UNIVERSITA' DEGLI STUDI DI PADOVA

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MSc in Environmental Engineering

Assessment of As contaminated soil stabilized with Iron amendments by laboratory and field experiments

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Table of Content

PART I

LITERATURE REVIEW

I. LITERATURE REVIEW

I.1 INTRODUCTION

The potential contaminated sites in Europe are estimated at 2.5 million, while there are around 342 000 sites in which the contamination has been verified and it is already confirmed that they represent a risk to human, water and ecosystem or other receptor are. Heavy metal and metalloid contaminations are the 35% of the overall amount. The management of contaminated site is estimated to cost around 6 billion euros per year (Panagos et al., 2013). The EPA has classified arsenic as a Class A human carcinogen. Ingestion of inorganic arsenic can result in both cancer and non-cancer health effects (NRC, 1999). Chronic exposure to low arsenic levels (less than 0.050 mg/L) has been linked to health complications, including cancer of the skin, kidney, lung, and bladder, as well as skin diseases and neurological and cardiovascular system conditions (US EPA, 2000). Common sources of contamination include the erosion of natural deposits, pesticide runoff from orchards, and runoff from glass & electronics production wastes (Treatment Technologies for Arsenic Removal EPA, 2005).

Arsenic contamination may occur due to a high natural abundance of this element in rocks and soils, but also as a result from human activities, such as mine tailings, coal combustion, CCA wood preservative or arsenic based pesticides (McAuley and Cabaniss, 2007; Nriagu et al., 2007).

Arsenic is present in more than 200 minerals; being arsenopyrite (FeAsS) the most abundant, but other sulphide minerals, phosphate minerals and oxide minerals are also common (Garelick et al., 2008; Klein, 1999). Volcanic rocks containing high arsenic concentration are another important source of arsenic (Garelick et al., 2008; Smedley and Kinnburgh, 2002; Nriagu et al., 2007).

Some of the main industrial processes that contribute to the arsenic contamination are mining, smelting of non-ferrous metals and combustion of fossil fuels (Garelick et al., 2008; ATSDR, 1997). Historically, arsenic pesticides have extensively polluted agricultural areas (Han et al., 2003; Nriagu et al., 2007). Another potential contributing source of arsenic in soil is the use of the arsenic in the wood preventive treatments (Stilwell and Gorny, 1996; Nriagu et al., 2007).

Chromate cooper arsenate (CCA) is a wood preservative that has been used since the 1930's (Hunt and Garratt, 1953). A common composition of CCA is 47.4 % CrO_3, 18.5 % CuO and 34 % As_2O_5. The soil contaminated by CCA can be found next to CCA-treated wood, near wood impregnation industries and at areas contaminated by accidental spillage of CCA (Dobran and Zagury, 2006).

I.2 ARSENIC CHEMISTRY

I.2.1 General information

As has the outer electron configuration of s^2p^3 and belongs to the subgroup V of the Periodic Table. Arsenic occurs in environmental system at oxidation state (III) and (V) as arsenite and arsenate. Since the mobility, solubility, bioavailability and toxicity of As is strongly influenced by its oxidation state, it is fundamental to study the speciation of arsenic to better understand the behavior of As in soil (Masscheleyn et al., 1991). In general As is more mobile under alkaline and more saline conditions (Matera and Le Hecho, 2001).

Soil is a complex system, the main factors influencing As chemistry in soils are soil solution chemistry, solid phase formation, adsorption and desorption, effect of redox conditions, biological transformation, volatilization and cycling of As in soil (Sadiq, 1997). Arsenic becomes an health issue when is in aqueous phase rather than solid phase. The processes involve in this transition are mainly adsorption/desorption, precipitation/co-precipitation and changing from aerobic to anaerobic condition (Fendorf et al., 2010). As can be strongly retained in soil, and the extent of retention influence its bioavailability and mobility. Understand the geochemical cycling leads to better assess the risk associate to different targets (Wilson et al., 2010).

As can be found as organic or inorganic forms. The most widespread species of organic forms are methylated ones, though organoarsenical complexes are a minor fraction of total dissolve As in soil solution (Sadiq, 1997).

The order of toxicity is given as:

organoarsenicals(methylated species) < arsenates (As(V)) < arsenites (As(II)) (Wilson et al., 2010).

The World Healt Organisation has set the Acceptable Daily Intake(ADI) for As at $2.1\ \mu g\ Kg^{-1}\ day^{-1}$ per Kg of body weight (WHO, 2011), while the limit of As concentration in drinking water is $0.01\ mg\ L^{-1}$.

I.2.2 Condition affecting arsenic valence and speciation

As oxidation state is largely influence by pH, redox potential and environment reaction in soil system as presence of iron, sulfur and calcium ions, and microbial activity (Sadiq, 1997; U.S. EPA, 2002).

The speciation of As in soil in essential to understand the behavior of arsenic compound in soil. In general As(V) dominate in oxidizing conditions, while As(III) prevail on reducing environment. In soil they coexist due to the variation of environmental condition and due to the slow transition rate from one species to the other. In general is demonstrate that As(V) is less biologically toxic (Mok and Wai, 1990), more soluble and mobile than As(III). Moreover As(V) sorbs more strongly than As(III).

Within an environmental acceptable pH range as 2 < pH < 9, the predominant arsenic species are:
H_3AsO_4 for As(III), $H_2AsO_4^-$ and $HAsO_4^{2-}$ for As(V). That means arsenic compound can be neutrally or negatively charges based on which As oxidation state (Matera and Le Hecho, 2001).
Arsenic occurs naturally in the environment deriving from weathering of soil parent materials, as arsenopyrite ($FeAsS$), orpiment (As_2S_3), realgar (AsS) and also As metal. Variation in background concentration is function of the presence of parent material and the mineralization (Wilson et al., 2010).
The combined values of pH and redox potential affect the As forms found in soil. As shown in fig. 1, under oxidizing condition the predominant species is As(V) with a charge that depend on pH value, but mainly negatively charged at feasible environmental condition. While under moderately reducing condition, As(III) is the predominant species, and it is present as uncharged form (Sadiq, 1997). In the graph the redox couple $Fe(OH)_3/Fe(II)$ and $MnOOH/Mn(II)$ are marked, since both can be involved and scavengers of As. Since both iron and manganese oxides and hydroxides exist in several degrees of crystallinity, those boundaries may vary among different soils (Masscheleyn et al., 1991).

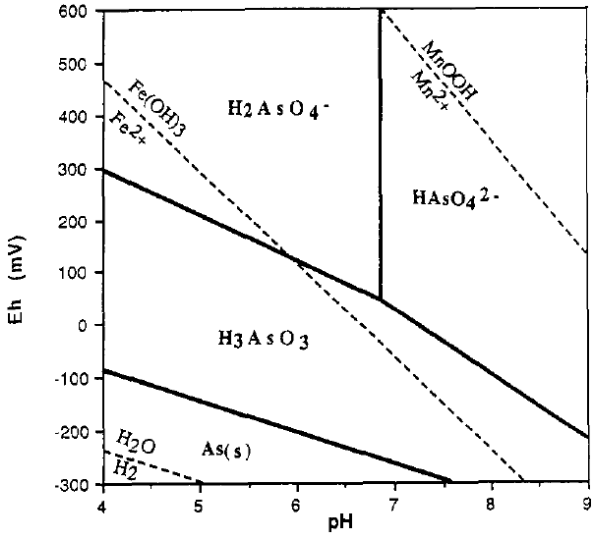

Figure. 1(I): Eh – pH diagram for As - H_2O system (Masscheleyn et al., 1991)

In environmental biogeochemical system the speciation is not at equilibrium, then the analysis of pH and Eh influence is not as sharp as shown in this type of diagram (Mok and Wai, 1990).
In this context the total As concentration is not a good indicator of mobility and toxicity of the contamination, thus it is important the sequential chemical soil extraction as tool to assess different As forms present (Ascar et al. 2008).

I.3 PROCESSES AFFECTING AS MOBILITY

I.3.1 Adsorption/desorption

These processes affect the As solubility and thus As mobility in the environment. The main compounds involved in the arsenic adsorption are clay, carbonaceous material, oxides of aluminum, iron and manganese (Sadiq, 1997; U.S. EPA,2002). The main factors affecting the adsorption are affinity of As with the adsorbent, temperature at which the process occurs and presence of competitive compounds for the available sites (Carabante et al., 2009). Furthermore the partitioning of arsenic onto soil solids depend on its oxidation state: As(V) can strongly bind to mineral and sediment in soil, while As(III) retention is related to specific soil condition (Fendorf et al., 2010).

I.3.2 Precipitation/co-precipitation

Direct precipitation of As solid phase can occur only in contaminated soil, but more common is the precipitation of soil colloid after As adsorption (Sadiq, 1997).
Adsorption and precipitation of As with Ca phases in neutral to alkaline calcareous soils is important retaining mechanism (Matera and Le Hecho, 2001). Both As(V) and As(III) may precipitate in soil, but the constituents inducing precipitation change. As(V), similar to phosphate, tends to precipitate with hard, multivalent cations such as aluminum and ferric-iron under acidic conditions and calcium and magnesium under alkaline conditions; arsenate may also replace SO_4^{2-} or, in particular, PO_4^{2-} in minerals due to similar size and charge characteristics (Smedley and Kinniburgh, 2002).

I.3.3 Aerobic/anaerobic condition

The greatest probability of As release in soil in when occurs the transition from aerobic to anaerobic condition. Indeed under saturated conditions, the consumption of O_2 by aerobic microbes combined with the decreasing of O_2 presence, induce anaerobic bacteria to utilize alternative electron acceptors. In this state arsenic may be displace through reduction from arsenate to arsenite or through mineralogical transformation of the soil matrix, as reductive dissolution of ferric oxides or hydroxides (U.S. EPA, 2002). In soil under anaerobic condition (100-200 mV) the prevalent species is arsenate, occurring in different oxianions based on pH value as already explained. When the soil undergo flooding condition, it may reach reducing condition (Eh < 100 mV), and the predominant species is arsenite (Ascar et al. 2008).

