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Risks associated with the transfer of toxic organo-metallic mercury from soils into the terrestrial feed chain

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ABSTRACT

Although the transfer of organo-metallic mercury (OrgHg) in aquatic food webs has long been studied, it has only been recently recognized that there is also accumulation in terrestrial systems. There is still however little information about the exposure of grazing animals to OrgHg from soils and feed as well as on risks of exposure to animal and humans.

In this study we collected 78 soil samples and 40 plant samples (*Lolium perenne* and *Brassica juncea*) from agricultural fields near a contaminated industrial area and evaluated the soil-to-plant transfer of Hg as well as subsequent trophic transfer. Inorganic Hg (IHg) concentrations ranged from 0.080 to 210 mg kg^{-1} d.w. in soils, from 0.010 to 84 mg kg^{-1} d.w. in roots and from 0.020 to 6.9 mg kg^{-1} d.w. in shoots. OrgHg concentrations in soils varied between 0.20 and 130 μg kg^{-1} d.w. representing on average 0.13% of the total Hg (THg). In root and shoot samples OrgHg comprised on average 0.58% (roots) and 0.66% (shoots) of THg. Average bioaccumulation factors (BAFs) for OrgHg in relation to soil concentrations were 3.3 (for roots) and 1.5 (for shoots).

The daily intake (DI) of THg in 33 sampling sites exceeded the acceptable daily intake (ADI) of THg of both cows (ADI = 1.4 mg d^{-1}) and sheep (ADI = 0.28 mg d^{-1}), in view of food safety associated with THg in animal kidneys. Estimated DI of OrgHg for grazing animals were up to 220 μg d^{-1} (for cows) and up to 33 μg d^{-1} (for sheep).

This study suggested that solely monitoring the levels of THg in soils and feed may not allow to adequately taking into account accumulation of OrgHg in feed crops and properly address risks associated with OrgHg exposure for animals and humans. Hence, the inclusion of limits for OrgHg in feed quality and food safety legislation is advised.

1. Introduction

Mercury (Hg) is an extremely toxic pollutant that poses global environmental and human health risks (Selin, 2009). During the last two centuries anthropogenic emissions of Hg, e.g. from chlor-alkali plants (Biester et al., 2002) and coal power stations (Kabata-Pendias, 2001) significantly exceeded the emission/inputs of natural sources (Peralta-Videa et al., 2009) resulting in severe soil contamination (Selin, 2009). Despite such contamination many of these sites are still being used for agriculture and pasture without a proper evaluation of Hg distribution and speciation in soils and without an assessment of risks to animals and humans. To assess such risks, it is imperative to consider the speciation of Hg, both in soils and in crops.

In the terrestrial and aquatic environment Hg occurs in both organic and inorganic forms (Challa et al., 2008), including elemental mercury (Hg^0), mercurous (Hg^+) and mercuric (Hg^{2+}) salts (Kabata-Pendias, 2001), cinnabar (HgS) and organo-metallic compounds, such as methylmercury(II) ($MeHg^+$) and ethylmercury(II) ($EtHg^+$) (Fernández-Martínez et al., 2005). The most common Hg mineral is HgS which is quite insoluble rendering this species rather unavailable for transport or plant uptake, at least in the short term (Rodrigues et al., 2010). Mercury in soil usually is strongly adsorbed onto organic matter and/or metal oxides which generally results in a large pool of mercury being relatively immobile (Kabata-Pendias, 2001). Inorganic mercury (IHg) species such as mercuric chloride ($HgCl_2$) are only weakly sorbed to soil components (Han et al., 2003) and can also be subject to mercury methylation processes resulting in highly mobile organic mercury species including methylmercury (MeHg) one of the most toxic organo-metallic forms of Hg (Han et al., 2003; Selin, 2009). Organo-metallic mercury (OrgHg) compounds are more toxic than IHg salts due to their ability to form water-soluble complexes with cysteine in body tissues (Clarkson and Magos, 2006) which increases the potential for uptake and accumulation by organisms (Fernández-Martínez et al., 2005).

Exposure to OrgHg mercury is of great concern due to its potential impact to the central nervous system that affects human and wildlife development and health (Mailman and Bodaly, 2005; Meng et al., 2011). For grazing animals, environmental exposure can also result in

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accumulation of OrgHg in kidneys of cows and sheep as well as in the transfer of OrgHg into animals' milk (Olkowski, 2009). Hence, along with animal health effects important aspects of OrgHg in livestock relate with potential risk of toxicity associated with the consumption of animal products by the human population.

This is relevant for terrestrial systems since recent studies showed that soil Hg can easily enter the food chain (Meng et al., 2010; Qiu et al., 2008; Rimmer et al., 2010; Shao et al., 2012). Risks for animals and humans particularly arise through dietary intake of organic forms of Hg present in edible plants (Gardea-Torresdey et al., 2005; Wang et al., 2011). Estimates indicate that in general, about 1 to 3% of total Hg (THg) in surface soils is in the organo-metallic form (Kabata-Pendias and Mukherjee, 2007) while in upland plants up to 2.5% of the THg can be in the organo-metallic form (Mailman and Bodaly, 2005). Recent studies have shown that rice grain is an intensive bioaccumulator of MeHg (up to 10–100 times greater than other crop plants (Qiu et al., 2008)) due to cultivation under flooded conditions where microbial Hg(II) methylation occurs (Rothenberg et al., 2011a) and that rice consumption is a significant pathway for human exposure to organic forms of Hg (Qiu et al., 2008; Rothenberg et al., 2011b; Zhang et al., 2010a,b). However, despite the recent documentation of uptake of OrgHg from contaminated soils for rice and other edible crops including white cabbage, spinach, radish and lettuce and maize (Qiu et al., 2008; Shao et al., 2012) as well as evidence of Hg bioaccumulation and trophic transfer to wildlife in forest ecosystems (Gnamus et al., 2000; Rimmer et al., 2010) relatively little is known about OrgHg uptake by feed crops and potential transfer and bioaccumulation for grazing animals or about OrgHg thresholds for animals' dietary intake to ensure food safety. To fill this gap studies on the exposure of grazing animals to organic forms of Hg in soils at polluted sites are necessary.

