Factors controlling the oral bioaccessibility of anthropogenic Pb in polluted soils

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HIGHLIGHTS

- Ingestion of soil is considered a major route of toxic Pb exposure.
- The oral bioaccessibility of soils polluted with various Pb sources was determined.
- The oral bioaccessibility was determined with an in-vitro test.
- Factors controlling the oral bioaccessibility were determined.
- Factors include pollution characteristics and soil composition.

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ABSTRACT

In human risk assessment, ingestion of soil is considered a major route of toxic Pb exposure. A large body of research has focussed on the measurement of the 'total' Pb contents in sediment, soil and dust as a measure for the exposure to lead. We report that Pb bioaccessibility (i.e. the maximum bioavailability), determined with an in vitro test, does not necessarily depend on the total Pb content. In contrast, the Pb bioaccessibility is initially controlled by the chemical form and particle size of the Pb source, which in turn determine its solubility. Furthermore, when anthropogenic Pb resides within the soil, it may form new, more stable, minerals and/or binds to organic matter, clay, reactive iron or other reactive phases, changing its bioaccessibility.

The bioaccessible Pb fraction of 28 soils, polluted with various Pb sources (including residues of Pb bullets and pellets, car battery Pb, city waste and diffuse Pb), was determined with an in vitro-test and varied from 0.5% to 79.0% of total Pb. The highest Pb bioaccessibility (60.7% to 79.0%) was measured in soils polluted with residues of Pb bullets and pellets (shooting range), while the lowest Pb bioaccessibility (0.5%–8.3%) was measured in soils polluted with city waste (including remnants of Pb glazed potsherds and rooftiles, Pb based paint flakes, and Pb sheets). Bioaccessibility of Pb was correlated with pH, organic matter and reactive Fe. These results indicate that soil characteristics play an important role in the oral bioaccessibility of lead in polluted soils. Instead of basing human risk assessment solely on total Pb contents we propose to incorporate in vitro bioaccessibility tests, taking factors such as soil pH, organic matter content and reactive iron content into account. This approach will result in a better insight into the actual risks of Pb polluted soils to children.

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1. Introduction

Egyptian, Greek and Roman Physicians were aware of the toxicity of lead (Pb) (Gilfillan, 1965). Despite this widely known information, it was not until the 1970s that governments started taking legislative measures to reduce the input of Pb and related heavy metals into the environment. The use of leaded gasoline has been reduced significantly worldwide, Pb in paints is officially banned in a large number of countries and active public information campaigns have been carried out to encourage the replacement of Pb water pipes. This raises the question; is the environmental Pb problem now solved? In general, the main problems have been addressed in most developed countries. Many sites in the world are, however, (still) heavily polluted with Pb. Should such soils be used for agriculture or residential building, they may pose a threat to human health.

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Children are particularly sensitive to the toxic effects of Pb, and environmental exposure may cause chronic health effects, such as disturbances to cognitive development and damage to the central nervous system (Elhelu et al., 1995). Due to the frequent hand-to-mouth behaviour of young children, soil ingestion is an important exposure route for Pb (Duggan and Inskip, 1985; Davis and Waller, 1990; Calabrese et al., 1997). In addition, children absorb higher percentages of metals through the digestive system into the blood stream than adults, which may leave them more susceptible to adverse health effects (Hamel et al., 1998 and references therein). Since for children ingestion of soil is considered a major exposure route for Pb, absorption and toxicity of ingested Pb has been studied extensively (Oomen, 2000 and references therein). It appears that less adsorption and toxicity is observed in animal tests when Pb is ingested with soil compared with Pb that is ingested with food, suspensions and liquids (Freeman et al., 1992; Dieter et al., 1993; Oomen, 2000 and references therein). This difference can, among others, be caused by variations in, 1) the chemical composition of the anthropogenic Pb source and its solubility (Steele et al., 1990; Cotter-Howells and Thornton, 1991; Davis et al., 1993; Ruby et al., 1992, 1996, 1999; Rieuwerts et al., 2000; Hettiarachchi and Pierzynski, 2004), 2) the specific reactive surface of Pb in soils (Steele et al., 1990; Ruby et al., 1992, 1999) and 3) the soil type, i.e., soil composition and properties, and capacity to form secondary Pb phases (Yang et al., 2003; Ruby et al., 1999; Davis et al., 1993; Casteel et al., 1997; Rieuwerts et al., 1998a,b, 2000; Hettiarachchi and Pierzynski, 2004; Selinius, 2005; Cave et al., 2011; Farmer et al., 2011). Current risk limits for Pb in soil are based on the measurements of oral bioavailability of Pb in food, suspension and liquids, consequently the oral bioavailability for Pb in soils can be substantially overestimated (Oomen et al., 2003).

Determination of the oral bioaccessibility of Pb (F_B) in soils using *in vitro* tests is an indication for the maximum oral bioavailability of Pb in soils. Although there is evidence that oral bioavailability of Pb in soils depends on soil matrix and type, particle size and chemical composition of the Pb source (e.g., Steele et al., 1990; Cotter-Howells and Thornton, 1991; Davis et al., 1993; Ruby et al., 1992, 1996, 1999; Rieuwerts et al., 2000; Hettiarachchi and Pierzynski, 2004; Selinius, 2005; Cave et al., 2011; Farmer et al., 2011), relatively little research has been performed to quantify these factors. The main aims of the current study are to determine: 1) the bioaccessibility of soils polluted with various Pb sources and 2) if Pb pollution characteristics (chemical composition and particle size of the anthropogenic Pb fraction) and soil characteristics (pH, total Pb, organic matter, clay, calcium carbonate, and reactive iron content) influence oral bioaccessibility. Lead polluted soils from The Netherlands were chosen for this study, because The Netherlands is one of the most densely populated countries in the world, with many lead polluted sites. In addition, the regional distribution of Pb in the environment has already been well studied by Walraven et al. (1997, 2013b, 2014a, submitted for publication).

For this study, 28 Pb polluted soils were selected and Pb isotope analysis was used to distinguish natural Pb and various anthropogenic lead sources. Electron microscopic images of selected samples were made to study the chemical composition and particle size of the anthropogenic Pb fraction. The soil composition and mineralogy were quantified using X-ray Fluorescence (XRF) and Thermal Gravimetric Analysis (TGA). Oral bioaccessibility of Pb in the selected polluted soils was determined with an *in vitro* digestion model developed and tested by Oomen et al. (2003). This method is reproducible, easy to perform and allows simultaneous determination of large numbers of samples (Oomen et al., 2003).

2. Background information

Geographical characteristics, general geology and general pedology of The Netherlands are described in detail in Van der Veer (2006) and Walraven et al. (2013a,b). The natural Pb content of soils (excluding peat) in The Netherlands varies from 3 to 53 mg/kg (Van der Veer,

2006). Natural Pb shows a strong correlation with Al due to its joint occurrence in aluminosilicates (e.g., Huisman, 1998; Van der Veer, 2006; Mol et al., 2012; Walraven et al., 2013a). In The Netherlands soils can be polluted with various Pb sources, in which diffuse and local sources can be distinguished. Diffuse Pb sources in The Netherlands include gasoline Pb, incinerator ashes, fertilisers and animal manure (Walraven et al., 2013b). Local Pb sources include residues of Pb bullets and pellets (henceforth referred to as Pb bullets and pellets), car battery Pb, made grounds and city waste (Walraven et al., 1997). Lead bullets and pellets are used for hunting, sports and military activities and can end up in soils after use. Car batteries often contain Pb. At car battery repair facilities, Pb entered the environment due to accidents, ignorance or indifference. Made grounds in The Netherlands were mainly formed between the 15th and the 19th century. In this period peat was extensively mined in The Netherlands. Boats that transported peat to the city, brought back city waste, manure, sludge to fill up the peat holes, and to raise and fertilize the land. These layers are called made grounds and have thicknesses typically between 15 and 50 cm (Bosveld and De Poorte, 1999). Due to the presence of among others paint flakes, remnants of Pb glazed potsherd, glass and Pb sheets in city waste, the Pb content in made grounds can be greater than the Dutch Intervention Value of Pb for standard soils (530 mg/kg). City waste was not only dumped in peat holes and in subsiding areas but it also ended up in city soils. Soils in Dutch cities and villages with a long habitation history often contain elevated contents of heavy metals, and Pb in particular (Walraven et al., submitted for publication). Lead contents in such soils can reach values of several thousand mg/kg Pb (Walraven et al., 1997).

Several researchers have demonstrated that Pb isotope analysis can be used to distinguish natural from anthropogenic Pb, but also to identify the anthropogenic Pb source in the environment (see review by Komárek et al., 2008 and references therein). In The Netherlands the Pb isotope composition of anthropogenic Pb sources differs clearly from natural Pb and various anthropogenic Pb sources have characteristic Pb isotope ratios (e.g., Walraven et al., 2013a,b, 2014a,b, submitted for publication). The principles of stable lead isotope analysis are described in detail in Faure (1986). In the present study we aim to use Pb isotope ratios to determine the specific anthropogenic Pb sources in soil samples.

3. Materials and methods

3.1. Sample selection

The selected 28 soil samples were collected in the framework of other studies (see Groot and van Swinderen, 1993; Oomen and Hagens, 2006; Van der Veer, 2006; Walraven et al., 2013b, submitted for publication). These samples were selected because they contain anthropogenic Pb from various (inferred) Pb sources (Pb bullets and pellets, car battery Pb, Gasoline Pb, diffuse Pb, made ground and city waste) and the characteristics of the soils (pH, total Pb, organic matter, clay, calcium carbonate, and reactive iron content) vary considerably. The sample locations are shown in Fig. 1. Description of the sample sites and sample depths are given in Table 1.

To determine if different anthropogenic Pb sources have different bioaccessibilities, only samples inferred to be polluted with one dominant anthropogenic Pb source were selected: Pb bullets and pellets (n = 2), car battery Pb (n = 2), gasoline Pb (n = 6), diffuse Pb (n = 5), made grounds (n = 7) and 'old' city waste (n = 6) containing various Pb-based artefacts (e.g. Pb glazed potsherds, Pb sheets and Pb-based paint flakes). These Pb sources were selected based on their difference in chemical composition and particle size.

To determine if bioaccessibility of Pb depends on soil characteristics, Pb polluted soil samples from the following soil types were selected: 1) aeolian sands (n = 12), 2) (peat-bearing) marine clays (n = 9) and 3) fluviatile clays (n = 7). These soil types were selected because they cover ~75% of the soils present in The Netherlands.
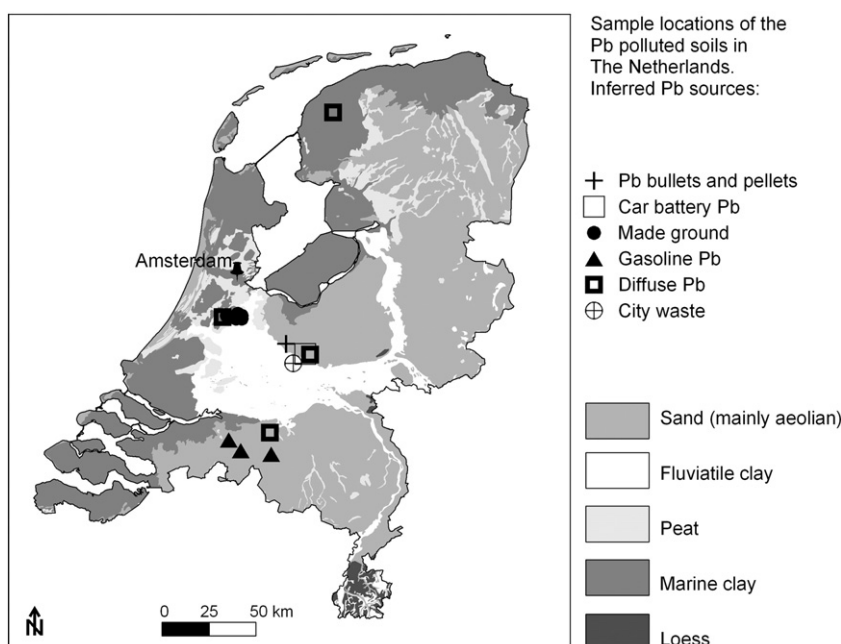
Fig. 1. Sample locations of the Pb polluted soils.