I.4 METAL-CONTAMINATED REMEDIATION TECHNIQUES

Remediation techniques used to treat contaminated soil can be classified in three main categories: in situ and ex situ treatments, and containment. In the last years has been studied how to enhance the first two options, try to overtake the landfilling or off-site disposal procedure due to economic and environmental reasons. The main ways to implement, both in end ex situ, are basically biological, physical-chemical or thermal treatments. Those principles are then apply differentially in or ex situ.

The advantage of in situ treatment with respect to the ex situ one is to avoid the excavation and transport of the material, and so the cost connected with these operations. For in situ application is important to control which are the by-products of the process apply, because they may be mobilized downward or being more soluble or toxic compound; it generally requires longer time periods, and the uniformity of the treatment is uncertain due to the heterogeneity of the soil and thus the effectiveness of the process is more difficult to monitor (FRTR, 2002).

The most commonly used treatment technologies for arsenic contaminated soil include solidification and stabilization (S/S), excavation and off-site disposal, and acid extraction.

Solidification/stabilization It will be discussed extensively later on.

I.5 SOIL WASHING OR ACID EXTRACTION

This technique is an ex situ technology that exploit the behavior of some contaminants to preferentially adsorb onto the fines fraction of soil. The pollutants tends to bind to clay and silt particles rather than sand and gravel ones. Physical methods are applied to separate fine particles from the larger ones, because they are attached through physical bonds. The separation of larger and finer particles lead consequently to concentrate the contaminant to the latter ones, thus achieving a reduced volume of soil, but with higher contaminant concentration, that it will need further treatment. After the first screening, the soil is washed in a solution of water with or without other chemicals, based on the target contaminants.

The solution used to wash the soil will need further treatment, but can be reused for more than one time. This process does not destroy the contaminant, so the overall amount of pollutant will be concentrate on the fine particles and in the washing solution, while is reduced the contaminant concentration in the remaining soil.

I.6 EXCAVATION AND OFF-SITE DISPOSAL

Contaminated soil excavation and removal are performed. Then the material is disposed in a suitable landfill. Before the final disposal some pretreatments could be required in order to meet land disposal restrictions (FRTR, 2002). This solution may not be feasible in many cases due to the huge volume of soil involved and thus the cost implies (Yukselen and Alpaslan, 2001).

I.7 OTHER TECHNIQUES

Beyond these techniques there are others that have been studied, and that could theoretically be applied.

I.7.1 Soil flushing

This technique is an in-situ treatment where an extractant solution is injected in the soil. It goes through the contaminated area carrying on the pollutant up to the well where the fluid is pumped up and collected. The contaminant-bearing fluid can be water with or without further additives, based on the type of pollutant dealing with, so that the pollutant can be dissolved and haul away. The result is to get a clean soil, and transfer the contaminant in the fluid that will need further treatment. The applicability of this technique depend on the hydraulic conductivity, indeed if it is too low the procedure will require a long time(U.S. EPA, 2002).

I.7.2 Electrokinetics

This treatment exploit the characteristic of the charged species to migrate due to a low-density current, that is applied to the contaminated area placed between two electrodes. The electrical field between the electrodes mobilizes the charged particles but also induce a water flow from the anode to the cathode that can carry on non-charged compound, this phenomena is called electroosmosis. The contaminant is collected surrounding the electrodes, then it can be remove by using electroplanting or electroposition, precipitation or co-precipitation, adsorption, complexing with ion exchange resins or by pumping the fluid surrounding the electrode (Virkutyte et al., 2002).

The main mechanism to extract the contaminant is desorption of arsenic species attached onto the surface soil. The efficiency of the treatment depends on several factors as soil pH, arsenic speciation and the influence due to the electroosmosis effect. The principal advantage is the applicability to low permeability soil and can be potentially applies to a wide range of pollutants, and arsenic is one of these (Kim et al, 2005).

I.7.3 Phytoremediation

This is an in-situ remediation exploiting the capability of some species of plants to uptake, gather and detoxify pollutants. The sequester is done by the plant itself, while root colonizing microbe degrade toxic compound to non-toxic metabolites in the rhizosphere (Peng et al., 2009).

The applicability is limited to shallow contaminated soil, because the depth should be reachable by plant roots.

The main advantages of this technique is the low capital and operation cost, and it needs only standard agricultural equipment, moreover it is a less destructive treatment that protects the soil from erosion minimizing the human from re-entrained particulates (Mench et al.,2005), while the main cons are the long treatment time required, and the higher availability of the pollutant to animals due to the translocation above the ground due to the plant uptake (U.S. EPA, 2002).

I.8 STABILIZATION

Stabilization techniques aim to rendering less available the metal(loid) fractions and thus decreasing the risks associated with their leaching, ecotoxicity, plant uptake and human exposure. The contaminant concentration will be the same after the remediation, but it is in less toxic and more inert forms. For this reason are required further studies about the stability in long term application of this treatment (Komarek et al., 2013), this is strictly related to the steadiness of new arsenic compound formed with time that depends on many factors as disposal site characteristics, particle crystallinity, grain size distribution and presence of other compounds (Miretzky and Cirelli, 2010), as well as the soil saturation degree (Kumpiene et al., 2009).

The contaminant's speciation becomes a key factor, and the target fractions include the mobile, soluble, bioavailable for biota or bioccessible for humans (Kumpiene et al., 2008).

The reduction of the leachability can be achieved through physical and chemical processes. In particular solidification process refers to a restraining physically in the soil matrix the contaminant, while stabilization treatment is related to a chemical reactions that aim to decrease the mobility of As, and switch to less toxic forms by adding and mixing different amendments to the contaminated soil. This technique can be applied in-situ or ex-situ (Mulligan et al., 2001; Yukselen and Alpaslan, 2001). It can be chosen as final or interim treatment, combined with others techniques or on his own. It can be applied to several target contaminants, but mainly inorganic ones (FRTR, 2002).

The stabilization can involve several processes as adsorption onto mineral surface, formation of stable complexes with organic ligands, ion exchange, precipitation as salt and co-precipitation with metal oxides (Kumpiene et al, 2008).

The main advantage of this remediation is that only needs to blend the ameliorant, or a combination of them, in the soil, therefore it is a cost-effective treatment and it is also consider non-disruptive to natural hydrological conditions than conventional ex situ extraction technologies (Peng et al., 2009). Moreover the amendments that have being studied are mainly by-products, thus it contribute to keep a low application cost, that will be strongly dependent on the type of chosen amendments.
Several amendments have been studied to reduce As contamination in soils, as Al and Mn oxides, clay, mineral oxyanions and organic matter, but the most extensively examined are oxides of Fe or combinations with them. Later on are presented some of them, focusing mainly in iron or combination with iron amendments.

I.9 ALUMINUM (AMORPHOUS AND OXIDES)

The information about implementation of Al compound as amendment in remediation is scarce, in spite of it showed significant adsorption capability. The maximum adsorption of As(V) on amorphous and crystalline Al oxides occurs within a pH range from 3 to 4, and it demonstrates an adsorption capacity decreasing over a pH increase(Moore et al., 2000).
Although synthetic amorphous Al oxide presents a specific area higher than some Fe oxides, it shows a similar immobilization potential to Fe oxides when it is applied as amendment in a contaminated soil (Komarek et al.,2013).

I.10 MANGANESE OXIDES

Although Mn oxides have shown to be an important scavenger, they are still not so many studies on its utilization as amendment for contaminated soil available in literature (Komarek et al, 2013). Its applicability as ameliorant is limited by the fact that it strongly affect the speciation of redox-sensitive contaminant influencing the mobility and toxicity of the pollutants present in the soil. So its applicability is strongly related to the pollutants present in soil (Manning et al.,2002).
Considering strictly As contaminated soil, the use of Mn oxides can significantly reduce As mobility and toxicity (Kumpiene et al., 2008).

I.11 FLY ASHES

Fly ashes are a pozzolanic material generated as residual product of combustion. Depending upon the source and makeup of the coal being burned, the components of fly ash vary considerably, but all fly ash includes substantial amounts of silicon dioxide SiO_2 (both amorphous and crystalline) and calcium oxide CaO. They have been studied as potential amendment for As contaminated soil. Since the final results is strongly dependent on both the nature of the fly ash and the type of soil, the

conclusions of researches conducted with amendment derived from different facilities, cannot be generalized. A detailed study required to provide a better evaluation of the treatment effectiveness.

The application of fly ashes can lead to a significant increase of pH. Moon and Dermatas (2007) have studied a class C fly ashes produced by coal burning at electric utility facilities. The objectives of this study were to determine the leaching behavior of As in field soils treated with this ameliorant. The experiment have shown a significant immobilization of As, that was supposed to be related to the alkalinity of fly ash leading to the formation of insoluble Ca-As precipitates.

I.12 CEMENT AND LIME

Cement and lime have been studied as amendments due to its high sorption capacity and its potential to form pozzolanic reaction products (Dermatas et al., 2004).

Dutrè et al. (1998) have treated arsenic contaminated soil and a rocklike with cement, lime and a combination of them. The solidification/stabilization treatments have shown a decrease on the arsenic leaching, but it was also demonstrate a strong increase of pH and Ca concentration in the leachate. In particular the rise of the former appeared to have a direct correlation with the amount of cement added, while with lime ameliorant the leachate pH was stable at 12.5. In this experiment was demonstrate that cement was a more effective amendment.

Also Dutrè and Vandecasteele (1996) have shown that cement and lime used as ameliorants decrease the arsenic leaching, but in this case lime is more effective than cement. The main mechanism is the formation of slightly soluble compound $CaHAsO_3$.

I.13 IRON AMENDMENTS

Although several amendments have been studied to remediate As contaminated soils to reduce As mobility and toxicity, iron minerals and iron industrial by-products show great potential for in situ remediation (Miretzky and Cirelli, 2010).

