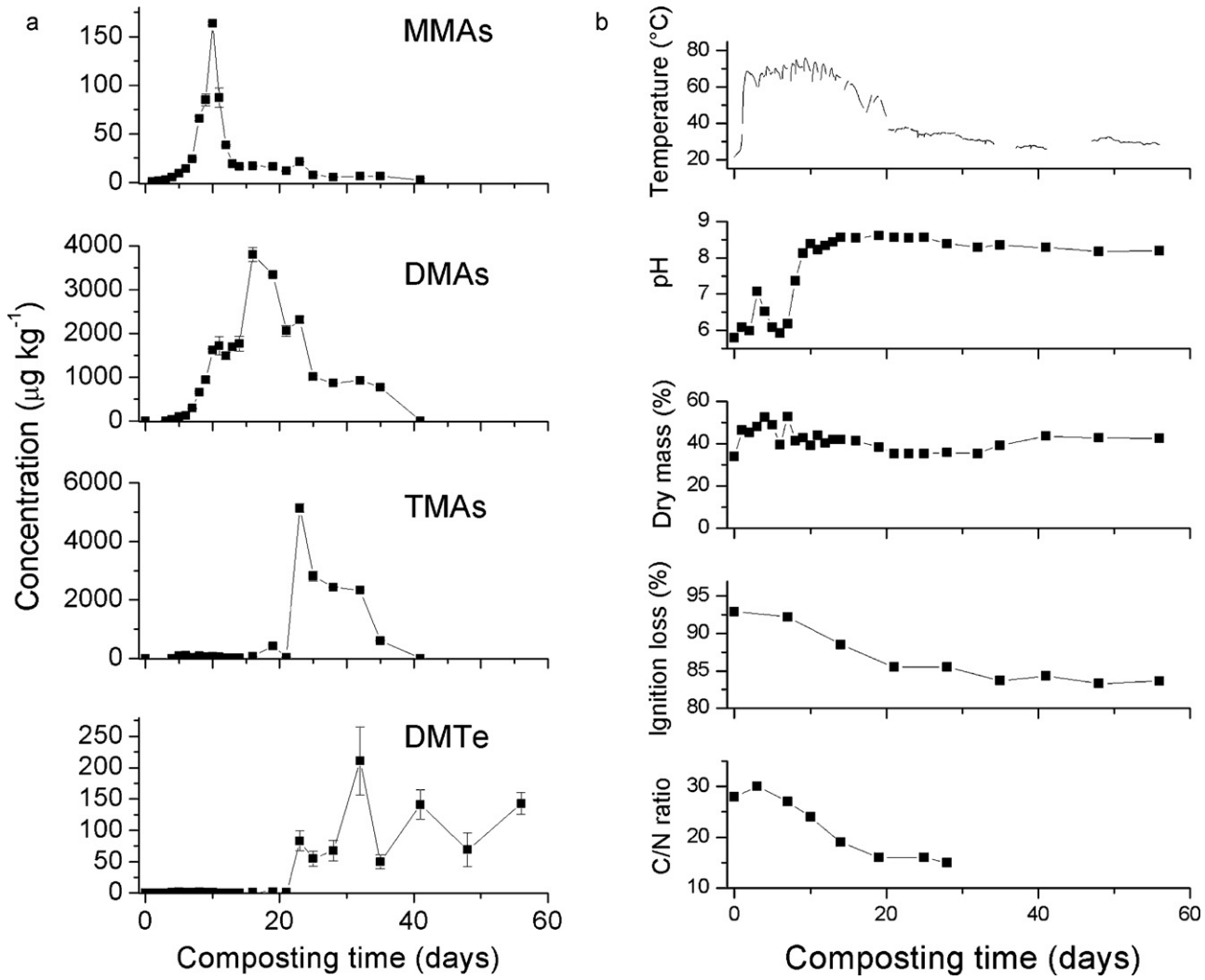

Fig. 2. (a) Time-resolved investigation of organometal(loid) species during 56 days of composting in a laboratory-scale reactor system. Values are means of triplicates ± SD. (b) Temperature [°C] at sensor T7, ignition loss [%], dry matter content [%], pH and C/N ratio during 56 days of composting in LSR. 10 mg L^{-1} Ge, Sb, Sn and Te as well as 20 mg L^{-1} sodium arsenate dissolved in 10 L of tap water were added to 10 kg of dry alfalfa hay and composted in the system shown in Fig. 1. Note that the temperature data between days 35 and 38 as well 43 and 48 was lost due to computer failure.

ment of mesophilic fungal species is plausible, as fungi mycelia were observable in the compost material after the hot phase and the ability to methylate tellurium has been shown for a number of fungal species [18], among them *Scopulariopsis brevicaulis*, which has been isolated during the maturation phase of composting [11].