3.2. Preparation and chemical analysis of the soil samples

All samples were dried at 105 °C. The soil samples were sieved over a 2 mm sieve to remove the gravel fraction. The 2 mm soil fractions were used to determine Pb bioaccessibility. For chemical and isotopic analysis the 2 mm fraction was ground (<15 μm), using an automated tungsten-carbide mill (Herzog HSM-HT). To determine oral bioaccessibility of Pb in the selected polluted soils, the *in vitro* digestion methodology developed and validated by Oomen et al. (2003) was used. The Pb content and isotopic ratios of the total sample, the chyme and the residual pellets were analysed with an Inductively Coupled Plasma –Mass Spectrometer (ICP-MS). Soil compositions were quantified using X-ray Fluorescence (XRF) and Thermal Gravimetric Analysis (TGA). Electron microscopic images of selected samples were made to study the chemical composition and particle size of the anthropogenic Pb fraction.

3.2.1. In vitro digestion model to determine Pb bioaccessibility

During the last decade many *in vitro* tests have been developed and evaluated to estimate the oral bioavailability of contaminants/compounds, among others Pb, in soils. The four most commonly used batch extraction methods are the physiologicaly based extraction test (PBET; Ruby et al., 1993), the *in vitro* gastrointestinal method (IVG; Rodriguez et al., 1999), The Dutch National Institute for Public Health and the Environment method (RIVM; Oomen et al., 2003) and the relative bioaccessibility leaching procedure (RBALP; Drexler and Brattin, 2007). The development of *in vitro* bioaccessibility test methods (for Pb) in soils has been reviewed thoroughly by Intawongse and Dean (2006) and Zia et al. (2011). The PBET, IVG and RIVM methods use extraction media that closely mimic the chemical environment of the human gastrointestinal system, i.e. they are physiologically based, whereas RBALP is conducted using a pH value that is physiologically relevant to that of the stomach but with a glycine buffer as the extraction medium (Farmer et al., 2011). Since the operating conditions of the *in vitro* methods can vary, the measured bioaccessibility values also vary substantially (Oomen et al., 2002; Van de Wiele et al.,2007). To harmonise the available physiologically based *in vitro* methods the Bioaccessibility Research Group of Europe (BARGE) developed an optimized method known as the Unified BARGE method which is based on the RIVM methodology (Wragg et al., 2009, 2011). The Unified

Barge method was not yet finalised when we conducted our research and consequently the RIVM *in vitro* model introduced by Oomen et al. (2003) was used. This model is based on Rotard et al. (1995). It is an *in vitro* gastrointestinal model in which the soil samples (fraction < 2 mm) are subjected to a number of stages simulating the human digestion process. Duplicate pre-treated subsamples of 0.6 g were weighed into centrifuge tubes and 9.0 ml of saliva (pH 6.5 ± 0.2) was added. This mixture was rotated for 5 min, end-over-end, at about 55 rpm at 37 °C. Then, 13.5 ml of gastric juice (pH 1.07 ± 0.07) was added, and the mixture was rotated at 37 °C. After 2 h, 27 ml of duodenal juice (pH 7.8 ± 0.2) and 9 ml of bile juice (pH 8.0 ± 0.2) were added. This mixture was rotated at 37 °C for 2 h and subsequently centrifuged at 3000 g for 5 min. The supernatant (total volume 58.5 ml) represents the chyme and should have a pH of at least 5.5 (pH adjustment is performed with an acid or base). The residual soil is called the residual pellet. A detailed description of the methodology, including constituents and concentrations of the various synthetic juices of the *in vitro* model (saliva, gastric juice, duodenal juice and bile), can be found in Oomen et al. (2002, 2003). Relative precision (2 RSD) of the bioaccessibility model for Pb (in chyme) was calculated to be 25% based on duplicate analysis of all studied soil samples.

3.2.2. Lead content and Pb isotope composition

Soil samples and residual pellets were analysed after HF-based sample digestion. Details of the HF-based digestion method can be found in Van der Veer (2006). The chyme samples were acidified with 16 M HNO_3 to final solutions of 1 M HNO_3. Lead contents of the digested soils samples, residual pellets and chyme were determined with an Agilent 7500a ICP-MS equipped with low uptake nebulizer. Details of the ICP-MS method for Pb content determination can be found in Huisman et al. (2009). The relative precision (2 RSD) for Pb based on sample duplicates is 3.6%. The accuracy for Pb as determined by soil standard ISE 921 is 0.7% (relative bias). Lead isotopes were also measured using an Agilent 7500a ICP-MS. A similar methodology to Krachler et al. (2004) was adopted to correct for mass bias discrimination by bracketing each 6 samples with the Pb isotope standard NIST 981. The effect of count rate on the mass discrimination was minimized by diluting all samples and standards to a lead concentration of 50 μg/kg. Full details of this method are described by Walraven et al. (2013a,b). $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ ratios were determined with a 2 RSD precision of

Table 1

Sample names, locations, inferred Pb sources, land use, lithology, sample depth and references wherein more detailed information about the sample locations and sample procedures can be found. (lit. = litter).

Sample name	Location	Inferred Pb source	Land use	Lithology	Depth (cm)	Sample origin (reference)
PU 1	Bornia	Pb bullets and pellets	Former shooting range	Aeolian sand	0–25	Oomen and Hagens (2006)
PU 2	Bornia	Pb bullets and pellets	Former shooting range	Aeolian sand	0–25	Oomen and Hagens (2006)
PU 3	Leersum	Car battery Pb	Car battery repair facility	Aeolian sand	0–25	Oomen and Hagens (2006)
PU 4	Leersum	Car battery Pb	Car battery repair facility	Aeolian sand	0–25	Oomen and Hagens (2006)
PU 6	Vinkeveen	Made ground (1500–1900 A.D.)	Grassland	Marine clay (+peat)	0–25	Oomen and Hagens (2006)
PU 7	Demmerik	Made ground (1500–1900 A.D.)	Grassland	Marine clay (+peat)	0–25	Oomen and Hagens (2006)
PU 8	Donkereind	Made ground (1500–1900 A.D.)	Grassland	Marine clay (+peat)	0–25	Oomen and Hagens (2006)
PU 9	Donkereind	Made ground (1500–1900 A.D.)	Grassland	Marine clay (+peat)	0–25	Oomen and Hagens (2006)
PU 10	Wilnis	Made ground (1500–1900 A.D.)	Grassland	Marine clay (+peat)	0–25	Oomen and Hagens (2006)
PU 11	De Hoef	Made ground (1500–1900 A.D.)	Grassland	Marine clay (+peat)	0–25	Oomen and Hagens (2006)
PU 12	Wilnis	Made ground (1500–1900 A.D.)	Grassland	Marine clay (+peat)	0–25	Oomen and Hagens (2006)
MOER 428	Moergestel	Gasoline Pb	Mixed forest	Aeolian sand (+litter)	–	Walraven et al. (2014a)
MOER 427	Moergestel	Gasoline Pb	Mixed forest	Aeolian sand (+litter)	–	Walraven et al. (2014a)
LBG 404	Moergestel	Gasoline Pb	Mixed forest	Aeolian sand (+litter)	–	Groot and Swinderen (1993)
LBG 408	Gilze	Gasoline Pb	Mixed forest	Aeolian sand (+litter)	–	Groot and Swinderen (1993)
LBG 413	Oosterhout	Gasoline Pb	Mixed forest	Aeolian sand (+litter)	–	Groot and Swinderen (1993)
MOER 415	Moergestel	Gasoline Pb	Mixed forest	Aeolian sand	0–1	Walraven et al. (2014a)
T270	Amerongen	Diffuse Pb	Mixed forest	Aeolian sand	0–20	Van der Veer (2006)
T772	Dronrijp	Diffuse Pb	Grassland	Marine clay (+peat)	0–20	Van der Veer (2006)
T052	Nieuwkoop	Diffuse Pb	Grassland	Marine clay (+peat)	0–20	Van der Veer (2006)
T380	Udenhout	Diffuse Pb	Corn	Aeolian sand	0–20	Van der Veer (2006)
WIJK 286	Wijk bij Duurstede	Diffuse Pb	Corn	Fluviatile clay	0–10	Walraven et al. (submitted for publication)
PU 5	Wijk bij Duurstede	City waste (1600-present)	Urban area	Fluviatile clay	0–25	Oomen and Hagens (2006)
WIJK 297	Wijk bij Duurstede	City waste (500–1000 A.D.)	Urban area	Fluviatile clay	50–60	Walraven et al. (submitted for publication)
WIJK 298	Wijk bij Duurstede	City waste (500–1000 A.D.)	Urban area	Fluviatile clay	80–90	Walraven et al. (submitted for publication)
WIJK 317	Wijk bij Duurstede	City waste (1600-present)	Urban area	Fluviatile clay	20–50	Walraven et al. (submitted for publication)
WIJK 312	Wijk bij Duurstede	City waste (1600-present)	Urban area	Fluviatile clay	0–30	Walraven et al. (submitted for publication)
WIJK 321	Wijk bij Duurstede	City waste (1800–1950 A.D.)	Waste disposal site in urban area	Fluviatile clay	100–120	Walraven et al. (submitted for publication)

<0.14% and 2 RSD <0.07% respectively. Soil standard ISE 921 was measured and yielded an average value and absolute precision (2 SD) of 1.166 ± 0.002 for $^{206}Pb/^{207}Pb$ and 2.444 ± 0.003 for $^{208}Pb/^{207}Pb$. Average and precision are based on the entire analytical procedure starting with the sample splits. Blanks and reagents used were also measured and appeared to contain negligible amounts of Pb (<20 ng/kg).

3.2.3. Reactive phase determination (clay, reactive iron, organic matter and calcium carbonate)

In Dutch soils Al and Fe are positively correlated with clay content and reactive Fe content respectively (Huisman, 1998; Huisman and Kiden, 1997). Therefore, Al and Fe are used in this study as a measure of the content of clay and reactive Fe (see Section 3.3.3). For Al and Fe analysis, subsamples (10 g) were ground in a tungsten carbide swing-mill to a grain size <15 μm and subsequently pressed with wax into pressed-powder tablets in an automated grinding- and pressing machine (Herzog HSM-HTP). Aluminium and Fe have been measured with an ARL9400 X-ray Fluorescence spectrometer (XRF) with a precision (1 RSD) of 0.5–1.0% based on replicate analysis. One reference sample (ISE 921) was added to each batch of 20 samples to determine accuracy. The certified Al and Fe content of ISE 921 is 5.7 wt.% and 3.2 wt.% respectively. The accuracy determined for Al and Fe is 4–6% relative bias. For details see Spijker (2005).