Due to their important sorption properties, Fe oxides have been extensively studied as potential stabilization amendments in soils contaminated with metals and As. Their application, either direct or indirect through the application of their precursors (e.g., iron grit or Fe sulfates) is supposed to decrease mobile, bioavailable and bioaccessible fractions of the As (Komárek, 2013).

Change from poorly crystalline form to more crystalline occur over time, influencing adsorption sites availability: higher the crystallization degree lower the density of adsorption site (Miretzky and Cirelli, 2010).

The mechanism involved in the adsorption of As species into iron oxides, is the replacement of OH2 and OH− for the anionic As species in the coordinate spheres of surface structural Fe atoms(Miretzky and Cirelli, 2010). The adsorption of As(V) and

As(III) anions (AsO3− 4 and AsO3− 3) on iron hydroxides depends also on pH: the oxides are positively charged for pH lower than 6, while above pH 8 the iron oxide surface is negatively charged. This mechanism, and how it affect the As adsorption, can be easily represent as follow:

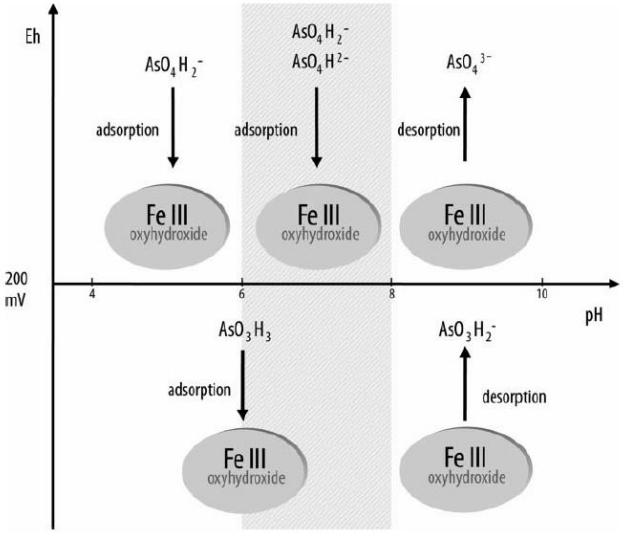

Figure 2(I): As adsorption mechanism onto Fe oxides (Miretzky and Cirelli, 2010)

The principal concern is that changing condition can significantly change the solubility of arsenic(Miretzky and Cirelli, 2010).

I.14 BIOACCESSIBILITY

The aim of stabilization technique is to lower the risk correlated to a contaminated site, and the target of major concern is the human being. There are several exposure pathways for human: ingestion, dermal contact, inhalation or dietary consumption, just to mention some of them. The oral swallow is one of the most important, due to the exposure of children to incidental ingestion of small soil particles, in particular particles with $Ø < 250\ \mu m$ are evaluated in all the procedures proposed. In this context is important to predict and assess the risk correlated to human exposure. Two fundamental definitions need to be pointed out:

- *bioaccessible fraction* is the contaminant amount available for systematic absorption, so the compounds soluble in gastric or gastrointestinal solution.
- *bioavailable fraction* is the amount of contaminant that is absorbed by humans or animals. In *in vivo* test is measured as the As concentration in blood plasma and urine, or accumulation in organs as kidney, liver or bone.

I.14.1 Regulation

So far the regulation establishes to consider the bioavailable amount equal to the total As concentration. Generally this quantity differs from the total concentration, because related to the free metal activity rather than the overall content, and only the bioavailable fraction is potentially toxic to human.
Considering bioavailable the total concentration leads to an overestimation of the risk assay. The only adjustment accepted by the regulation is through *in vivo* bioassessability test. In particular juvenile swine have been suggested because of its digestive mechanism, close to the pediatric one.
In vivo assay is a long and expensive assessment, and animals are used by definition. A cost and time effective alternative is *in vitro* assay, and lately several tests have been proposed. Since they should substitute the *in vivo* ones, those tests have to be validated, showing a good predictive ability of animal As bioavailability.
In vitro test has already been establish for bioaccessibility of lead in contaminated soil risk assessment (US EPA method 9200), while for As methods are needed further validations.

I.14.2 Methods

Several methods have been proposed, but it is possible to subdivide in two main groups: physiologically based test and glycine-buffered method (Musier et al., 2010).
The first method proposed was PBET, physically based extraction test (Ruby, 1996).
This type of method try to simulate the gastro or gastrointestinal condition to assess the bioaccessibility of As. Several parameters are monitored, solution composition, pH, anaerobic condition, mixing mechanism, transition time, temperature. The digestive system is so complicated that actually is not possible to recreate the same environment. The studies show a strong results dependency on stomach pH, but also on L/S ratio applied, in particular for glycine-buffer tests. The method has been simplified, one of the most famous is proposed by Basta et al, 2007, so called IVG-OSU, in vitro gastrointestinal Ohio State University. Juhasz et al., 2009, evaluate the performance of four different assay. Those methods were validated, comparing the in vitro and in vivo results, and linear regression models were proposed for each test.
This study shows the effectiveness of SBRC and IVG method over PBET.
So far all the studies present procedures for both gastro and gastrointestinal tract, but it is demonstrated that the gastric phase is the worst case scenario for As bioaccessibility (Basta et al., 2007; Juhasz et al., 2009; Rodriguez and Basta, 1999). In particular in presence of iron, the extension to gastrointestinal method shows a decrease of As bioaccessibility due to precipitation of amorphous Fe, dissolved at acid pH during the gastric phase, and consequent availability of adsorbent sites on its surface, following the same mechanism of remediation process (Juhasz et al., 2009).
The parameters of the main important methods are summarized in Table 1.

Table 1 (I): SBRC, IVG-OSU, PBET in vitro method parameters (Juhasz et al., 2009)

Method/phase	composition (g L^{-1})	pH	L/S ratio	Extraction time (h)
	SBRC			
gastric	30.03 of glycine	1.5	100	1
intestinal	1.75 g of bile, 0.5 g of pancreatin	7.0	100	4
	IVG			
gastric	10 g of pepsin, 8.77 g of NaCl	1.8	150	1
intestinal	3.5 g of bile, 0.35 g of pancreatin	5.5	150	1
	PBET			
gastric	1.25 g of pepsin, 0.5 g of sodium citrate, 420 µL of lactic acid, 500 µL of acetic acid	2.5	100	1
intestinal	1.75 g of bile, 0.5 g of pancreatin	7.0	100	4

SBRC = Solubility Bioaccessibility Reaserch Consortium (Kelley et al., 2002); IVG = In Vitro Gastrointestinal, Ohio State University (Rodriguez and Basta, 1999; Basta et al., 2007); PBET = Physiologically Based Extraction Test (Ruby et al., 1996)

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PART II

LABORATORY AND FIELD WORK

II. LABORATORY AND FIELD WORK

II.1 FIELD EXPERIMENT DESCRIPTION

The examined soil was collected from a former industrial site in Northern Sweden, at Solgårdarna belonging to Boden municipality. The site was used for timber treatment with chromated copper arsenate chemical, thus the soil was contaminated mainly with As.

Two different types of amendments were applied:
1) spent blasting sand (BS) from SSAB, Luleå, containing 98.3% of Fe^0 and some impurities;
2) peat, obtained from Geogen production AB, Arieplog. Peat was used in combination with Fe^0.
The total amount of soil (800 t) was homogenized and divided in three parts, the first was kept untreated (ca. 267 t), while the remaining quantity was amended with 1% of Fe^0 (by weight). A half of this volume (one third of the overall amount) was further mixed with 5% of peat (by weight). The soil was mixed with a scoop tractor.
On 17th June three samples for each treatments were collected. The soils were sampled at 15 cm depth, to avoid mixing the contaminated soil with clean soil that could cover the original heap.

Soil pH and electrical conductivity (EC) of the soil samples from the field were measured in suspensions of fresh soil and distilled water in the ratio 1:2. The samples for the other tests were air dried and homogenized.

Figure 3 (II). To measure the pH and EC in soil samples the suspension soil and distilled water was prepared

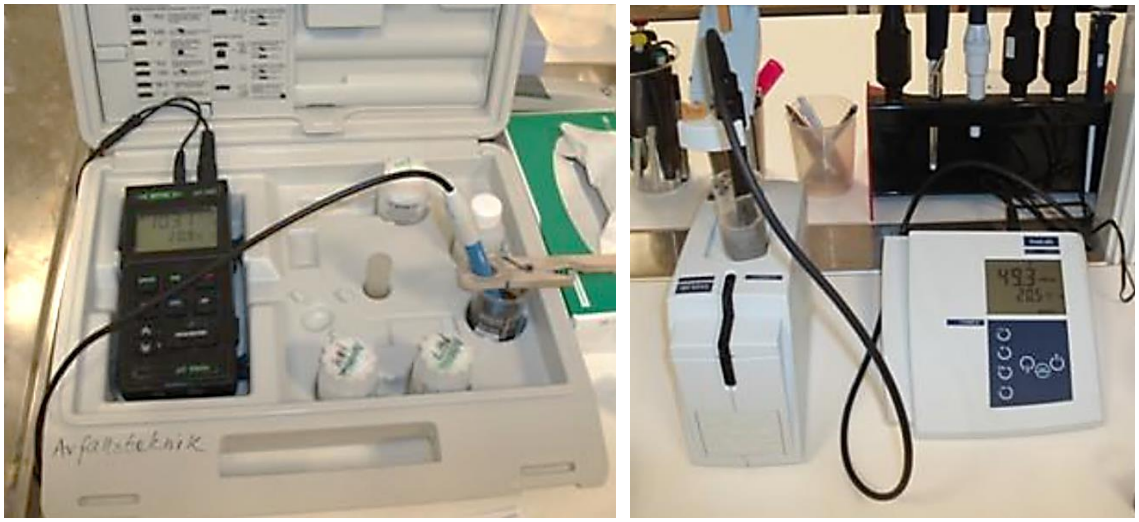

Figure 4 (II). The pH-meter and EC-meter were used to evaluate pH and EC right after the sampling. The values calculated are summarized in Table 1 at the end of the thesis.