3.2. Influence of metal(loid) concentrations on biomethylation during composting

Composting experiments with different concentrations as well as combinations of metal(loid)s were conducted using temperature insulated vessels (TIV) (Table 1). The metal(loid)s were added to humidified alfalfa hay, which showed intensive self-heating similar to the LSR with maximum temperatures between 65 and 70 °C and a 7–10 days hot phase above 60 °C with a subsequent slow decline. The samples were taken after the hot composting phase.

After composting, the material in the temperature insulated vessels showed a stratification with a dry and cooler top and a damp and hotter bottom layer. In case of the concentration rows with multielement as well as arsenic amendment, only the bottom layer was sampled. Total metal(loid) contents showed a significant upconcentration exceeding the levels expected by dry matter loss due to metabolism of organic matter to CO_2. Presumably, water vapor from the bottom layer condensed in the top layer and translocated the metal(loid) amendment to the bottom layer material. In the experiments studying the interelement cross-influence, in which the entire material was sampled, this phenomenon was not observed.

In all experiments, arsenic was readily methylated. Maximum concentrations of methylated arsenic of up to 150 mg kg^{-1} were detected with relative methylation yields up to 50%. The concentration levels as well as methylation rates are among the highest reported for arsenic so far [19]. The efficient methylation is also indicated by the species pattern, trimethylarsine oxide was the most important methylated arsenic species, which is unusual in comparison to other environmental samples, where dimethyl or monomethyl species dominate.

For the background levels of arsenic in alfalfa hay not amended with arsenite, relatively low proportion of methylated species was found (1.7%), indicating that the arsenic found in alfalfa hay is significantly less available in comparison to the arsenite amended. With exception of the lowest levels amended, similar relative methylation yields were found for arsenic amended indicating only little influence on the concentration applied. Likewise, methylation in experiments with arsenite and arsenate were very similar, even though arsenite is significantly more toxic to

bacteria. Similar relative methylation yields were found in experiments with 50,000 $\mu g_{As}\ L^{-1}$ with and without amendment of other metal(loid)s, indicating that arsenic methylation is also not inhibited by these meta(loid)s. In comparison to the arsenic amended as soluble species, the arsenic present in alfalfa hay was metabolized to a significantly lower extent.

For tellurium, low methylation yields were found in experiments amended with tellurium alone (0.14%) or together with Ge, Sn and Sb (0.24%). In the multielement concentration row, methylation yields rose together with the concentration applied up to 0.64%. When composted together with either 50,000 $\mu g_{As}\ L^{-1}$ arsenite or arsenate, up to 120 $\mu g_{Te}\ kg_{DW}^{-1}$ DMTe were detected resulting in a methylation yield of 3 resp. 4.7%. This indicates towards a cross influence of arsenic on biomethylation of tellurium. An enhancement of methylation of one metal(loid) in presence of higher concentrations of another metal(loid) has been previously reported for tellurium methylation enhanced by selenium [20] as well as antimony methylation enhanced by arsenic [21,22].

The induction of tellurium methylation by arsenic hints towards a methylation mechanism for tellurium triggered by arsenic. For arsenic, it has recently been shown that bacteria containing the As^{III}-S-adenosylmethionine (SAM) methyltransferase (*arsM*) gene are able to methylate inorganic arsenic [23]. This gene is organized in the arsenic-resistance (*ars*) operon together with genes encoding for proteins involved in arsenic recognition (*arsR*) and arsenic reduction (*arsC*). While very little experimental evidence for enzymatic mechanism of tellurium exists, a stepwise methylation mechanism similar to arsenic has been proposed [18]. A gene involved in tellurium resistance has been identified, for which a SAM-dependent methyltransferase activity has been proposed [24].

For antimony, significant concentrations of methylated species were detected in non-amended compost ($15.0 \pm 2.4\ \mu g\ kg_{DW}^{-1}$ methylated Sb-species) comprising 19.5% of the total antimony (see supplemental information). These levels are similar to those reported for real compost samples [9]. Surprisingly, concentrations of methylated species did not significantly increase after addition of antimony. This indicates that the antimony salt used (potassium antimony(V) oxide tartrate) was not available for biomethylation, even though the same compound was found available in methylation experiments with pure microbial cultures [22]. Likewise, a similar phenomenon was reported for biotransformation of hexahydroxy-antimonate(V) by intestinal microorganisms [25].