Organic matter (OM) and carbonate (CO_{2carb}) content were analysed with a LECO TGA 601. Temperatures were varied from 105 °C to 550 °C for organic matter content determination, 550 °C to 800 °C for carbonate content (mainly $CaCO_3$) and from 800 °C to 1000 °C for clay bound water content. The relative precision (2 RSD) for OM and CO_{2carb} based on sample duplicates is 3.8% and 4.5% respectively. The accuracy for OM and CO_{2carb} as determined by soil standard ISE 921 is 0.2% and 13% respectively (relative bias).

3.2.4. pH-KCl

Five ml of treated soil sample (dried at 105 °C; fraction <2 mm; not milled) was transferred to a glass beaker with a volumetric spoon. Twenty-five ml 1 M KCl was added. This solution was shaken for 5 min. After a settling period of two hours the pH of the solution was measured with a WTW Multi 340i.

3.2.5. Electron-microprobe analysis (EMPA)

Three samples were selected for electron-microprobe analysis (EMPA): one soil inferred to be polluted with gasoline Pb (MOER 415), one with Pb bullets and pellets (PU 2) and one made ground (PU 6). The treated soil samples (dried at 105 °C; fraction <2 mm; not milled) were manually split into two subsamples. One subsample was density separated using an aqueous solution of sodium polytungstate to extract the fraction heavier than 2.8 g/cm³, presumable containing the anthropogenic Pb fraction, and removing the silicates, clay minerals and organic matter particles. Both sub-samples were cast in epoxy resin. Polished sections were made and carbon coated. An electron-microprobe (JEOL JXA-8600 Superprobe) equipped with a WD-XRF and ED-XRF was used for qualitative chemical analysis and imaging.

3.3. Calculations and statistical analysis

3.3.1. Natural and anthropogenic Pb content and Pb isotope composition

The Pb content of polluted soil samples consists of natural Pb and anthropogenic Pb. If the natural Pb content is known, the anthropogenic Pb content can be calculated. The natural Pb content (Pb_n) in sediments and soils can be calculated based on the common relationship between Pb and Al in unpolluted soils and sediments (e.g., Huisman, 1998; Van der Veer, 2006; Walraven et al., 2013a). Walraven et al. (2013a)

established the following relationship for unpolluted sedimentary soils in The Netherlands:

$$Pb_n = 3.69 \times Al + 1.75 \qquad (1)$$

in which Pb_n = calculated 'natural' Pb content (mg/kg) and Al = measured Al content in wt.% (n = 303; R^2 = 0.89; Standard error of estimate is 2.6 mg/kg).

Based on Eq. (1), it is possible to calculate the anthropogenic Pb content in polluted soil samples according to Eq. (2):

$$Pb_a = Pb_t - Pb_n \qquad (2)$$

in which Pb_n = calculated 'natural' Pb content (mg/kg), Pb_t = measured total Pb content (mg/kg) and Pb_a = calculated anthropogenic Pb content (mg/kg).

If Pb_t and Pb_n are known, the enrichment factor (EF) can be calculated according to Eq. (3):

$$EF = Pb_t / Pb_n. \qquad (3)$$

If both natural and anthropogenic Pb are present, the total (measured) Pb isotope composition in the soil is a mixture of the isotope composition of natural and anthropogenic Pb. To derive the Pb isotope composition of the anthropogenic Pb fraction, it is necessary to account for the amount of natural Pb present in the soil. The Pb isotope composition of the natural lead fraction of the soil samples in the present study is unknown, since only polluted soil samples were taken from the sample sites. It was calculated, however, that any correction for natural Pb is irrelevant if the amount of natural Pb is negligible compared with the total Pb content. Based on a simple sensitivity analysis, it is calculated that if EF is >20, the contribution of natural Pb, with a mean Pb isotope composition of 1.197 and 2.468 for $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ respectively (Walraven et al., 2013a), is negligible (within the range of the precision of the $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ measurements). For the samples with EF < 20 (n = 7) a correction for the natural Pb content is made according to Eq. (4):

$$(^xPb/^yPb)_a \times Pb_a = (^xPb/^yPb)_t \times Pb_t - (^xPb/^yPb)_n \times Pb_n \qquad (4)$$

in which the letters a, t and n indicate the Pb concentration and Pb isotope ratio ($^xPb/^yPb$) of the *a*nthropogenic, *t*otal and *n*atural Pb fraction, respectively. The mean natural Pb isotope composition in Dutch soils is 1.197 and 2.468 for $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ respectively, with an absolute standard deviation (1 SD) of 0.009 and 0.010 respectively (Walraven et al., 2013a).

3.3.2. Bioaccessibility

Provided the sum of the Pb content in the chyme and the Pb content in the residual pellet does not differ significantly from the total Pb content of the soil sample, the bioaccessibility of Pb can be calculated accurately according to Eq. (5).

$$F_{B-Pb} = \left(Pb_{chyme} / Pb_t \right) \times 100\% \qquad (5)$$

in which F_{B-Pb} is the calculated bioaccessibility of Pb (%), Pb_{chyme} is the measured Pb content determined in chyme (mg/kg) and Pb_t is the measured total Pb content in the soil samples.

3.3.3. Reactive phases: clay and reactive iron

In this study, clay content is calculated based on the Al content. In Dutch soils, Al shows a positive correlation with the clay content according to Eq. (6) (Huisman, 1998).

$$Clay\ (wt.\%) = 3.0 \times Al_2O_3(wt.\%) - 8.0 \quad if \quad Al_2O_3 > 3.5\ wt.\% \qquad (6)$$

in which Al_2O_3 content of the soil samples was determined by XRF.

In general soils with Al_2O_3 contents below 3.5 wt.% are virtually free of clay minerals. In these samples Al is commonly present in the feldspars (Huisman, 1998; Mol et al., 2003).

The content of reactive iron (Fe_{react}) is calculated according to Eq. (7) (Huisman and Kiden, 1997).

$$Fe_{react}(wt.\%) = (Fe_2O_3(wt.\%) - (Al_2O_3(wt.\%) / 4.0))/1.4297 \qquad (7)$$

in which Al_2O_3 and Fe_2O_3 content of the soils samples were determined by XRF.

3.3.4. Spearman correlation

Data correlations are assessed on the non-parametric Spearman tests rather than Pearson correlations, because the number of observations is low and normal distributions could not be assumed. Moreover, Spearman correlation analysis was performed separately on the samples from the three distinct soil types because of multimodality. Based on the Grubb's test, sample WIJK 321 is identified as an outlier (high Fe_{react} content) and is therefore excluded from the Spearman correlation analysis of the fluviatile clays. Sample T 7721 (marine clay) was found to be unpolluted and is also excluded from Spearman correlation analysis. The approach resulted in three groups, each consisting of a limited number of samples, and the results of the Spearman analysis should therefore be regarded as only indicative. The Spearman tests were conducted with the software package Statistica version 12 and the Grubb's test with GraphPad software.

4. Results

4.1. Chemical composition of the soil samples

The chemical and Pb isotope compositions of the studied soils are listed in Table 2. For convenience inferred Pb sources are used for identification rather than the sample numbers. In Section 5.1 the actual pollution sources are critically assessed.

The total Pb content of the soil samples ranges from 11 to 2362 mg/kg. All soil samples, except sample T 772 and T 380, have Pb contents above the upper confidence level of the relationship between Pb and Al contents in natural Dutch soils and sediments (Fig. 2). Soil samples polluted with Pb bullets and pellets, car battery Pb and Pb in made grounds, all have Pb contents higher than the Dutch intervention value for Pb, which is 530 mg/kg for standard soils (Fig. 2). The soils polluted with Pb bullets and pellets are acidic sandy soils (pH-KCl = 3.9–4.1) and contain negligible quantities of reactive phases. Only minor amounts of organic matter (2.0–3.1 wt.%) are present in these soils. Soils polluted with car battery Pb are also sandy soils with negligible amounts of reactive phases, but are buffered (pH-KCl = 7.4–7.5) due to the presence of calcium carbonate (1.3 wt.%). These soils contain organic matter amounts (2.0–3.1 wt.%) comparable to the soils polluted with Pb bullets and pellets. The soils polluted with gasoline Pb are sandy soils, contain 129 to 565 mg/kg Pb and are the most acidic (pH-KCl = 2.5–2.8) of the studied soils. The dominant reactive phase in these soils is organic matter (22.2–71.1 wt.%). All other reactive phases are negligible. The made grounds and soils polluted with city waste are clayey soils with a Pb content of 129–652 mg/kg and 129–1006 mg/kg respectively. The pH-KCl values of these soils range from 4.8 to 5.4 (slightly acidic) for the made grounds and from 6.0 to 7.4 (slightly acidic to neutral) for the soils polluted with city waste. The dominant reactive phases in these soils are organic matter (20.2–39.5 wt.%) and clay (9.4–25.6 wt.%). The soils polluted with city waste contain significant amounts of three reactive phases, from which clay (7.0–21.1 wt.%) and organic matter (4.9–18.4 wt.%) are the most dominant. The content of reactive Fe in these soils is negligible. The Pb content of the rural soils polluted with diffuse Pb sources varies from 11 to 192 mg/kg. These soils are the least enriched with Pb of the studied soils. Enrichment factors vary from 1.2 to 12. The content of reactive phases of the rural soil

Table 2
Average chemical composition (Pb, Al, Fe), soil characteristics (clay, Fe_react, OM, CaCO_3 and pH) and Pb isotope composition (^{206}Pb/^{207}Pb and ^{208}Pb/^{207}Pb) of the studied soils. (Pb)_t, (Pb)_n and (Pb)_a = total, natural and anthropogenic lead content (Eqs. (1) and (2)); EF = enrichment factor (Eq. (3)); Clay = clay content (Eq. (6)); Fe_react = reactive iron content (Eq. (7)); OM = organic matter content.