Total solids (TS) were assessed after drying at 105°C for 24 hours, according to Swedish Standard SS 02 81 13. Volatile solids (VS) were measured after ignition at 550°C for 2 hours. All the measurements were made in triplicates.
In particular the determination corresponds to the Swedish Standard SS 02 81 13 is given by the following procedure:

- Control that porcelain crucibles are cleaned and marked (Sample#), always use metal pliers when moving crucibles.
- Place crucibles into a drying oven set at 105°C.

- leave crucibles in drying oven at least one hour.
- Place hot crucibles into a desiccator with pressure prelease valve and let crucibles cool down to room temperature (about 1 h). Be careful when closing the desiccator.
- Weight empty crucibles (that are now at room temperature)
- Transfer fresh samples (20-30 grams are the best , or crucible about half full) into crucible and weight crucible plus wet sample; keep track of measurements and sample ID's.
- Collect weighted crucibles on a table and transfer them into a drying oven set at 105°C and let samples dry for 24 h.
- Place the crucibles in the desiccator and cool them down to the room temperature (about 1 h).
- Determine the weight of the cooled crucibles including the dried sample.
- After the drying, the mass of the residue remaining in the crucibles correspond to the TS content. The TS is the expressed as g kg^{-1}

$$TS\ (g\ kg^{-1} soil) = \frac{1000 \cdot (weight\ of\ soil\ after\ 105°C\ drying, g)}{Wet\ soil\ before\ drying, g}$$

Determination of the content of volatile solids (VS), also called: Loss of ignition (LOI).The VS is a common and widely used method to estimate the organic content of a sample. The organic matter is oxidized at 550°C to CO_2 and ash. The VS procedure usually follows the TS analysis because the sample has to be dry.
Below is described the steps that follow the TS procedure:

- Transfer the crucibles with samples into a muffle furnace set at 550°C; leave the samples in the furnace after the temperature has been reached 550°C for 2 h.
- Open the furnace door and very carefully transfer hot crucibles into the desiccator using metal pliers and fire protection gloves as well. Avoid direct contact of crucible and desiccator walls.
- Let samples in the desiccator cool to room temperature and at that temperature carefully open the pressure valve at the desiccator lid to release pressure differences.
- Weight crucibles with the ignited samples and record the weight.

$$LOI_{550} = ((DW_{105} - DW_{550}) / DW_{105}) \cdot 100 \qquad [g/kg] \text{ or } [\%\ of\ TS]$$

Where:
DW_{105} is the dry weight of the sample after over drying at 105°C
DW_{550} is the dry weight of the sample after furnace burning at 550°C

The values calculated are summarized in Table 2 and 3 at the end of the thesis.

II.2 SEQUENTIAL EXTRACTION

The sequential extraction procedure was performed on three soils: untreated As contaminated soil, soil amended with Fe^0 and soil amended with Fe^0 and peat.
A six steps sequential extraction was performed, following the procedure given by Kumpiene et al., 2012. The method given by Dold (2003) was modified with an additional step to assess the fraction bound to Fe - Mn oxides, since not all goethite was dissolved during the crystalline Fe (III) (oxyhydr)oxides dissolution step (Kumpiene et al., 2012).
The extraction was carried out on 1 g of air dried, homogenized and sieved to <2mm size soil in a 50 ml Teflon centrifuge tube. The soils were sieved before performing the sequential extraction. The value of the sieved fraction are reported in Table 5.
The sequential extraction was performed according to the following procedure:

I. Exchangeable fraction: 1 M NH_4-acetate pH 4.5 at liquid to solid ratio (L/S) 25, shaking for 2 h at room temperature (RT), followed by centrifugation at 10000 rpm for 15 min, and rinsing with 10 mL of deionized water (centrifuged and discarded).
II. Fe(III) oxyhydroxide fraction: 0.2 M NH_4-oxalate pH 3.0, L/S 25, shaking for 2 h at RT in darkness, centrifugation at 10000 rpm for 15 min. For the washing step the same solution, i.e. 0.2 M NH_4-oxalate pH 3.0, at L/S 12.5 and, after centrifugation and filtration, was combined with the previously extracted portion (making in total L/S 37.5).
III. Fe(III) oxide fraction: 0.2 M NH_4-oxalate pH 3.0, L/S 25, heated in water bath at 80°C for 2 h, centrifugation at 10000 rpm for 15 min, washing with 10 mL deionized water (centrifuged and discarded).
IV. Fe-Mn oxide fraction: 0.04 M NH_2OH-HCl in 25% (v/v) HO-acetate pH 2, L/S 20, heated in water bath at 96°C for 2 h, centrifugation at 10000 rpm for 15 min, rinding with 10 mL of deionized water (centrifuged and deiscarded)
V. Organic matter and secondary sulphide fraction: 35% H_2O_2, heated in water bath at 85°C for 1 h, L/S 25, centrifugation at 10000 for 15 min, rinsing with 10 mL of deionized water (centrifuged and discarded)
VI. Residual fraction: aqua regia (HNO_3:HCl, 1:3 v/v), L/S 15, in a microwave digester (CEM Microwave Sample preparation System, Model MARS 5) at 195°C for 10 min. Filter through a funnel paper filter, bring to volume of 100 mL.

All extracts were filter through 0.45 µm syringe filters and stored at 4 °C prior to analyses by ICP-OES. The samples were acidify and diluted before the analysis. The procedure was performed in triplicates.
The values calculated are summarized at the end of the thesis. In Tables 6-11 are reported the different As fractions. In Table 12 there is the values of As total

concentration in untreated and treated soils, calculated as sum of all the fractions. The SD is calculated as the square root of the sum of SD of each fraction elevated to the second power.

In Tables 12-17 are reported the different Fe fractions. In Table 18 there is the values of As total concentration in untreated and treated soils, calculated as explained for As.

II.3 WATER HOLDING CAPACITY

The WHC was measured in soils stored in the Environmental laboratory at Luleå University of Technology, to avoid that the contaminated soil could be mixed with clean soil. The same soils were used for the phytotoxicity test. The calculation of the WHC was done to keep the water moisture fixed, taking into consideration the diversity between soils.

Water holding capacity (WHC) is the amount of water that the studied material can keep against gravity. For its determination the sample must be first saturated with water. The procedure is described below:

- Weight the empty cylindrical beakers with a filter paper inside. Register the weight.
- Place a triple set of material into beakers with the filter paper inside.
- Place the filled beakers into a vessel filled with water. The water level has to be equal to that of the material in the beaker.
- After one hour lift the saturated beakers from the vessel with water and place them into a vessel with sand. Cover the beakers.
- Leave beakers for 3 hours for excess of water to run out.
- Weight the beakers with the water saturated material. Register the weight.
- Place the beakers into an oven and dry the samples for 20 h at 105°C.
- Place the beakers into a desiccator for 1 h.
- Weight the beakers with the dried material. Register the weight.
- Calculate the water holding capacity according to the following formula:

$$\frac{Water\ content\ in\ the\ saturated\ sample}{Weight\ of\ dry\ soil} \cdot 100 = WHC(\%)$$

Figure 6 (II). Pictures of the imbibition phase and the beakers left on the sand to let the excess water to drain

The values calculated are summarized in Table 19 at the end of the thesis.

II.4 PHYTOXICITY TEST

Figure 7 (II). Pots at the beginning of the phytotoxicity test.

Figure 7 (II). Pots during the phytotoxicity test.

TS and VS were measured before preparing the pots for the phytoxicity test, to assess the effective amount of TS in each pot. Knowing these values together with WHC were necessary to keep the soil moisture constant during the whole test.
Phytotoxicity was assessed using the method described by Vangronsveld and Clijsters (1992). Seeds of dwarf beans (*phaseolus vulgaris*) were left for 1 day in refrigerator, for vernalization, and then they were submerged into distilled water for 4 hours, for the imbibition phase. Four pots (Ø=120 mm, volume 0.9 L) were prepared for each type of soil studied and a rhizon soil moisture sampler was placed in each pot. The soils were kept humid for 1 week before sowing, to recover the balance of nutrients and microbiological system. Four seeds were sown in each pot. The experiment lasted 14 days, during which the water moisture was kept between 47% and 53% of soil water holding capacity, and 12 h of artificial light were supplied.
After 14 day morphological parameters were measured for each plant: shoot length, fresh shoot weight and primary leaf area.

Plant shoots were harvested and fresh weight of the above ground parts was measured. Plant then were washed with distilled water, dried at 50°C for 96 h, and weight for dry weight determination before sending the samples to element concentrations analysis. The same procedure was applied for plant roots. The element concentrations in biota were analyzed by ALS Scandinavia AB.
The morphological parameters measured are summarized in Tables 24-32.

Soil pore water was collected the third day and the last one to assess the solubility, i.e. mobility, of contaminant, and soil pH and EC were measured. The samples were stored at 4°C until element concentration analysis was performed, using ICP-OES.
The pore water samples were collected three days after the beginning of the phytotoxicity test and the last day of the experiment. 100 mL acid washed bottles were used. To performed the element analysis is necessary to collect at least 20-30 mL of pore water. The bottles were wrapped with aluminum sheet to avoid changing in the samples. Indeed it has been observed in previous studies that the pore water rich in Fe changed color and some solids precipitated during the sampling, due to the light.
The pH and EC were measured right after the samples have been collected, and the values obtained are written in Tables 37 and 39, in the third part of the thesis.
The values measured in both sampling, i.e. on 12th and 23rd June, are reported in Tables 38 and 40 in the last part of the thesis.

II.5 LEACHATE ANALYSIS

1 m^2 lysimeters were placed below the top layer, built with untreated and treated soils. All the lysimeters were collected though pipes to a shed placed closed to the heap. Each collecting point had two tubes each. From the first one was sucked the leachate collected by the lysimeter. The second one was connected to a bag filled with N_2, to avoid to lead the area under atmospheric pressure. Three sampling where performed on 4th and 27th June, and 8th August.
The pH and EC of the samples were measured in situ after the sampling with a mobile pH/EC-meter. The values obtained are reported in Tables 41 and 43. The samples were then analyze to evaluate the element concentrations. The As and Fe concentration are written in Table 42 and 44.