Hence, the scientific objectives of this study were: (1) to evaluate speciation of Hg in soils from an historically contaminated industrial site in Portugal and to assess factors affecting the geographical variability of OrgHg in soils; (2) to evaluate soil-to-feed transfer of IHg and OrgHg; (3) to estimate the animal exposure to IHg and OrgHg from contaminated soils and feed; and (4) to assess potential risks for animal health and food safety.

2. Materials and methods

2.1. Study area

The Estarreja Chemical Complex (ECC) is located 1 km north from the village of Estarreja (North-West Portugal). According to the Köppen classification (Peel et al., 2007) the region has a warm-summer Mediterranean climate (Cbs). The annual average temperature is 14.6 °C and the average precipitation per year is 914 mm (Rodrigues et al., 2006). The fields surrounding the complex are mostly used for agricultural and livestock purposes. The predominant vegetation includes ryegrass and mustard greens. The ECC dates back to the 1950s and among other industrial units it includes a chlor-alkali plant with production of chlorine and sodium hydroxide from rock salt and which used electrolytic cells with mercury cathodes for several decades (Costa and Jesus-Rydin, 2001). Since the 1950s and until the late 1970s, liquid effluents from the Chemical Complex were discharged into the "Esteiro de Estarreja", which is a river branch of a nearby lagoon ("Ria de Aveiro"), through open streams that crossed the surrounding agricultural fields, such as "Vala de S. Filipe" (Fig. 1). In 1986 the plant consumed 28 ton/year of Hg as a raw material and it was estimated that around 12% of annual consumption of Hg was released to the atmosphere (Rodrigues et al., 2006).

Although the chlor-alkali plant completely ceased the use of mercury cathodes in 2002, severe contamination by this pollutant still remains in the surrounding environment (Reis et al., 2009; Rodrigues et al., 2012a).

2.2. Sampling and sample preparation

Sampling was carried out at 39 sites along agricultural/pasture fields located in the vicinity of the chlor-alkali plant, as showed in Fig. 1. Samples were collected:

- along the former effluent stream ("Vala de S. Filipe") — from site 1 to site 8,
- at the former effluent stream — site 9,
- away from the former effluent stream — from site 10 to site 13.

At each site, soil samples were taken at two depths (0–10 cm and 10–20 cm) using a plastic spade. Afterwards, soils were air dried at room temperature until constant weight and sieved to <2 mm using a nylon sieve. Visible stones and biological debris were removed during the drying stage. The following soil properties were measured: pH (according to ISO 10390:1994 procedure), soil organic carbon content (Org C) (Elemental Analysis, LECO CNH-2000, according to ISO 10694:1995) and particle size distribution (using a Coulter LS230 laser diffraction particle size analyzer).

A total of 40 plant samples, including ryegrass (*Lolium perenne*, n = 35) and mustard greens (*Brassica juncea*, n = 5) were collected. All samples were placed in plastic bags during transport to the laboratory. At the laboratory, plant samples were washed with tap water and thoroughly rinsed with distilled water using a brush to remove all traces of dust and soil from the roots and shoots. Both root and shoot samples were dried at 40 °C until constant weight, as several authors agree that this temperature can be applied to avoid loss of mercury due to volatilization (Kamal et al., 2004; Loredo et al., 2003), and milled.

2.3. Total mercury concentration

Total mercury (THg) concentrations in soil, roots and shoots were measured by thermal decomposition atomic absorption spectrometry with gold amalgamation (LECO model AMA-254), as described by Costley et al. (2000). The analysis is performed directly on the sample (sample mass varied in the range 5–150 mg d.w.), without digestion or specific pre-treatment, avoiding mercury losses or contamination as well as matrix interferences. At least three replicate measurements were carried out for each sample (acceptable relative standard deviation among replicates: <10%). Several blanks (i.e. an empty sample nickel boat) were run before and between sample analyses to ensure that mercury was not being carried over between samples. Detection and quantification limits obtained through blank measurements (n = 20) were 0.01 ng Hg (defined as the mean value plus 3.3 standard deviation) and 0.03 ng Hg (defined as the mean value plus 10 standard deviation), respectively.

Analytical quality control was performed by using Certified Reference Materials (CRMs):

- BCR 060 — Aquatic plant (*Lagarosiphon major*); 0.34 ± 0.04 mg kg^{-1} (total Hg)
- CRM 021 — USEPA certified soil reference; 4.7 ± 0.4 mg kg^{-1} (total Hg)
- TORT 2 — *Lobster Hepatopancreas*; 0.27 ± 0.06 mg kg^{-1} (total Hg).

The CRMs were analyzed every day prior to the beginning of the analysis and repeated at the end of the day. All percentages of recovery for THg were within the range of 87–113% (n = 19).

2.4. Organic and inorganic mercury concentration

Organo-metallic mercury (OrgHg) concentrations in soil, roots and shoots were assessed through a method described by Válega et al. (2006). Extraction of OrgHg mercury compounds from the sample matrix was achieved through digestion of 100–200 mg d.w of each sample with a mixture of 18% KBr in 5% H_2SO_4 with $CuSO_4$