Sample name	Inferred Pb source	(Pb)_t	Al	Fe	(Pb)_n	(Pb)_a	EF	Clay	Fe_react	OM	CaCO_3	pH	(^{206}Pb/^{207}Pb)		(^{208}Pb/^{207}Pb)	
		(mg/kg)	(wt.%)	(wt.%)	(mg/kg)	(mg/kg)		(wt.%)	(wt.%)	(wt.%)	(wt.%)		(Total)	(antro)	(total)	(antro)
PU 1	Pb bul./pel.	1432	1.16	0.07	6	1426	237	0.0	0.0	2.0	0.2	3.9	1.133	1.133	2.407	2.407
PU 2	Pb bul./pel.	2362	1.38	0.14	7	2355	346	0.0	0.0	3.1	0.2	4.1	1.133	1.133	2.407	2.407
PU 3	Car bat. Pb	2049	1.80	0.98	8	2041	244	0.0	0.4	3.1	-	7.4	1.152	1.152	2.431	2.431
PU 4	Car bat. Pb	1049	1.64	0.63	8	1041	134	0.0	0.1	2.0	1.3	7.5	1.147	1.147	2.419	2.419
PU 6	Made ground	1452	3.07	2.87	13	1439	111	9.4	1.9	32.0	2.1	5.4	1.177	1.177	2.448	2.448
PU 7	Made ground	1014	4.71	4.55	19	995	53	18.7	3.0	33.9	3.0	5.3	1.173	1.173	2.445	2.445
PU 8	Made ground	648	4.71	3.78	19	629	34	18.7	2.2	37.8	2.0	5.1	1.177	1.177	2.448	2.448
PU 9	Made ground	918	4.82	4.20	20	898	47	19.3	2.6	34.4	2.9	5.3	1.174	1.174	2.446	2.446
PU 10	Made ground	744	5.40	4.97	22	722	34	22.6	3.2	30.0	1.6	4.8	1.176	1.176	2.446	2.446
PU 11	Made ground	595	5.93	3.92	24	571	25	25.6	2.0	20.3	2.3	5.1	1.176	1.176	2.446	2.446
PU 12	Made ground	557	4.02	3.29	17	540	34	14.8	2.0	39.5	1.9	5.3	1.175	1.175	2.444	2.444
MOER 428	Gasoline Pb	565	0.85	1.40	5	560	116	0.0	1.1	71.1	0.3	2.5	1.125	1.125	2.401	2.401
MOER 427	Gasoline Pb	652	1.38	0.84	7	645	95	0.0	0.4	38.2	0.7	2.6	1.135	1.135	2.414	2.414
LBG 404	Gasoline Pb	227	1.27	0.63	6	221	35	0.0	0.2	45.1	0.1	2.6	1.144	1.144	2.423	2.423
LBG 408	Gasoline Pb	280	0.69	0.84	4	276	65	0.0	0.6	66.1	0.0	2.8	1.114	1.114	2.387	2.387
LBG 413	Gasoline Pb	129	0.90	0.35	5	124	25	0.0	0.1	25.8	0.1	-	1.117	1.117	2.392	2.392
MOER 415	Gasoline Pb	561	1.53	0.56	7	554	76	0.0	0.1	22.2	0.9	2.8	1.138	1.138	2.419	2.419
T270	Diffuse Pb	28	1.32	0.49	7	21	4.2	0.0	0.1	7.0	0.3	3.3	1.160	1.149	2.438	2.429
T772	Diffuse Pb	27	7.30	4.48	29	(−2)	-	33.4	2.1	5.6	1.6	6.3	1.198	-	2.473	-
T052	Diffuse Pb	192	5.13	4.76	21	171	9	21.1	3.1	37.8	1.9	5.5	1.167	1.163	2.448	2.446
T380	Diffuse Pb	11	1.32	0.07	7	4	1.7	0.0	0.0	3.5	0.3	4.9	1.165	1.116	2.443	2.405
WIJK 286	Diffuse Pb	44	5.66	2.87	23	21	1.9	24.1	1.0	10.2	1.2	5.6	1.181	1.164	2.458	2.447
PU 5	City waste	1006	3.65	2.24	15	991	66	12.7	1.0	6.9	5.0	6.9	1.161	1.161	2.433	2.433
WIJK 297	City waste	194	5.08	2.80	20	174	9	20.8	1.1	6.7	2.5	6.8	1.145	1.139	2.425	2.420
WIJK 298	City waste	129	5.13	2.59	21	108	6.2	21.1	0.9	5.2	1.9	6.7	1.178	1.174	2.460	2.458
WIJK 317	City waste	416	3.60	1.96	15	401	28	12.4	0.8	5.3	7.0	7.1	1.176	1.176	2.453	2.453
WIJK 312	City waste	222	2.65	1.40	12	210	19	7.0	0.5	4.9	4.0	7.4	1.173	1.172	2.454	2.453
WIJK 321	City waste	774	2.96	5.11	13	761	61	8.8	4.1	18.4	4.9	6.0	1.160	1.160	2.435	2.435

samples is highly variable due to the difference in lithologies of the samples.

4.2. Lead isotope composition of the soil samples

The (^{206}Pb/^{207}Pb)_t and (^{208}Pb/^{207}Pb)_t ratios of polluted soils vary from 1.114 to 1.198 and 2.387 to 2.473 respectively (Table 2). The lowest ratios are measured in the roadside soils ((^{206}Pb/^{207}Pb)_t = 1.114–1.138 and (^{208}Pb/^{207}Pb)_t = 2.387–2.423) and the highest (most radiogenic) values in the rural soils polluted with diffuse Pb sources ((^{206}Pb/^{207}Pb)_t = 1.160–1.198 and (^{208}Pb/^{207}Pb)_t = 2.438–2.473). The inferred Pb sources, with the exception of the diffuse Pb and city

waste, have distinct Pb isotope compositions (e.g., the (^{206}Pb/^{207}Pb)_t values for the made grounds only vary from 1.173 to 1.177).

4.3. Electron-microprobe analysis

The EMPA results of the untreated and treated samples (Section 3.2) are presented in Table 3 and Fig. 3. For sample PU 2 and PU 6 the anthropogenic Pb-bearing phases were most easily examined in the >2.8 g/cm^3 fraction, whereas for sample MOER 415 anthropogenic lead was best preserved in the untreated sample.

Sample PU 2, polluted with Pb bullets and pellets, consists mainly of quartz minerals, organic matter particles and Pb bullets and pellets (Fig. 3a). The Pb bullets and pellets vary in size from approximately 5
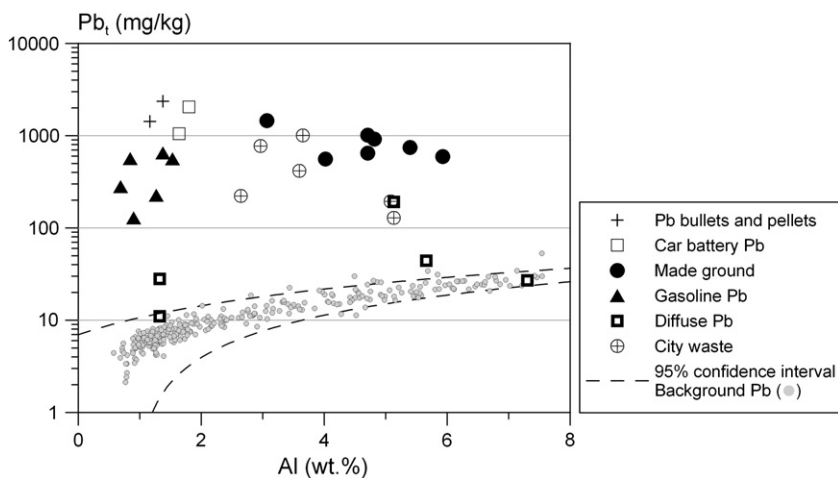

Fig. 2. Pb_t (mg/kg) versus Al (wt.%) content of soil samples polluted with Pb bullets and pellets, car battery Pb, Pb in made grounds, gasoline Pb, diffuse Pb in rural areas, and Pb containing city waste; and of natural (unpolluted) subsoils in The Netherlands. The regression line (±95% confidence levels) is formed by the relationship between the Pb and Al contents in natural Dutch soils (Walraven et al., 2013a).

Table 3
EMPA results. Chemical composition and grain size of the primary and secondary Pb phases in sample PU 2, PU 6 and MOER 415.

Sample	Pb source	Primary Pb phases		Secondary Pb phases	
		Chemical composition	Grain size (μm)	Chemical composition	Grain size (μm)
PU 2	Pb bullets and pellets	• Native Pb	• 5–150	• Pb oxide (up to 70 wt.%) (Fig. 3-A) • Pb (0.5–17 wt.%) adsorbed to OM and/or coatings/infillings of $PbCO_3$ and/or $PbSO_4$ (Fig. 3-D)	• 5–150 (size of bullets) • 20–600 (size of OM particles)
PU 6	Toemaakdek	• Pb aluminosilicates (Fig. 3-B) • Native Pb • Pb oxide	• 1–280 (all, particles)	• Pb (up to 0.5 wt.%) adsorbed to OM and/or coatings of $PbCO_3$ and/or $PbSO_4$	• 20–330 (size of OM particles)
Moer 415	Gasoline Pb	• Not found	• If present <1	• Pb (0.5–1.5 wt.%) adsorbed to OM and/or coatings of $PbCO_3$ and/or $PbSO_4$ (Fig. 3-C)	• 20–140 (size of OM particles) (Fig. 3-C)
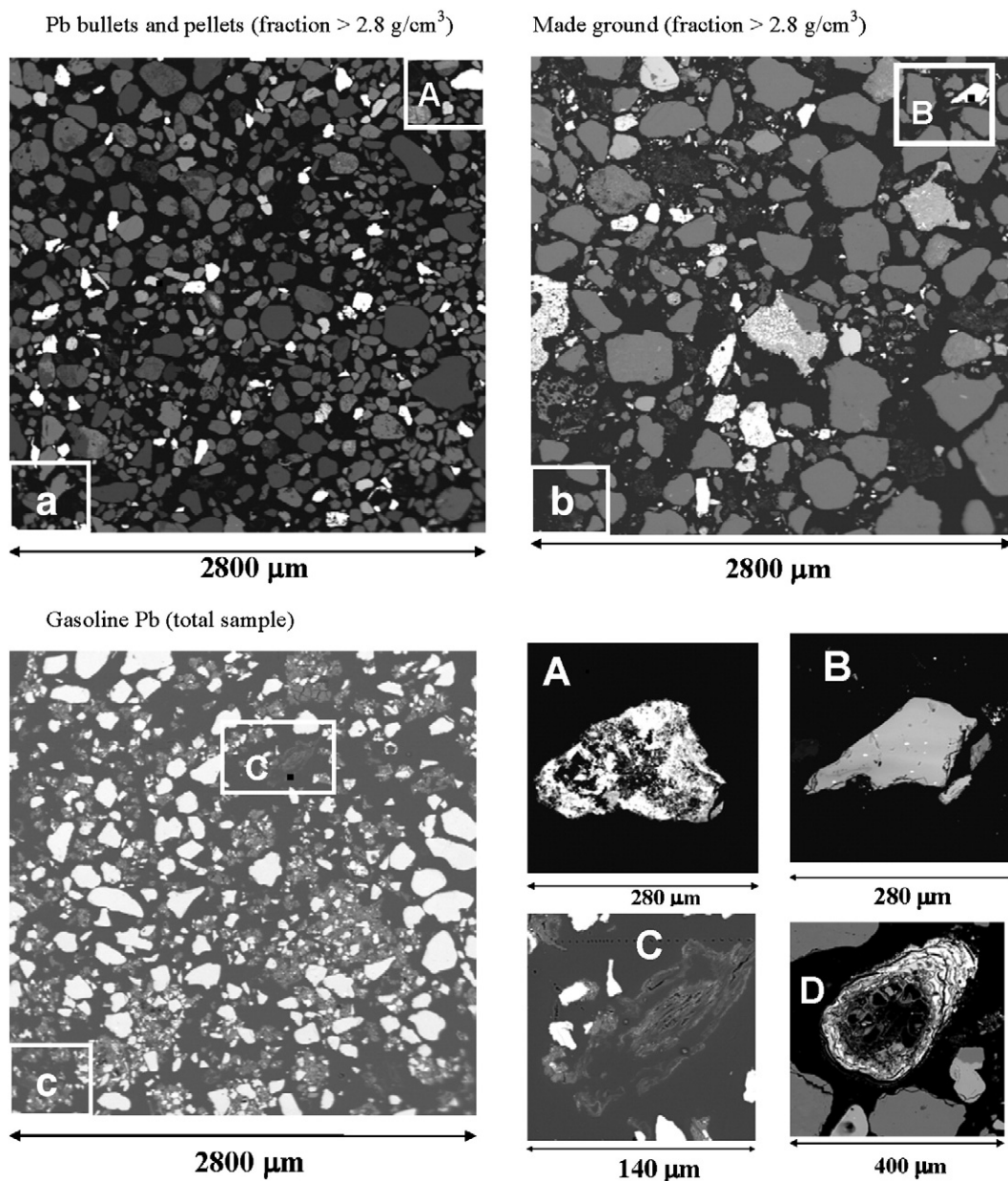
Fig. 3. EMPA back scatter images (a, b and c) of soils polluted with Pb bullets and pellets (PU 2), made ground (PU 6) and gasoline Pb (MOER 415). A = Pb bullet showing dissolution holes; B = Pb aluminosilicate (Pb glazed potsherd); C, D = organic matter associated with Pb.

to 150 μm (Table 3). Most bullets and pellets show dissolution holes (Fig. 3-A) and are oxidized (native Pb transformed to Pb oxide). Most organic matter particles, varying in size from 20 to 600 μm, have a light grey colour in the backscatter images. These particles have Pb contents up to 2.5 wt.% Pb. The cell structure of some organic matter particles is filled with secondary Pb minerals (most likely Pb sulphate or Pb carbonate) containing up to 17 wt.% Pb (Fig. 3-D).