II.6 BIOACCESSIBILITY TEST AND BIOAVAILABILITY

The bioaccessibility of As on untreated and treated soils was evaluated using in vitro SBRC method. Only the gastric phase was applied, because as showed by Juhasz et al. (2009), Basta et al. (2007), Rodriguez and Basta (1999) extending the procedure to intestinal-phase do not increase the As bioaccessibility. A literature study about bioaccessibility methods was done. The concept of bioaccessible test was introduce by Ruby et al. (1996) that introduced the Physiologically based extraction test (PBET). The chosen method was validated by Juhasz et al. (2009), comparing in vitro assay and measured As concentration in swine's blood after oral administration of contaminated soil (Juhasz et al., 2007).
Air dried bulk soils were sieved, and only Ø < 250 µm particles size were used for this analysis, because it is considered that these particles can adhere to children hands and be ingested.
1 g of soil and 100 ml of gastric solution, consisting of 0.4 M of glycine solution at pH 1.5, were put in high density polyethylene bottles. The samples were intermittent shaken for 1 hour in a water bath at 37°C. The samples were filtered through 0.45 µm cellulose acetate syringe filters and stored at 4°C before element concentrations were measured with ICP-OES. The procedure was performed in triplicates.

The bioaccessible fraction is calculated as follow:

$$in\ vitro\ bioaccessibility\ (\%) = \frac{in\ vitro\ As}{total\ As} * 100$$

To estimate the bioavailable fraction was applied a linear regression function model, proposed by Juhasz et al. (2009):

$$in\ vivo\ relative\ As\ bioavailability\ (\%) = 1.656 + 0.992 * gastric\ (\%)$$

The values obtained are summarized in Tables 45-47.

PART III

SCIENTIFIC PAPER

III. SCIENTIFIC PAPER: ASSESSMENT OF AS CONTAMINATED SOIL STABILIZED WITH IRON AMENDMENT BY LABORATORY AND FIELD EXPERIMENTS

III.1 ABSTRACT

The aim of this work was to assess the effectiveness of chemical stabilization technique on As contaminated soil, amended with Fe^0 and combination of Fe^0 and peat by laboratory and pilot scale field experiments. The used amendments were spent blasting sand (BS), a by-product from a steel industry containing 98.3% of Fe^0, and its combination with peat.

It was evaluated if the stabilized material could be used as a final landfill cover. The field experiment reproduced a landfill cover, where the untreated and treated soils made up the 2 m thick top layer. It was assess the change in As solubility and mobility analyzing pore water and leachate samples. The results showed the effectiveness of chemical stabilization in oxidizing condition. While it was demonstrated a limit of this technique that is the adverse effect on the As solubility when reducing condition occurs, i.e. thick soil layer are considered. It was measured an higher As concentration in the leachate percolating from both treated soil profile in the long term, when compared to the one collected where untreated soil was used as the top layer. It was also studied how the As fractionation, species bound to different a compound, changed applying the chemical stabilization.

The main exposure pathways concerning public health and environmental pollution were studied using pore water analysis, phytotoxicity and bioaccessibility tests. The stabilization with Fe^0 and peat significantly reduced the As uptake by plants and an improvement of the main morphological parameters. It also reduced the bioaccessible and the assessed bioavailable fraction.

III.2 INTRODUCTION

The potential contaminated sites in Europe are estimated at 2.5 million, while there are around 342 000 sites in which the contamination has been verified and it is already confirmed that they represent a risk to human, water and ecosystem. 35% of the sites are contaminated with heavy metals and metalloids (Panagos et al., 2013).

A rank of hazardous substances was drawn up by Johonson and DeRosa (1995), based on three criteria: frequency of occurrence of a substance at contaminated sites, the substance's toxicity, and the potential for human exposure. Arsenic is at the second place of that list. Moreover, WHO also declares As among the elements of major concern for human health, due to its acute toxicity. Thus, As contaminated soil have been extensively studied considering that the remediation priority should be given to the pollutants on the basis of toxicity, environmental persistence, mobility, and bioaccumulation (WHO, 2000).

There are several techniques to clean up the polluted area, but the most extensively applied is excavation and landfilling. This method is expensive and it requires the availability of large land areas and volume to confine the wasted material. A feasible alternative is chemical stabilization technique, that aims at rendering the metal(loid) to the less available forms and thus decreasing the risks associated with their leaching, ecotoxicity, plant uptake and human exposure. The contaminant concentration will be the same after the remediation, but it is in less toxic and more inert forms (Komarek et al., 2013). The main advantage of this remediation is that the contaminated soil is blended with the selected stabilizing material or their combination, therefore it is relative simple to implement. If the suitable amendments are industrial by-products, this technique can become a cost-effective treatment. If the soil is treated on site, the method can also be considered as less disruptive to the soil ecosystem than conventional excavation technologies (Peng et al., 2009).

Although several amendments have been studied to remediate As contaminated soils to reduce As mobility and toxicity, iron minerals and iron-containing industrial by-products show a great potential for in situ remediation (Miretzky and Cirelli, 2010). Due to their strong binding capacities, Fe oxides have been extensively evaluated as potential stabilization amendments in soils contaminated with metals and As. Application of Fe oxides, either direct or indirect through the application of their precursors (e.g., iron grit or Fe sulfates) is supposed to decrease mobile, bioavailable and bioaccessible fractions of As (Komárek et al., 2013). In particular several studies showed high efficiency for As immobilization applying zerovalent iron (Fe^0). Oxidation of Fe^0 does not lead to a strong fluctuation of pH in soil that could remobilize contaminants and lower the soil quality (Boisson et al., 1999; Mench et al., 2006; Lidelöw et al., 2007; Maurice et al., 2007; Kumpiene et al., 2008). The application rate

for Fe^0 ameliorant usually range from 0.5% to 5% by dry weight , but applying 2% to 5% do not usually improve the contaminant retention (Mench et al., 2000)

On the other hand, Kumpiene et al. (2013) demonstrates that the effectiveness of the stabilization is verified only in the upper soil layer, where oxidizing condition prevail.

In this study the efficiency of stabilization using Fe^0 and its combination with peat are investigated. Peat supports the plant growth and improves the soil texture, and is expected to maintain a high redox potential along the soil layer (Kumpiene et al., 2013). Since the mobility, solubility and toxicity of As is strongly influenced by its oxidation state (Masscheleyn et al., 1991), chemical fractionation using sequential extraction was performed to evaluate distribution of As between various soil fractions and better understand the changes in As binding caused by soil treatment.

Leachate percolating though a 2 m thick layer of treated and untreated soil was collected from the pilot scale field experiment in Boden to evaluate the solubility of As along the soil profile.

The main exposure pathways concerning public health and environmental pollution were studied using pore water analysis, phytotoxicity and bioaccessibility tests.

III.3 SCOPE OF THE THESIS

The aim of this work was to assess the effectiveness of chemical stabilization technique on As contaminated soil, amended with Fe^0 and combination of Fe^0 and peat by laboratory and pilot scale field experiments. The questions are:

- how stabilization with chosen ameliorants affect As solubility and mobility in soil layer used as a final landfill cover;
- how the soil treatment affect As bioaccessibility to humans and availability to plants.

III.4 MATERIALS AND METHODS

III.4.1 Soil and Amendments

The examined soil was collected from a former industrial site in Northern Sweden, at Solgårdarna belonging to Boden municipality. The site was used for timber treatment with CCA chemical, thus the soil was contaminated mainly with As.

Two different types of amendments were applied: 1) spent blasting sand (BS) from SSAB, Luleå, containing 98.3% of Fe^0 and some impurities; 2) peat, obtained from Geogen production AB, Arieplog. Peat was used in combination with Fe^0.

The total amount of soil (800 t) was homogenized and divided in three parts, the first was kept untreated (ca. 267 t), while the remaining quantity was amended with 1% of Fe^0 (by weight). A half of this volume (one third of the overall amount) was further mixed with 5% of peat (by weight). The soil was mixed with a scoop tractor.

The untreated and treated soil was used to build a pilot scale field experiment in Brännkläppen, waste management facility in Boden, on September 2012. A small volume of soil was brought to the Environmental laboratory at Luleå University of Technology for additional tests.

Figure 1 illustrates the schematic drawing of the field experiment. The heap consists of a 2 m thick top layer of untreated and stabilized As contaminated soil, a drainage layer and a liner. In this work only the top layer was studied. The heap can be divided in three parts, the first one is untreated soil, the second one is soil amended with Fe^0 and the last one is soil mixed with a combination of Fe^0 and peat. 1 m^2 glass fiber lysimeters were used to collect leachate, placed below the soil layer, three for each type of soil.