For Ge and Sn, no methylated species were detected in all experiments indicating that the metal(loid) salts used are not or only poorly methylated during composting.

3.3. Biomethylation of arsenic during composting of *P. vittata* and *P. cretica*

For assessment of the availability of arsenic accumulated by *P. vittata* and *P. cretica* to methylation during composting, both ferns were grown on soils amended with arsenic. Arsenic was efficiently taken up by both *P. vittata* and *P. cretica* (see Table 2 and supplemental information). Arsenic concentrations in the fronds of both ferns grown at a soil concentration of 1000 $mg_{As}\ kg^{-1}$ were more than threefold higher than the soil concentration. Arsenic concentrations in the fronds of both plants were significantly higher than the concentration in roots and shoots of the plants (see supplemental information). The relative fraction of methylated species determined for *P. cretica* was negligible (0.002%).

Self-heating in composting experiments with fern material was lower with maximal temperatures of 47 °C. However, significant arsenic methylation was found in compost experiments with both *P. vittata* and *P. cretica* with maximal concentrations of several $mg_{As}\ kg_{DW}^{-1}$ methylated arsenic species. For *P. cretica*, 4–5% of the arsenic was methylated, which is higher than the methylation rate found for non-amended alfalfa hay (1.7%) but tenfold lower than in the experiments with alfalfa hay amended with similar levels of arsenite (Table 1). This indicates towards a limited availability of arsenic in *P. cretica* towards methylation. Methylation rates were lower for *P. vittata*. The decline of the concentration of methylated species after 21 days indicates towards an inhibition due the high arsenic levels. Similar to experiments with arsenic-amended alfalfa hay, TMAs was the dominant species with exception of the 21 days composting of *P. vittata*.

Very recently, Cao et al. [26] studied the arsenic transformation during composting of *P. vittata* collected from a As-contaminated site with a similar total arsenic content as in this study (4589 $mg_{As}\ kg_{DW}^{-1}$). In contrast to the present study, composting was conducted for 120 days in a temperature controlled reactor at a temperature of 45–50 °C. MMAs and DMAs, but not TMAs were analyzed in compost leachate after 1, 4, 8, 16 weeks composting as well as water-soluble arsenic in the final compost product. MMAs and DMAs comprised up to 4% of total arsenic, which is similar to the level found in this study. By using of chemical trapping, an absolute arsenic volatilization of 12 μg_{As} was determined during the composting period, which corresponds to 7 ppm relative to the total content of 1.67 g_{As} per composter.

4. Conclusions

Overall, these studies indicate that metal(loid)s can undergo intensive biomethylation during composting. While both arsenic and tellurium were found to be readily methylated, the factors influencing the formation of methylated selenium, tin, antimony, lead and bismuth species observed in compost from source-separated organic household waste and duck manure [9,10], needs to be further investigated. Furthermore, biotransformation from other metal(loid) contaminated input materials needs to be studied, in particular arsenic bearing sludge generated from an arsenic treatment process. Noteworthy, maximal levels of arsenic in compost materials are regulated in USA ($10\ mg\ kg_{DW}^{-1}$), but not in European countries and the other above mentioned metal(loid)s with exception of lead are not regulated in the USA or Europe [9]. The conversion of inorganic metal(loid)s to methylated species is relevant for biological waste treatment of metal(loid) contaminated materials in several aspects. At first, organometal(loid) species are highly mobile not only under aerobic, but also under anaerobic conditions or even volatile in case of permethylated species. The malodorous exposure in particular to tellurium and selenium species has been discussed [10]. Finally, toxicological aspects of this process need to be considered. As the hydride generation method used in this study allows only the differentiation between different degrees of methylation, but not different redox states or oxo- and thioforms of arsenic, little conclusions on the toxicicological relevance of arsenic methylation can be drawn. However, the detection of dimethyl-methylthio-arsine, $(CH_3)_2AsSCH_3$ in compost samples [8] as well as dimethylmonothioarsinic acid ($DMMTA^V$) and dimethyldithioarsinic acid ($DMDTA^V$) in municipal landfill leachate [27] indicates that highly toxic thiolated species can be formed in organic waste treatment and therefore need to be considered in future studies. Furthermore, volatile trimethylarsine has been reported to be genotoxic [28] while neurotoxic effects have been shown for dimethyltellurium [29]. Therefore, further studies elucidating the involved microbial species and the parameters influencing biomethylation in biological waste treatment are necessary.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2010.12.011.

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