The Pb polluted made ground sample PU 6 consists mainly of quartz grains, clay minerals, organic matter and anthropogenic Pb phases (Fig. 3b). The primary Pb phases are native Pb, Pb oxides and Pb aluminosilicates, most likely Pb glazed potsherds (Fig. 3-B).

The size of these Pb phases ranges from approximately 1 to 280 μm. As in sample PU 2 (Pb bullets and pellets), some organic matter particles, varying in size from 20 to 330 μm, have elevated Pb contents, up to 0.5 wt.%.

Sample MOER 415, polluted with gasoline Pb, consists mainly of quartz grains and organic matter particles (Fig. 3c). No primary Pb phases were found (Table 3). Most organic matter particles (20–140 μm) have a light grey colour in the backscatter images (Fig. 3-C) with Pb contents between 0.5 and 1.5 wt.%.

4.4. In vitro digestion model

The Pb content and Pb isotope composition of the chyme and residual pellets, and the calculated bioaccessibility of Pb (Eq. (5)) are listed in Table 4. The Pb content of the chyme (bioaccessible Pb) varies from 1 to 1865 mg/kg. The lowest chyme Pb contents are measured in soils polluted with diffuse Pb (2–19 mg/kg) and the highest chyme Pb contents in the soils polluted with Pb bullets and pellets (869–1865 mg/kg). The $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ values of the chyme samples range from 1.114 to 1.191 and from 2.390 to 2.460 respectively (Table 4). The Pb content of the residual pellets varies from 9 to 1482 mg/kg and the Pb isotope ratios from 1.115 to 1.199 for $^{206}Pb/^{207}Pb$ and from 2.384 to 2.477 for $^{208}Pb/^{207}Pb$.

Since the sum of Pb present in the chyme and the residual pellet does not differ significantly from total Pb content present in the soil samples ($\log(Pb_t) = 1.0 \pm 0.1 \times \log(Pb_{chyme} + Pb_{pellet}) + 0.1 \pm 0.2$; $R^2 = 0.96$; $p < 0.05$), bioaccessibility can be calculated quantitatively according to Eq. (5). Bioaccessibility ranges from 0.5% (sample polluted with city waste) to 79.0% (sample polluted with Pb bullets and pellets) (Table 4). The highest bioaccessibility values are measured in samples polluted with Pb bullets and pellets (56.2–79.0%) and relatively low values are measured in samples polluted with city waste (0.5–8.3%), with the exception of sample WIJK 321, which was taken from a landfill (43.8%). The data clearly show that the bioaccessibility of samples polluted with a specific Pb source can vary considerably. For example, bioaccessible Pb in soils polluted predominantly with gasoline Pb varies from 2.5% (sample MOER 427) to 32.6% (sample LBG 413).

5. Discussion

5.1. Anthropogenic Pb sources

To determine if the studied soils are actually polluted with the inferred Pb sources the calculated anthropogenic Pb isotope compositions are presented in Fig. 4. This figure also includes Pb isotope data of known anthropogenic Pb sources in The Netherlands, indicated with the ellipses. Unfortunately Pb isotope data are not available for the sources 'Pb bullets and pellets' and 'car battery Pb', sold and used in The Netherlands. Fig. 4 shows that made grounds have a very distinct Pb isotope composition that matches with coal/galena and with household waste that includes Pb-containing artefacts. Both coal and Pb containing artefacts such as remnants of Pb glazed pottery and roof tiles, Pb-based paint and Pb sheets (e.g., used for roofing), are visible in made grounds (Walraven et al., 1997, submitted for publication).

The Pb isotope composition of the soil samples inferred to be polluted with gasoline Pb (except sample LBG 408 and LBG 413), fall within the range of gasoline Pb used in The Netherlands (Walraven et al., 2014a). Sample LBG 408 and 413 fall just outside this range, but
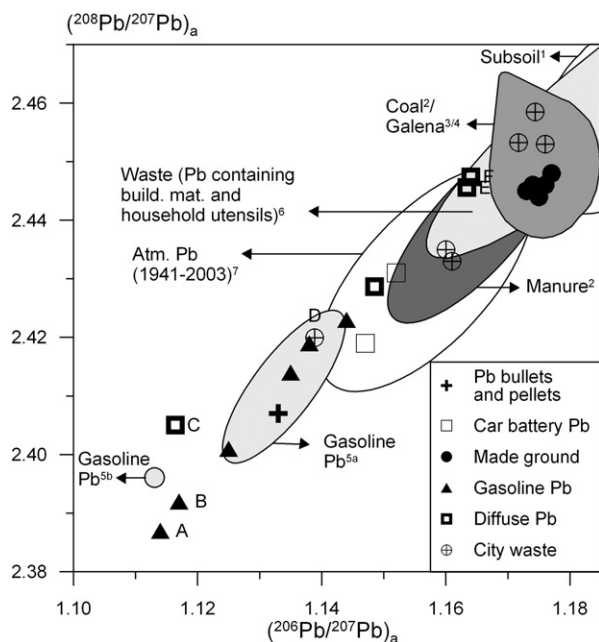
Table 4
Average Pb content and Pb isotope composition of the chyme and pellets, and calculated F_B-Pb (see text, Eq. (5)) of the studied soils.

Sample name	Inferred Pb source	Chyme				Pellet		
		Pb (mg/kg)	$^{206}Pb/^{207}Pb$	$^{208}Pb/^{207}Pb$	F_{B-Pb} (%)	Pb (mg/kg)	$^{206}Pb/^{207}Pb$	$^{208}Pb/^{207}Pb$
PU 1	Pb bul./pel.	869	1.148	2.414	60.7	322	1.136	2.424
PU 2	Pb bul./pel.	1865	1.138	2.412	79.0	746	1.131	2.421
PU 3	Car bat. Pb	406	1.155	2.434	19.8	1482	1.152	2.441
PU 4	Car bat. Pb	502	1.157	2.424	47.9	465	1.145	2.432
PU 6	Made ground	303	1.176	2.456	20.9	1023	1.175	2.460
PU 7	Made ground	155	1.171	2.451	15.3	764	1.172	2.455
PU 8	Made ground	74	1.176	2.455	11.4	414	1.176	2.457
PU 9	Made ground	102	1.183	2.451	11.1	628	1.173	2.454
PU 10	Made ground	71	1.181	2.452	9.5	612	1.174	2.453
PU 11	Made ground	120	1.181	2.455	20.2	578	1.175	2.453
PU 12	Made ground	70	1.172	2.452	12.6	413	1.173	2.450
MOER 428	Gasoline Pb	21	1.122	2.398	3.7	387	1.121	2.393
MOER 427	Gasoline Pb	16	1.141	2.415	2.5	403	1.133	2.409
LBG 404	Gasoline Pb	12	1.142	2.421	5.3	237	1.144	2.419
LBG 408	Gasoline Pb	20	1.114	2.390	7.1	237	1.115	2.384
LBG 413	Gasoline Pb	42	1.115	2.392	32.6	165	1.115	2.398
MOER 415	Gasoline Pb	72	1.137	2.419	12.8	294	1.141	2.422
T270	Diffuse Pb	9	1.156	2.433	32.1	19	1.166	2.451
T772	Diffuse Pb	2	1.191	2.460	7.4	23	1.199	2.477
T052	Diffuse Pb	19	1.171	2.445	9.9	183	1.173	2.467
T380	Diffuse Pb	3	1.156	2.431	27.3	9	1.168	2.452
WIJK 286	Diffuse Pb	3	1.170	2.444	6.8	37	1.181	2.460
PU 5	City waste	83	1.173	2.437	8.3	806	1.160	2.446
WIJK 297	City waste	11	1.124	2.406	5.7	179	1.141	2.413
WIJK 298	City waste	2	1.172	2.448	1.6	64	1.185	2.458
WIJK 317	City waste	7	1.167	2.447	1.7	194	1.178	2.452
WIJK 312	City waste	1	1.170	2.445	0.5	216	1.177	2.452
WIJK 321	City waste	339	1.154	2.435	43.8	708	1.161	2.436

Fig. 4. $(^{208}Pb/^{207}Pb)_a$ versus $(^{206}Pb/^{207}Pb)_a$ in Pb polluted soils in The Netherlands. Ellipses represent the natural Pb isotope composition of Dutch soils (subsoil) and the Pb isotope composition of potential anthropogenic Pb sources (gasoline Pb, atmospheric Pb, manure, waste, coal and galena ore). [1]Walraven et al., 2013a; [2]Walraven et al., 1997, 2013b; [3]Pasteels et al., 1980; [4]Cauet et al., 1982; Walraven et al., 2014a 1; [6]Walraven et al., 1997, submitted for publication; [7]Walraven et al., 2014b. A = sample LBG 408; B = sample LBG 413; C = sample T 380; D = sample WIJK 297; E = sample T 052; F = sample WIJK 286. The internal errors of analysis (2 SD) are smaller than the symbols.

match with gasoline Pb in neighbouring countries (Belgium: Petit et al., 1984; Germany: Krause et al., 1993). The Pb isotope composition of gasoline Pb in The Netherlands – as determined by Walraven et al. (2014a) – is based on roadside soil samples (n = 24) covering a limited proportion of the centre of The Netherlands. In addition all samples inferred to be polluted with gasoline Pb are taken within a distance of ~10 m from major Dutch highways close to the Dutch–Belgian border (Fig. 1; ~15 km). Consequently, we consider that samples LBG 408 and LBG 413 do contain gasoline Pb and that their Pb isotope compositions should be used to extend the range in Pb isotope compositions of gasoline Pb occurring in The Netherlands. Sample T 380, polluted with diffuse Pb, has a Pb isotope composition similar to gasoline Pb.