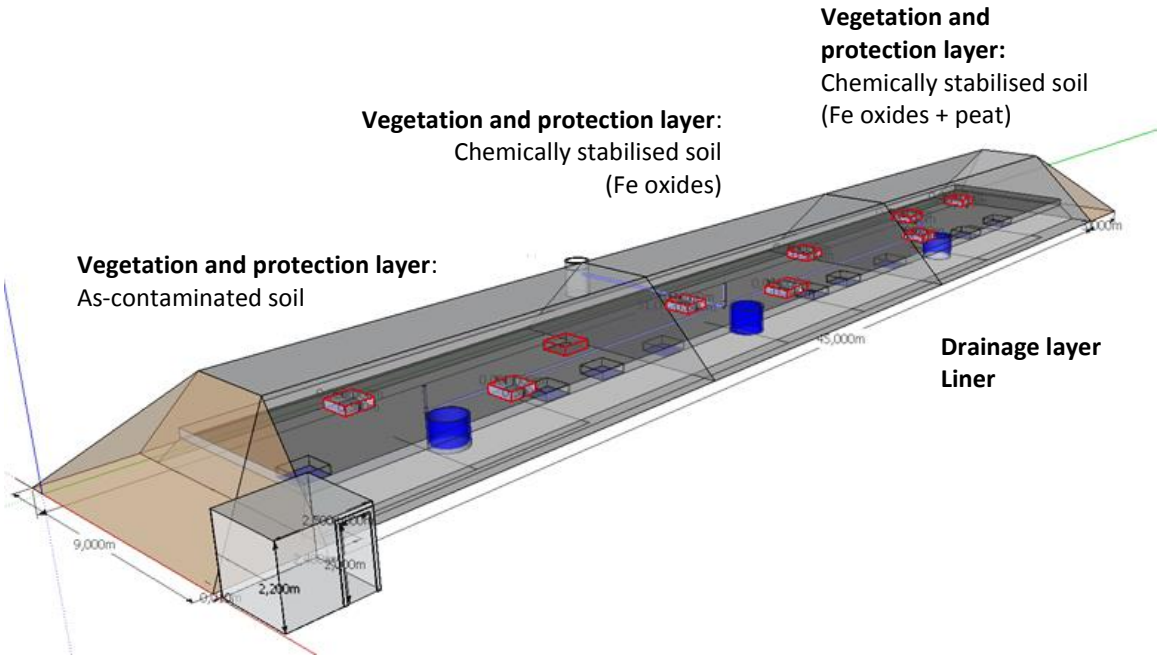

Figure 1 (III). Sketch of the pilot field experiment in Brännkläppen. The top layer consists of untreated, and amended soil as indicate in the drawing. The leachate lysimeters are highlight in red

To perform the sequential extraction soils samples were collected in June 2013 from the field. Three composite samples were taken with a spade at different spots of the surface of each area. Leachate samples were collected from lysimeters by pumping out the accumulates water.

For phytotoxicity test, pore water analysis and bioaccessibility test the soils collected directly after mixing the materials in field and stored in laboratory were used.

III.4.2 Soil basic characterization

Soil pH and electrical conductivity (EC) of the soil samples from the field were measured in suspensions of fresh soil and distilled water in the ratio 1:2. The samples for the other tests were air dried and homogenized.

Total solids (TS) were assessed after drying at 105°C for 24 hours, according to Swedish Standard SS 02 81 13. Volatile solids (VS) were measured after ignition at 550°C for 2 hours. All the measurements were made in triplicates.

Water holding capacity (WHC) is the amount of water that the studied material can keep against gravity. To determine WHC, a filter paper was place on the permeable bottom of cylindrical bakers and then weighted. The three types of soils were place into the bakers and left into a vessel filled with water for one hour, in this way the samples were humified from the bottom. Then the saturated samples were placed into a vessel with wet sand for 3 hours to let the excess water to drain. The weight of all samples was registered and then they were placed in the oven at 105°C for 20 hours.

The procedure was performed in triplicates. An empty baker was used to adjust the calculation of the saturated samples, taking into account the water held by filter paper and baker bottom itself. The dry weight was recorded and WHC was measured as the ratio between the water content in saturated sample and the weight of dry soil, all multiply by one hundred.
Soil density was measured by weighting a known volume, filled with loose air dried soil. The measurements were done in triplicates.

III.4.3 Evaluation methods

III.4.3.1 Sequential extraction

Distribution of As between soil fractions and sorption to Fe compounds largely affect the accomplishment of stabilization. Knowing the total concentration is not sufficient to assess the environmental impact of contaminated soil, and through sequential extraction it is possible to examine the association between Fe and As.
The basic idea of sequential extraction procedure is to use different chemical reagents to obtain the release of different metal(loid) fractions from soil by destroying the bond between metal(loid) and soil solids. This method can be a good indication of metal(loid) partitioning in soils by analyzing the extracted supernatant, thus giving an estimation of their potential mobility (Balasoiu et al., 2001).
The sequential extraction procedure was performed on three soils: untreated As contaminated soil, soil amended with Fe^0 and soil amended with Fe^0 and peat.
A six steps sequential extraction was performed, following the procedure given by Kumpiene et al., 2012. The method given by Dold (2003) was modified with an additional step to assess the fraction bound to Fe - Mn oxides, since not all goethite was dissolved during the crystalline Fe (III) (oxyhydr)oxides dissolution step (Kumpiene et al., 2012).
The extraction was carried out on 1 g of air dried, homogenized and sieved to <2mm size soil in a 50 ml Teflon centrifuge tube. The method was applied in triplicates and is summarized in Table 1. Separation of remaining chemical extractants from soil sample after the extraction step was performed by centrifuging at 10 000 rpm for 15 minutes. The only exception was for step (IV), that was centrifuged at 10 000 rpm for 30 minutes. The solid residue, after the washing phase, was used in the next step. The last step (VI) was not centrifuged but was filtered through paper funnel and then diluted to total volume of 100 mL using deionized water.
All the extracts were filtered through 0.45 μm cellulose acetate syringe filters and stored at 4°C prior to analyses by ICP-OES.
In some fractions the element concentrations were below instrument detection limits. In these cases, the detection limit value was taken to calculate means and standard deviations.

Table 1 (III). Sequential extraction steps

	Dissolved Fraction	Chemical reagent	L/S	Extraction procedure	Washing step
I	**Exchangeable**	1 M NH_4 acetate, pH=4.5	25	shaking for 2 h at room temperature	10 mL of deionized water
II	**Poorly crystalline Fe(III)-oxyhydroxide**	0.2 M NH_4-oxalate, pH=3	25	shaking for 2 h at room temperature in darkness	12.5 mL of 0.2 M NH_4-oxalate, pH=3
III	**Crystalline Fe(III) (oxyhydr)oxide**	0.2 M NH_4-oxalate, pH=3	25	heated in water bath at 80°C for 6 h	12.5 mL of 0.2 M NH_4-oxalate, pH=3
IV	**Fe – Mn oxide**	0.04 M NH_2OH-HCl in 25% HO-acetate pH=2	20	heated in water bath at 96°C for 6 h	10 mL of deionized water
V	**Organic matter and secondary sulphide**	35% H_2O_2	25	heated in water bath at 85°C for 1 h	10 mL of deionized water
VI	**Residual**	Aqua Regia ($HCl:HNO_3$, 1:3 v/v)	15	Digestion in microwave at 195°C for 10 min	-

The total As concentration in soil from the field was calculated as a sum of concentrations of all the fractions determined by the sequential extraction. The total As concentration in soil samples used for the phytotoxicity tests was determined by digesting 1 g of soil in 15 ml of aqua regia (HCl-HNO3, 3:1, v/v) using microwave digester at 195°C for 10 min. The samples were filtered through 0.45 µm filter prior analysis. All the measurements were performed in triplicates.

III.4.3.2 Phytotoxicity test and pore water analysis

Phytotoxicity was assessed using the method described by Vangronsveld and Clijsters (1992). Seeds of dwarf beans (*phaseolus vulgaris*) were left for 1 day in refrigerator, for vernalization, and then they were submerged into distilled water for 4 hours, for the imbibition phase. Four pots (Ø=120 mm, volume 0.9 L) were prepared for each type of soil studied and a rhizon soil moisture sampler was placed in each pot. The soils were kept humid for 1 week before sowing, to recover the balance of nutrients and microbiological system. Four seeds were sown in each pot. The experiment lasted 14 days, during which the water moisture was kept between 47% and 53% of soil water holding capacity, and 12 h of artificial light were supplied.

After 14 day morphological parameters were measured for each plant: shoot length, fresh shoot weight and primary leaf area.
Plant shoots were harvested and fresh weight of the above ground parts was measured. Plant then were washed with distilled water, dried at 50°C for 96 h, and weight for dry weight determination before sending the samples to element concentrations analysis. The same procedure was applied for plant roots. The element concentrations in biota were analyzed by ALS Scandinavia AB.
Soil pore water was collected the third day and the last one to assess the solubility, i.e. mobility, of contaminant, and soil pH and EC were measured. The samples were stored at 4°C until element concentration analysis was performed, using ICP-OES.

III.4.3.3 Bioaccessibility test and Bioavailability

The bioaccessibility of As on untreated and treated soils was evaluated using in vitro SBRC method. Only the gastric phase was applied, because as showed by Juhasz et al. (2009), Basta et al. (2007), Rodriguez and Basta (1999) extending the procedure to intestinal-phase do not increase the As bioaccessibility. The chosen method was validated by Juhasz et al. (2009), comparing in vitro assay and measured As concentration in swine's blood after oral administration of contaminated soil (Juhasz et al., 2007).
Air dried bulk soils were sieved, and only Ø < 250 µm particles size were used for this analysis, because it is considered that these particles can adhere to children hands and be ingested.
1 g of soil and 100 ml of gastric solution, consisting of 0.4 M of glycine solution at pH 1.5, were put in high density polyethylene bottles. The samples were intermittent shaken for 1 hour in a water bath at 37°C. The samples were filtered through 0.45 µm cellulose acetate syringe filters and stored at 4°C before element concentrations were measured with ICP-OES. The procedure was performed in triplicates.
The bioaccessible fraction is calculated as follow:

$$in\ vitro\ bioaccessibility\ (\%) = \frac{in\ vitro\ As}{total\ As} * 100$$

To estimate the bioavailable fraction was applied a linear regression function model, proposed by Juhasz et al. (2009):

$$in\ vivo\ relative\ As\ bioavailability\ (\%) = 1.656 + 0.992 * gastric\ (\%)$$

III.5 RESULTS

III.5.1 Soil Characterization

The main soil characteristics of the samples collected in the field experiment are summarized in Table 2.

Table 2 (III). Main characteristics of untreated and treated soil, samples from the field experiment (n = 3, ± SD)

Soil Proprieties	Unit	Untreated As soil	Soil + Fe	Soil + Fe + peat
pH (1:2 H_2O)	-	7.85 ± 0.13	7.89 ± 0.06	7.72 ± 0.15
Electrical conductivity (EC)	µS cm^{-1}	403.5 ± 10.6	297.3 ± 36.5	272.3 ± 45.5
TS (bulk soil)	wt.%	99.0 ± 0.2	98.2 ± 0.6	97.5 ± 0.8
VS (bulk soil)	% of TS	1.3 ± 0.4	3.7 ± 1.1	7.3 ± 0.5

The soil samples were air dried before this characterization, thus the high percentage of TS are measured as expected. The soil amended with peat shows a higher presence of volatile solids, due to the addition of organic matter.