The main sources of diffuse Pb pollution in rural areas in The Netherlands are atmospheric deposition (including gasoline Pb) and manure (Groot et al., 1998; Walraven et al., 2013b). The Pb isotope composition of 3 (T 270, T 380 and Wijk 286) of the 5 soils inferred to be polluted with diffuse Pb actually match with the Pb isotope composition of atmospheric Pb and manure (Fig. 4). The Pb isotope compositions of manure and atmospheric Pb, however, overlap with Pb containing waste. In other words, two samples (T 052 and WIJK 286) could also contain city waste or made ground Pb instead of diffuse Pb. Based on the origin of sample T 052 (Nieuwkoop, situated in the so-called made ground area in The Netherlands) and the relatively high Pb content of this sample (192 mg/kg), it is highly likely that this sample is polluted with made ground Pb instead of diffuse Pb. The same argument does not apply to sample WIJK 286. Of the 2 other samples inferred to be polluted with diffuse Pb, sample T 380 is most likely polluted with gasoline Pb (see above) and T 772 is not polluted (see Table 2).

Five out of the 6 samples inferred to be polluted with city waste have the expected Pb isotope compositions (e.g. coals ashes and Pb-containing artefacts in waste, such as remnants of Pb glazed pottery and roof tiles, Pb-based paint and Pb sheets; Walraven et al., 1997, submitted for publication). The Pb isotope composition of three city waste samples (WIJK 298, WIJK 317 and WIJK 312) is very distinct

and matches with that of coal ashes and galena Pb. These values also agree well with the Pb isotope composition of manure, however, no significant amounts of manure are applied in the sampled urban areas. The Pb isotope composition of anthropogenic Pb in sample WIJK 297 does not match with Pb known to be present in city waste but corresponds with atmospheric Pb and gasoline Pb. This sample, however, is taken from a depth of 50 to 60 cm below surface, and since migration rates of atmospheric Pb or gasoline Pb in clayey soils are most likely low (ATSDR, 2007 and references therein), atmospheric Pb and gasoline Pb as the source of the pollution are highly unlikely. Hence the exact anthropogenic Pb source of this sample is unknown, but it is most likely Pb containing city waste with a Pb isotope composition not previously encountered (in The Netherlands).

The $^{206}Pb/^{207}Pb$ ratios of bullets sold in Israel and Norway vary from 1.10 to 1.30 and from 1.04 to 1.23 respectively (Zeichner et al., 2006; Sjåstad et al., 2014). The $^{206}Pb/^{207}Pb$ values of the soils inferred to be polluted with Pb bullets and pellets fall within these ranges (Table 2). Although Pb isotope data are not available for the sources 'Pb bullets and pellets' and 'car battery Pb', sold and used in The Netherlands, the EMPA results confirm (Fig. 2 and Table 3) that Pb bullets and pellets are present in the soil. Such evidence is not available for the soils inferred to be polluted with car battery Pb, except that the soil samples were taken at the premises of a company restoring and recycling car batteries.

Soil Pb isotope ratios are highly correlated ($p < 0.05$) with chyme Pb isotope ratios ($R^2 = 0.87$ for $^{206}Pb/^{207}Pb$; $R^2 = 0.86$ for $^{208}Pb/^{207}Pb$) and residual pellet Pb isotope ratios ($R^2 = 0.98$ for $^{206}Pb/^{207}Pb$; $R^2 = 0.89$ for $^{208}Pb/^{207}Pb$). This is an indication for the presence of a single (dominant) anthropogenic source in each soil samples and confirms the initial selection criteria of the sample sites was correct. In summary, except for sample T 052, all studied soil samples appear to contain the inferred single dominant anthropogenic Pb source.

5.2. Factors controlling Pb bioaccessibility

Before anthropogenic Pb enters the soils, its bioaccessibility is initially controlled by the chemical form and particle size distribution (Fig. 5; adjusted after Ruby et al., 1999). When anthropogenic Pb enters the soil, however, it may dissolve and be redistributed via adsorption on, or precipitation with other soil constituents, such as organic matter, reactive iron or other reactive phases (Ruby et al., 1999). These processes may drastically change the (orginal) bioaccessibility of Pb.

5.2.1. Mineralogy and particle size of anthropogenic Pb sources

Combustion of gasoline produces Pb halides (e.g. PbBrCl) in fresh exhaust that alter to Pb carbonates, Pb oxides, and Pb sulphates due to photochemical induced reactions during atmospheric transport (Olson and Skogerboe, 1975). Primary vehicle exhaust Pb, comprises particles of around 0.015 μm in diameter, and may be attached to particles of carbon of similar dimensions. (Chamberlain et al., 1979). These particles combine with other particles in the atmosphere and grow to around 0.1 to 1.0 μm (Chamberlain et al., 1979). According to Fig. 5 , the relative soluble Pb halides, oxides and sulphates (Olson and Skogerboe, 1975), and their small particle size (Chamberlain et al., 1979), would make combusted gasoline Pb the most bioaccessible Pb source in our study.

Diffuse Pb in The Netherlands consists of 65–95% atmospheric Pb and 5–35% of Pb in manure/fertiliser (Groot et al., 1998). Since atmospheric Pb also contains combusted gasoline Pb, with particle sizes in the same range, it is assumed that this fraction is equally bioaccessible as gasoline Pb. Although not studied, the fraction of diffuse Pb, originating from manure, is most likely not very bioaccessible since it was excreted and not taken up by the animal that produced the manure. Therefore, diffuse Pb is expected to be less bioaccessible than gasoline Pb.

Made ground and city waste can contain various anthropogenic Pb sources like paint flakes, remnants of Pb glazed potsherds, glass and Pb sheets. The most common forms of Pb in paint are red lead [Pb_3O_4],
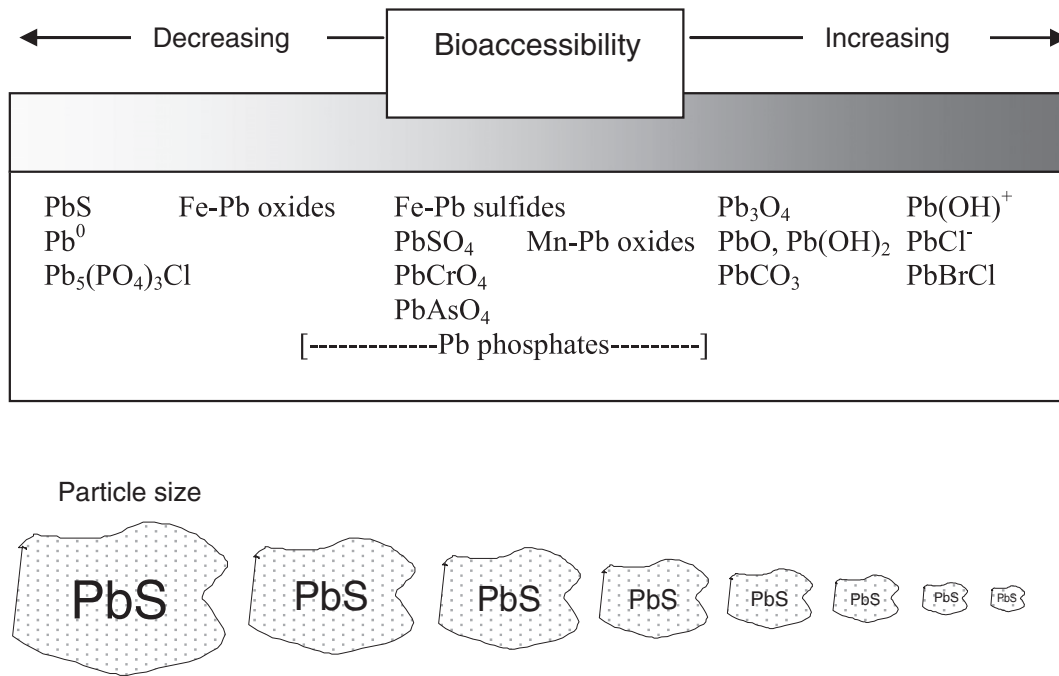
Fig. 5. Schematic overview of how different lead compositions and particle sizes affect Pb bioaccessibility. (Adapted from Ruby et al., 1999, with permission).

white Pb [$2PbCO_3.Pb(OH)_2$], Pb sulphate [$PbO_2.PbSO_4$] and Pb chromate [$PbCrO_4$] (Heaton, 1940). Lead paints were manufactured by drying the Pb pigments in sheets, stamping out small particles (approximately 1–10 μm) of the material, and then mixing these particles in a binding material. Consequently, when paints weather in soil they release small particles of Pb, which are likely to be highly bioavailable due to the solubility of Pb oxide and Pb carbonate species and the small particle size (Ruby et al., 1999). Lead oxide (PbO and Pb_3O_4) is added to ceramic glaze to obtain a low melting point and the greatest possible luster as well as to stabilize glass (Grandjean, 1975). Lead sheets used for gutter and water linings also weather when used and end up in soils where weathering continues. Pb based gutters and water linings mainly consist of native Pb. In summary, Pb phases in made ground and city waste can vary from native lead with low bioaccessibility to Pb oxides that are more bioaccessible. The Pb phases in made grounds and city waste are larger in size (Table 3) than gasoline Pb and diffuse Pb, and hence less bioaccessible.

A typical Pb-acid battery contains ~10 kg of Pb (Waste360, 2012). The Pb species present in Pb-acid batteries are both native Pb and Pb sulphate. In contrast a new Pb pellet contains mainly Pb (97 wt.%), Sb (2 wt.%), As (0.5 wt.%) and sometimes Ni (0.5 wt.%) (Lin et al., 1995). The metals of jacketed bullets contain Pb (90 wt.%), Cu (9 wt.%) and Zn (1 wt.%) (Tanskanen et al., 1991). The most common form of Pb in bullets and pellets is native lead (Ruby et al., 1999). Car battery Pb and Pb bullets and pellets are assumed to be the least bioaccessible of the Pb sources under consideration, since they consist largely of native lead, which is relatively non-bioaccessible (Fig. 5). In addition, the size of the bullets and pellets is larger than that of gasoline Pb and diffuse Pb (Table 4).

Based on the above discussion the bioaccessibility of the Pb sources (before entering the soil) is expected to decrease in the following order:

Gasoline Pb > Diffuse Pb > Made ground ≈ City waste > Car battery Pb ≈ Pb bullets and pellets

To visualise the measured bioaccessibility, the Pb isotope composition of the various anthropogenic Pb sources, is plotted against calculated bioaccessibility (F_B-Pb) in Fig. 6. This figure shows that the highest bioaccessibilities are measured in the samples polluted with Pb bullets and pellets (F_{B-Pb} = 60.7–79.0%) and the lowest bioaccessibilities in the samples polluted with gasoline Pb (F_B-Pb = 2.5–32.6%) and city waste (Fb = 0.5–8.3%). The measured bioaccessibility decreases in the following order:

Pb bullets and pellets > Car battery Pb > Made ground ≈ Gasoline Pb ≈ Diffuse Pb > City waste

The measured bioaccessibilities are different from the presumed bioaccessibilities of the studied Pb sources before entering the soil, based on chemical composition and particle size. These observed differences, are a strong indication of the importance of the influence of soil composition on bioaccessibility of Pb. When anthropogenic Pb phases enter the soil, weathering, dissolution and, new mineral formation starts, resulting in the formation of smaller particles and the formation of more stable minerals under the prevailing soil conditions (e.g., Ruby et al., 1999). Reactive phases present in the soil (e.g. organic matter, reactive Fe, clay and calcium carbonate) play an important role in these processes (e.g., Ruby et al., 1999; Hettiarachchi and Pierzynski, 2004; Finžgar et al., 2007; Zia et al., 2011).

Others also determined the Pb bioaccessibility of Pb polluted soils (e.g., Madrid et al., 2008; Smith et al., 2011; Appleton et al., 2012; Luo et al., 2012;). Comparison studies have shown that the bioaccessibility methodologies used in these studies differ substantially (Oomen et al., 2002; Van de Wiele et al., 2007), hence a quantitative comparison between the various studies cannot be made. Nevertheless, general comparisons can be made. Smith et al. (2011), for example, assessed Pb bioaccessibility in a range of Pb contaminated peri-urban soils, including shooting range soils, and soils affected by incinerator, historical fill, mining/smelting, and gasworks activities. Generally, Pb bioaccessibility was highest in the shooting range soils, intermediate in the historical fills and slightly lower in the incinerator impacted soils (50–105.2%, 35.7–85.1% and 60.9–64.1% respectively in the gastric phase; Smith et al., 2011). This finding is in agreement with our observations, assuming that incinerator impacted soils are comparable with diffuse Pb (atmospheric Pb) and landfills are comparable with made grounds and city waste. In most other studies, anthropogenic Pb sources were not identified or other Pb sources were involved (e.g. mining and smelting
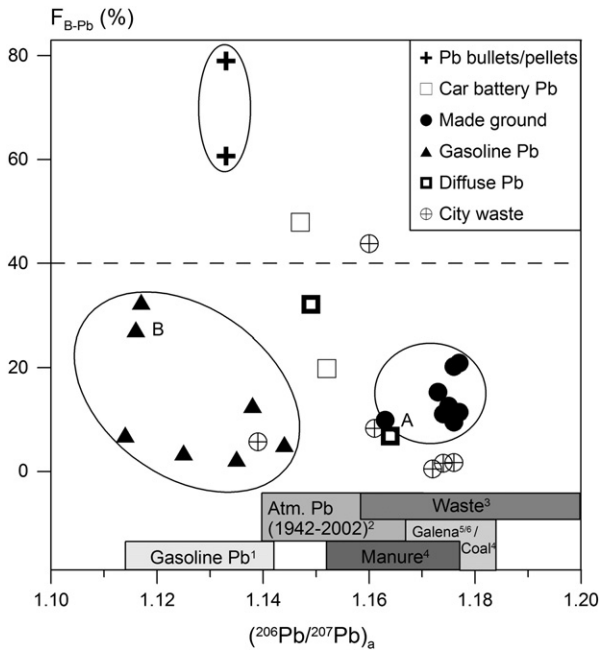
Fig. 6. F_{B-Pb} versus $(^{206}Pb/^{207}Pb)_a$ of soils polluted with either Pb bullets and pellets, car battery Pb, gasoline Pb, made ground, diffuse Pb or city waste. Ellipses indicate samples polluted with Pb bullets and pellets, gasoline Pb and made ground. [1]Walraven et al., 2014a; [2]Walraven et al., 2014b; [3]Walraven et al., 1997, submitted for publication; [4]Walraven et al., 2013b; [5]Pasteels et al., 1980; [6]Cauet et al., 1982. A = sample T 052; B = sample T 380. The internal errors of analysis (2 SD) are smaller than the symbols.

impacted soils) and therefore a general comparison with the Dutch (urban) soils cannot be made.

5.2.2. Role of soil characteristics

To assess the influence of soil characteristics on Pb bioaccessibility, F_B-Pb is compared to different soil characteristics in Fig. 7. In the aeolian sands there is a significant ($p < 0.05$) negative correlation between F_{B-Pb} and the OM content ($r = -0.81$) and Fe_{react} content ($r = -0.81$), and a positive correlation between F_{B-Pb} and pH ($r = 0.77$) (Fig. 7a–c; Table 5). These soil characteristics, however, also show significant inter-correlations (Table 5). This means that one, two or all three characteristics can influence bioaccessibility. The Spearman correlations do not give a definitive answer to this question.

EMPA provide evidence that organic matter influences F_{B-Pb}. Fig. 3-C and -D show that Pb is associated with organic matter in soils polluted with Pb bullets and pellets (PU 2) and gasoline Pb (MOER 415). Organic matter particles/flakes in these samples contained organically complexed Pb and secondary Pb minerals in the organic cell structure — with contents ranging from 0.5 wt.% up to 17 wt.%. Morin et al. (1999) recognized organic matter as the main sink of Pb in organic-rich soil in France and Zimdahl and Skogerboe (1977) found that most of the lead fixed in USA soil is associated with organic matter.

According to Eq. (7) reactive Fe is (nearly) absent in sample PU 2 and MOER 415 (Table 2). Nevertheless, these samples were also analysed with EMPA for the presence of reactive Fe and the association with Pb. However, no reactive Fe was encountered with the EMPA in these samples. In other studies, it was found that Pb has a strong affinity for Fe-oxides in soils. Based on a sequential extraction, Emmanuel and Erel (2002) summarized the affinity of Pb in Czech soils as Fe-oxides > organic matter > silicates and the order in Mediterranean soils appears to be Fe-oxides > carbonates > organic matter > silicates. In our study the association of reactive Fe with Pb is (only) observed in the Spearman correlation (Fig. 7b; Table 5).

Several researchers have emphasized the influence of soil pH on the bioaccessibility of Pb. Ruby et al. (1999) state that mineral phases that form under acidic conditions (e.g. Pb sulphate, Fe-Pb sulphate) will tend to be more stable in the acidic conditions of the stomach and hence less bioaccessible. In contrast, mineral phases that form under alkaline conditions (e.g. Pb carbonate, Pb oxide) will be less stable in the acidic conditions of the stomach and more bioaccessible. Although the correlation coefficient is not very high (Table 5; $r = 0.77$), Fig. 7c shows that the lowest F_B-Pb values are measured in the most acidic
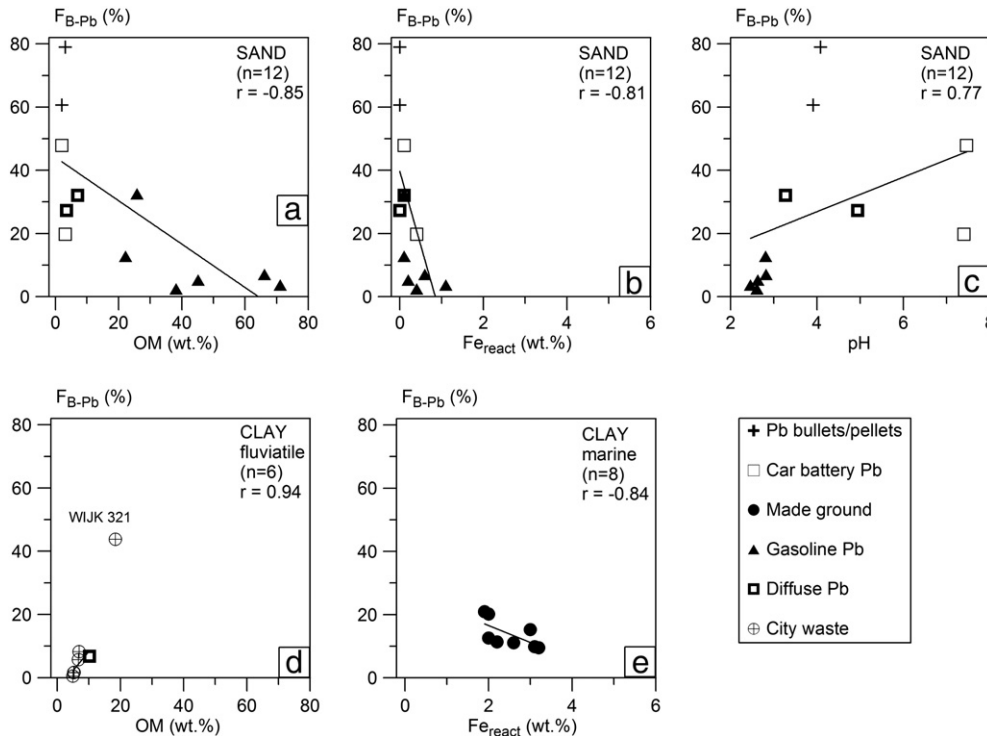

Fig. 7. Soil characteristics that show a significant correlation ($p < 0.05$) with Pb bioaccessibility, versus F_B-Pb for the three soil types (aeolian sands, fluviatile clay and marine clay) polluted with the various anthropogenic Pb sources.

Table 5
Spearman correlation matrix of $F_{B\text{-}Pb}$, soil pH and content of Fe_{react}, OM, $CaCO_3$, total Pb and clay for the three soil types (aeolian sand, fluviatile clay and marine clay). Significant correlations ($p < 0.05$) are indicated in italics.

Sand (n = 12)	Fe_{react}	OM	$CaCO_3$	pH	Total Pb
$F_{B\text{-}Pb}$	−0.81	−0.81	−0.06	0.77	0.26
Fe_{react}	******	0.72	−0.08	−0.55	0.00
OM		******	−0.34	−0.85	−0.45
$CaCO_3$			******	0.11	0.20
pH				******	0.30

Marine clay (n = 8) Fluviatile clay (n = 6)	$F_{B\text{-}Pb}$	Fe_{react}	OM	$CaCO_3$	pH	Total Pb	Clay
$F_{B\text{-}Pb}$	******	−0.84	−0.26	−0.56	0.26	0.38	−0.46
Fe_{react}	0.75	******	0.00	−0.27	−0.11	−0.16	0.53
OM	0.94	0.75	******	−0.23	0.24	−0.44	−0.49
$CaCO_3$	−0.03	−0.38	−0.26	******	0.31	0.54	−0.12
pH	−0.43	−0.64	−0.60	0.83	******	0.10	−0.36
Total Pb	−0.14	−0.23	−0.14	0.94	0.77	*******	−0.40
Clay	0.43	0.64	0.60	−0.83	−1.00	−0.77	*******

sandy soils (pH-KCl = 2.5–2.8), which are the soils polluted with gasoline Pb. Higher bioaccessibilities are measured in less acidic soils such as the soils polluted with Pb bullets and pellets (pH-KCl = 3.9–4.1) and with car battery Pb (pH-KCl = 7.4–7.5). These results indicate that pH might influence $F_{B\text{-}Pb}$ in (acidic) sandy soils in The Netherlands.

There is also a significant ($p < 0.05$) negative correlation between Fe_{react} and $F_{B\text{-}Pb}$ ($r = -0.84$) in the marine clays (Fig. 7e; Table 5). EMPA were performed on marine clay sample PU 6 but no reactive Fe was encountered. The Fe_{react} content of this sample is 1.9 wt.% (Table 2). The particle size of the reactive Fe phases appears to have been too small to be detected (<1 μm). A significant negative correlation between Fe_{react} and $F_{B\text{-}Pb}$ was also observed in the aeolian sands (Fig. 7b). This supports the indication that reactive Fe might influence oral bioaccessibility of Pb in Dutch soils.