The pH did not have strong fluctuations. The measured pH values for treated soil do not deviate significantly from untreated one. It was measured quite low value of EC, order of magnitude of µS cm^{-1}. The EC values decreased for treated soils.

The main properties of soils used to perform the phytoxicity test are presented in Table 3.

Table 3 (III). Main characteristics of untreated and treated soils, samples stored in laboratory (n = 3, ± SD)

Soil Proprieties	Unit	Untreated As soil	Soil + Fe	Soil + Fe + peat
TS (bulk soil)	wt.%	90.8 ± 0.2	89.2 ± 0.2	90.0 ± 0.3
VS (bulk soil)	% of TS	1.2 ± 0.0	1.3 ± 0.0	6.6 ± 0.2
Water holding capacity (WHC)	%	19.7 ± 0.3	22.2 ± 1.4	33.3 ± 1.1
Density	g cm^{-3} dw	1.35 ± 0.01	1.33 ± 0.01	1.25 ± 0.01
Elements total concentration				
As	mg kg^{-1} dw	136.71 ± 39.84	148.75 ± 4.36	125.07 ± 6.66
Fe	g kg^{-1} dw	23.23 ± 3.75	36.92 ± 3.63	38.32 ± 4.82

The TS are lower than of the former samples, because these measurements were performed on samples that were not previous air dried.

WHC was improved for both treated soil. The one stabilized with Fe^0 has WHC increase of 13% with respect to the untreated soil, while Fe^0 -peat treated soil has WHC 70% greater than untreated one.
The soil density did not significantly change from untreated and Fe^0 treated soil, but it was lower for Fe^0 -peat stabilized soil.

III.5.2 Sequential extraction

The total element concentration, calculated as the sum of each fraction, is shown in Figure 2.
The total arsenic concentration was on average higher in the soil stabilized with Fe^0 and peat (224.56 ± 43.97 mg kg^{-1} dw) than in the untreated soil (189.72 ± 12.06 mg kg^{-1} dw) and soil treated only with Fe^0 (191.75 ± 6.29 mg kg^{-1} dw). The data variability in soil with Fe^0 and peat was quite high, which made the differences between all the samples statistically not significant.
As shown in Figure 2, the Fe amount in significantly lower in the untreated soil, compare to the concentration in the treated soil where iron grit ameliorant was added. The total Fe concentration in both samples containing Fe^0-amendment increased by ca 1.6% compared with the untreated soil (Fig. 7).

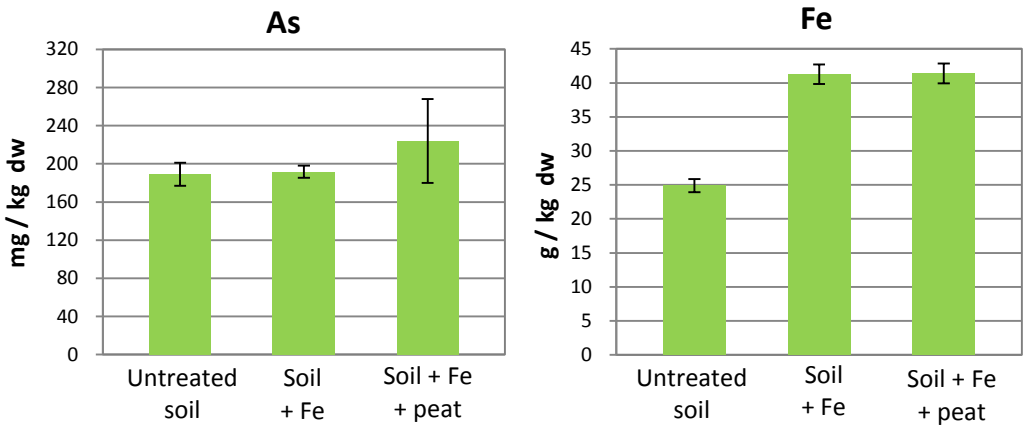

Figure 2 (III). Total arsenic and iron concentration calculated as sum of each fraction measured by the sequential extraction (n=3)

Arsenic and iron fractionations are shown in Figures 3 and 4, respectively.
The exchangeable As fraction in both stabilized soils decreased when compared to the untreated soil (Fig. 3). The decrease was larger in Fe^0-peat containing soil (46% lower concentration than in the untreated soil) than in soil amended only with Fe^0 (28% lower concentration than in the untreated soil).
No differences between the soil samples regarding the concentrations determined in all the other fractions were found. The Fe^0-peat amended soil shows on average an increase in the fraction bound to crystalline Fe oxides (Fig. 3). But as mentioned above, Fe^0-peat containing samples had the highest variability between the replicates, which

made this difference insignificant. Arsenic bound to organic matter/secondary sulphide fraction was in most cases undetectable.

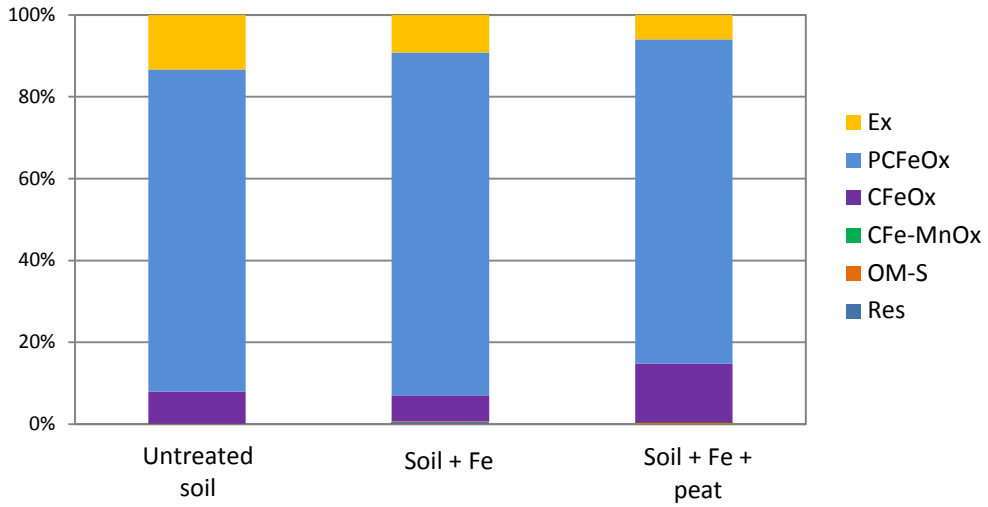

Figure 3 (III). Arsenic speciation in untreated and stabilized soils. (Ex) exchangeable fraction, (PCFeOX) bound to poorly crystalline Fe(III)-oxyhydroxides, (CFeOx) bound to crystalline Fe(III)-(oxyhydr)oxides, (CFe-MnOx) bound to Fe-Mn oxides, (OM-S) bound to organic matter and secondary sulphides, (Res) residual fraction.

The Fe exchangeable fraction increase in both treated soil, from 0.272 ± 0.015 g/kg in the untreated soil to 1.377 ± 0.358 g/kg, and 0.890 ± 0.048 g/kg in Fe^0 and Fe^0-peat stabilized soils respectively. The fraction of Fe poorly crystalline shows the greatest difference between treated and untreated soils (both concentrations are between 3.5-4 fold greater). All the soils showed the presence of Fe crystalline fraction: untreated soil 7.303 ± 0.174 g/kg, Fe^0 treated 6.547 ± 0.158 g/kg, Fe^0-peat stabilized 7.066 ± 0.726 g/kg. This fraction did not significantly change with treatments (Fig. 4). No differences between the soil samples regarding the concentrations determined in the Fe-Mn fraction and the residue one. The concentration on Fe bound to organic matter increased 6-fold in Fe^0-peat treated soil and 2-fold in Fe^0 treated soil. But this fraction is much lower compared to the others, the Fe^0-peat treated soil concentration is 0.261 ± 0.082 g/kg (Fig. 4).

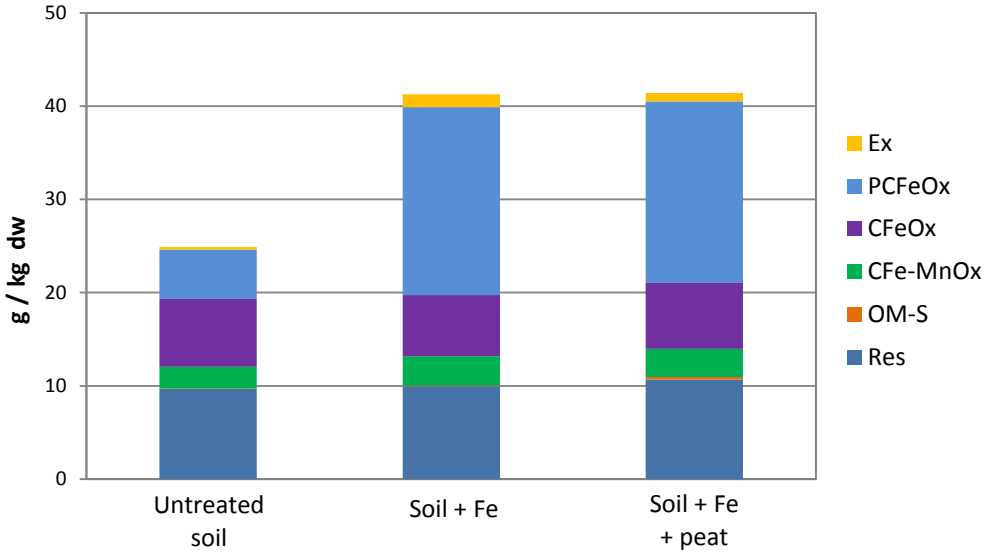

Figure 4 (III). Iron speciation in untreated and stabilized soils. (Ex) exchangeable fraction, (PCFeOX) poorly crystalline Fe(III)-oxyhydroxides, (CFeOx) crystalline Fe(III)-(oxyhydr)oxides, (Cfe-MnOx) Fe-Mn oxides, (OM-S) bound to organic matter and secondary sulphides, (Res) residual fraction.