Fluviatile clays record a significant ($p < 0.05$) positive correlations between $F_{B\text{-}Pb}$ and OM ($r = 0.94$). It is notable that OM shows a positive correlation with $F_{B\text{-}Pb}$ in the fluviatile clays and a negative correlation in the sandy soils. It is noted, however, that only 6 fluviatile clays were studied (n = 6) and there is a limited range of the $F_{B\text{-}Pb}$ and OM values. We conclude that further study of fluviatile clays polluted with Pb is needed before significance can be given to the observed relationships.

Although the number of samples studied is limited, this study shows that bioaccessibility of Pb in soils (both sands and clays) is correlated with pH, organic matter and reactive Fe. This is in agreement with the findings of others who have shown that lead bioavailability is largely controlled by phosphate, iron oxides, organic matter and pH (Zia et al., 2011; and references therein). The presence of iron(oxy)hydroxides – among others, goethite and ferrihydrite – and organic matter creates surface sorption or chelation sites for binding Pb^{2+}, which results in binding and precipitation of Pb (e.g., Finžgar et al., 2007; Zia et al., 2011). In a similar way, an increase in pH also decreases Pb mobility and bioavailability as fewer H^+ ions are available to compete with Pb^{2+} ions for binding sites (Hettiarachchi and Pierzynski, 2004; Zia et al., 2011). Rieuwerts et al. (1998a,b) demonstrated that pH is a consistent predictor for the extractability of Pb from soils. These studies support our research findings. Further study is needed, however, to determine if the observed correlation between oral bioaccessibility of Pb and soil characteristics holds on a larger scale.

5.2.3. Additional factors controlling the Pb bioaccessibility of soils

Three Pb sources (Pb bullets and pellets, gasoline Pb and made ground Pb) were examined in more detail. Based on the EMPA results and the indications that bioaccessibility in the studied soils is influenced by pH, reactive Fe and organic matter, the measured bioaccessibilities of these samples can be better understood. The actual bioaccessibility of

soils polluted with Pb bullets and pellets was higher than expected based on chemical composition and particle size. Although Pb bullets and pellets consist of relatively insoluble native Pb (Fig. 5), native Pb was transformed to more soluble Pb oxides after entering the soil (Table 3; Fig. 3-A). Dissolved Pb migrated through the soil and formed new, more stable, Pb phases associated with organic matter under acidic conditions (Fig. 3-D). The partly dissolved Pb bullets and pellets would be most likely bioaccessible after oral ingestion, whereas the secondary Pb phases associated with organic matter are not. It is proposed that bioaccessibility of Pb in these samples (polluted with Pb bullets and pellets) will decrease in time when the bullets and pellets dissolve and more stable (under acidic conditions) organo-Pb phases are formed.

Comparable processes are also observed in soils polluted with gasoline Pb. After combustion of leaded gasoline, relatively mobile Pb phases entered the roadside soils (e.g., Pb halides, Pb oxides and Pb sulphates). These phases either dissolved and migrated towards the groundwater (Walraven et al., 2014a) or formed more stable phases in association with organic matter (Fig. 3-C). No primary gasoline Pb phases (Table 3) were encountered with the EMPA and Pb is now only present in secondary Pb phases. The bioaccessibility of gasoline Pb was most likely very high when it entered the soils (mainly in the 1960s and 1970s) and decreased after the transformation of the soluble primary Pb phases to more stable secondary organo-Pb phases, i.e., aged gasoline Pb (Laxen and Harrison, 1977; and references therein).

Soils polluted with made ground Pb contain glazed ceramics, glass, painted wood, metal slag and native Pb sheets. EMPA confirmed the presence of Pb aluminosilicates, native Pb and Pb oxides. High Pb contents in organic matter particles are less frequently encountered in the made grounds compared with soils polluted with Pb bullets and pellets and gasoline Pb. This observation implies that the primary Pb phases in made grounds are not very soluble. Since made ground Pb was present in the soils for centuries (100 to 400 years) this argues for a low solubility of made ground Pb, despite of the low soil pH (pH-KCl = 4.8–5.5). The low soil pH might explain the relatively low bioaccessibility of made ground Pb. Mineral phases that do not dissolve under acidic conditions will probably also be stable in the acidic conditions of the stomach and hence less bioaccessible (e.g., Ruby et al., 1999).

Time is probably also an important factor in determining oral bioaccessibility of Pb. Assuming that oral bioaccessibility of Pb eventually decreases in time, samples WIJK 297 and 298 are expected to have among the lowest $F_{B\text{-}Pb}$ values since these soils were polluted between 500 and 1500 A.D (Walraven et al., submitted for publication; and references therein). With bioaccessibility values of 5.7% and 1.6% this suggestion is confirmed. We conclude that the pollution date or period is an interesting factor to include in future research into factors controlling bioaccessibility.

5.3. Implications for risk assessment

The Dutch intervention value for Pb is 530 mg/kg for standard soils (25% clay and 10% organic matter). Soils with a higher Pb content are classified as 'seriously' contaminated (Swartjes, 1999). In case of a serious soil contamination, the contaminated site has, in principle, to be remediated. The need for remediation, however, is decided on the basis of actual risk to humans and ecosystems and the actual risk due to migration of the contamination. Dutch risk assessment for Pb is based on criteria laid down by FAO/WHO (1993) and IPCS (1995). It is recommended to avoid Pb blood levels above 50 μg/l, resulting in a provisional tolerable weekly intake (PTWI) of 25 μg/kg body weight per day, which is based on absorption ($F_B \times F_A$) of 40% dietary Pb (Oomen et al., 2003). This value for the absorption of dietary Pb is based on toxicity studies. Media that are typically employed in toxicity studies are food, suspensions, and liquids such as water (Mushak, 1991; Paustenbach et al., 1997). Freeman et al. (1992) and Dieter et al. (1993), however, showed that the oral bioavailability of Pb in soil

determined with *in vivo* studies using laboratory animals can be significantly less than measured for contaminants in matrices such as food and liquids. Therefore, current health risk assessment may cause a substantial overestimation of the oral bioavailability for Pb in soils.

Several researchers (e.g., Ruby et al., 1999; Oomen et al., 2003) introduced a relative bioavailability factor ($F_{relative}$) to compare oral bioavailability of Pb in soils with bioavailability of Pb based on toxicity studies. $F_{relative}$ is calculated according to Eq. (8).

$$F_{relative} = F_{soil} / F_{tox.studies}$$
$$= (F_{A-soil} \times F_{B-soil} \times F_{H-soil}) / (F_{A-tox.studies} \times F_{B-tox.studies} \times F_{H-tox.studies})$$
(8)

in which $F_{relative}$ is the relative bioavailability of Pb, F_{soil} is the bioavailability of Pb in soils, F_{B-soil} is the bioaccessible Pb fraction in soil (the fraction that is mobilized from soil into the digestive juice, i.e., chyme), F_{A-soil} is the bioaccessible Pb fraction from the soil entering the portal vein or lymph, F_{H-soil} is the Pb fraction that entered the portal vein or the lymph and passes through the liver without being metabolized (this may exert toxicity in organs and tissues), $F_{tox.studies}$ is the bioavailability of Pb determined in toxicity studies (mainly matrices such as foods and liquids), $F_{B-tox.studies}$ is the bioaccessible Pb fraction determined in toxicity studies, $F_{A-tox.studies}$ is the bioaccessible Pb fraction entering the portal vein or lymph determined in toxicity studies and $F_{H-tox.studies}$ is the Pb fraction that entered the portal vein or the lymph and passes through the liver without being metabolized, determined in toxicity studies.

Due to their frequent hand-mouth behaviour, children are the most vulnerable group with respect to soil ingestion. No *in vivo* studies, however, are described in literature in which F_{A-soil} is established for children. Therefore, F_{A-soil} is assumed to be 1 for children, as a worst case scenario. Since inorganic Pb is not metabolized in the liver (Oomen et al., 2006), F_H in both soil and toxicity studies is 1. In current risk assessment $F_{B-tox.studies} \times F_{A-tox.studies}$ is set at 0.4 (based on absorption of 40% dietary lead). Based on these values, Eq. (8) becomes:

$$F_{relative} = (F_{B-soil} \times 1 \times 1) / (0.4 \times 1) = F_{B-soil} / 0.4.$$

The implication is that if F_{B-soil} is lower than 40%, the bioavailability of Pb in the polluted soils is overestimated in current risk assessment ($F_{relative} < 1$). If F_{B-soil} is higher than 40%, the risk for children are underestimated in current risk assessment ($F_{relative} > 1$). Fig. 6 shows that 24 samples have a bioaccessibility lower than 40%. This means that the risk for children may be overestimated in current risk assessment in The Netherlands. However, 4 soil samples (2 Pb bullets and pellets, 1 Pb car battery and 1 city waste), have Pb contents >530 mg/kg and bioaccessibilities >40%. For this reason, the risk for children at sites like these may be underestimated at the moment and hence the urgency of remediation of these highly polluted sites should be reconsidered.

6. Conclusions

The oral Pb bioaccessibility (F_{B-Pb}), determined with an *in vitro* test, of soils (aeolian sands, and marine and fluviatile clays) polluted with various anthropogenic Pb sources varies from 0.5 to 79% and decreases in the following order:

Pb bullets and pellets > Car battery Pb > Made ground Pb ≈ Gasoline Pb ≈ Diffuse Pb > City waste

This inferred bioaccessibility order differs from that predicted based on the chemical composition and particle size of the anthropogenic Pb sources alone. This implies that soil composition (pH, and organic matter, clay, reactive Fe and calcium carbonate content) – and possibly some other factors – influences oral bioaccessibility. Statistical analyses

indicate that oral bioaccessibility of Pb depends on soil pH, and the content of organic matter and reactive Fe.

This study shows that in current risk assessment in The Netherlands, the risk of Pb polluted soil to children may be generally overestimated. In 85% of the studied samples (24 out of 28) the 'in vitro based' bioavailability is lower than the bioavailability based on the toxicity studies – in which food, suspensions and liquids are used as media containing Pb – which underlie the current Dutch risk assessment. In 15% of the studied samples (4 out of 28), however, the risk to children may be underestimated in current Dutch risk assessment. For this reason, the remediation urgency of such sites should be reconsidered.

The results of this study indicate that oral bioaccessibility of Pb depends on 1) the chemical composition of the anthropogenic Pb source and its solubility, 2) the specific reactive surface of Pb in soils and 3) soil type, and capacity to form secondary Pb phases. We propose to base future human risk assessment of Pb on *in vitro* bioaccessibility tests, taking factors such as soil pH, and organic matter and reactive Fe content into account, instead of basing risk on total Pb content, land use and toxicity studies based on media such as food, suspensions and liquids alone.

Due to the limited number of samples analysed this study is only of an indicative character. Further study is needed to determine if the observed relations between bioaccessibility of Pb and soil characteristics hold. The bioaccessibility, soil characteristics and chemical composition of primary and secondary Pb phases of more soils polluted with various anthropogenic Pb sources need to be determined. Interesting polluted soils for study would be: clayey soils polluted with gasoline Pb, Pb bullets and pellets and car battery Pb and (aeolian) sand polluted with made ground or city waste. It would also be potentially useful to determine the chemical composition of the secondary Pb phases in more detail to provide a more detailed understanding of Pb mobility in soils.

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