III.5.3 Leachate analysis

Leachate samples were collected on the 4th and the 27th of June 2013. The pH and EC were measured immediately and values are summarized in Table 4. All the pH values measured were included between 7 and 8. No significant changes occurred between the two different sampling on the pH values.

The EC increased of almost one order of magnitude in untreated soil. For Fe^0 treated soil the mean value did not change but in both sampling the standard deviation (SD) is quite high due to variability among replicates. In the first sampling for leachate collected from Fe^0-peat treated soil the measured EC was quite high, but it decreased 13-fold in the second sampling (Tab. 4).

Table 4 (III). pH and EC of leachates collected in field (n=3, ± SD).

		Unit	Untreated As soil	Soil + Fe	Soil + Fe + peat
1st sampling (130604)	pH	-	7.60 ± 0.21	7.06 ± 0.03	7.15 ± 0.23
	EC	mS cm^{-1}	0.28 ± 0.249	7.513 ± 2.757	13.05 ± 0.226
2nd sampling (130604)	pH	-	7.83 ± 0.18	7.27 ± 0.09	7.03 ± 0.29
	EC	mS cm^{-1}	2.285 ± 0.694	7.630 ± 3.212	1.467 ± 0.285

The total As and Fe concentrations in leachate are shown in Figure 5. The As concentration significantly increase in Fe^0 treated soil (3-fold greater concentration than in the untreated soil). The concentration from the Fe^0-peat treated soil profile

does not change compared to the untreated soil, 4.37 ± 0.93 µg/l and 4.39 ± 1.17 µg/l respectively (Fig. 5).

The trend of Fe concentration is similar to As one. Fe concentration significantly increase in Fe^0 treated soil (16-fold greater concentration than in the untreated soil). While the Fe concentration from the Fe^0-peat treated soil profile does not change compared to the untreated soil 0.162 ± 0.105 mg/l and 0.211 ± 0.046 mg/l respectively (Fig. 5).

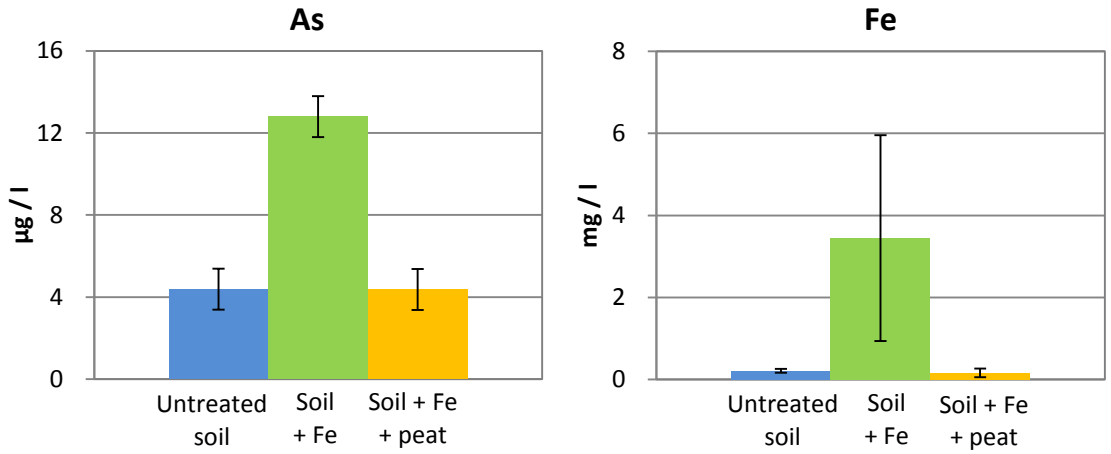

Figure 5 (III). As and Fe concentration in leachates collected on 4th June 2013 (n=3)

The analysis of the second sampling, on 27th June, show an outlier data in Fe^0-peat treated soil leachate. The As concentration is consistent with the one measured in the leachate collected on 6th June, considering the value of one of the samples as an outlier. The concentration in this sample is equal to 23.3 µg/L, while the mean value is 4.8 µg/L. This average does not differ from the As average concentration of leachate from untreated soil profile. Fe^0 treated soil has an As average concentration higher than untreated soil, but the high SD made this difference statistically not significant (Fig. 6).

The Fe concentration decrease significantly from the previous sampling, about 3 order of magnitude lower than the samples collected on 4th June 2013. Standard deviation in concentration of leachate percolating from Fe^0 treated soil profile is high due to the strong variability of the measured values (Fig. 6).

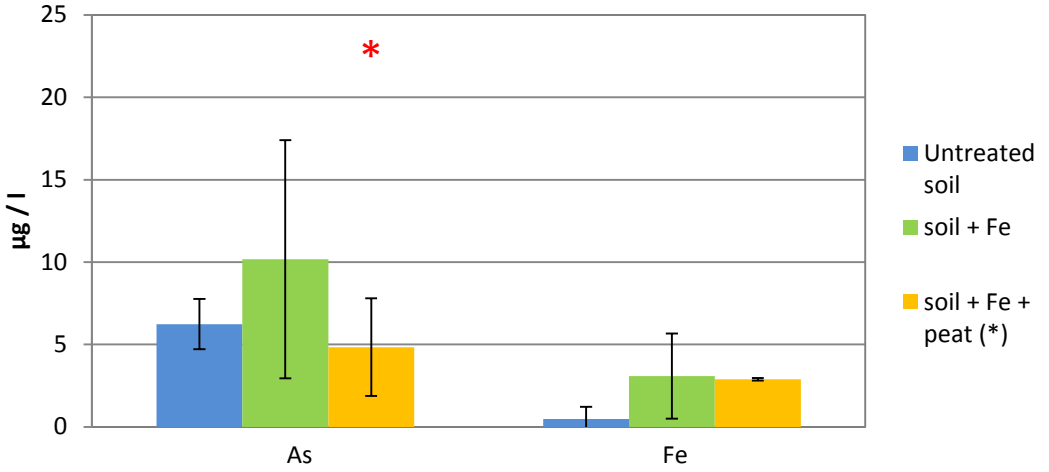

Figure 6 (III). As and Fe concentration in leachates collected on 27th June. The mean value for Fe-peat treated soil is done considering 2 values. (n =3)

The samples collected on 21st August showed a different situation with respect to the previous sampling (Fig. 7). The average As concentration in leachate percolating from Fe^0 and Fe^0-peat top layer soils are equal. The former treatment lowered the As concentration if compared to the previous sampling, while the Fe^0-peat slight increase the element concentration. Not outlier was detected, the sample with the greatest As concentration has a value equal to 9.9 µg/L. The SD for this treated soil was still large, highlighting the variability of As concentration. Both treated soil doubled the As concentration when compared to the concentration in leachate collected below the untreated cover layer (Fig. 7), but these values were still below the As concentration limit for drinking water that is 10 µg/L.

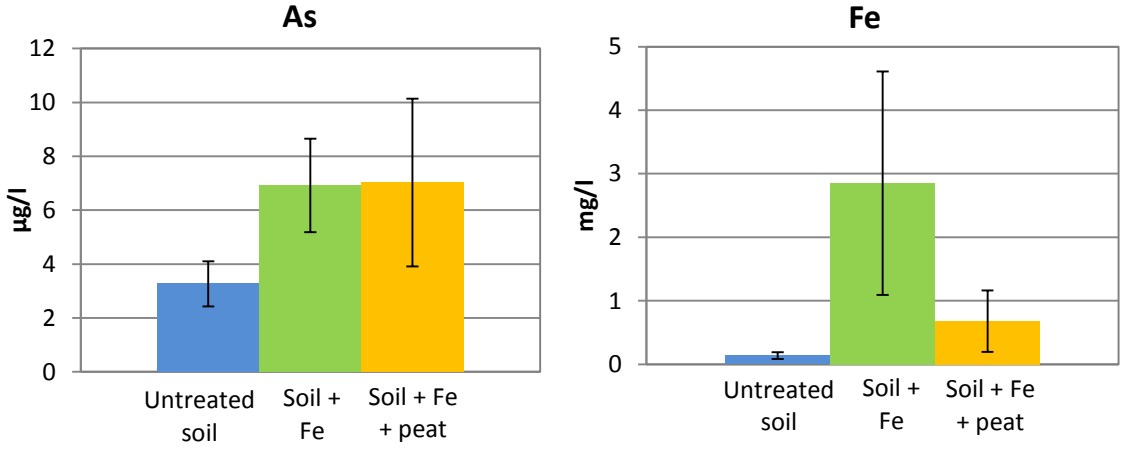

Figure 7 (III). As and Fe concentration in leachates collected on 21st August (n =3).

Considering the three sampling the As concentration in the leachate from Fe^0 treated soil is the highest, but it has a progressively decreasing of concentration. The Fe^0-peat treated soil had As concentration comparable to the untreated one in the first two

sampling, but in the last sampling showed an increase of the concentration. The leachate of the untreated soil presented a quite stable values (the lowest and the highest As concentrations measured were 3.27 µg/L and 6.24 µg/L, respectively measured on 21st August and on 27th June).

III.5.4 Phytotoxixity test

III.5.4.1 Morphological parameters

Morphological parameters where determined right after harvest, reported in Figure 8. The general trend for all the parameters are an improvement for the plants grew on Fe^0-peat treated soil, and a reduction of the characteristics for plants grew on Fe^0 treated soil if compared to the same parameter for plants grew on untreated soil. The high variability between the replicates made the differences of shoot length and root dry weight not significant. Primary leaf area, fresh biomass and dry shoot weight showed a similar trend among the different soils. These parameters decreased for plants grew on Fe^0 treated soil of 46%, 40% and 43% respectively when compared to the plants grew on untreated soil. The plants raised on Fe^0-peat treated soil increased these parameter of 2-fold compared to the untreated soil (Fig. 8).

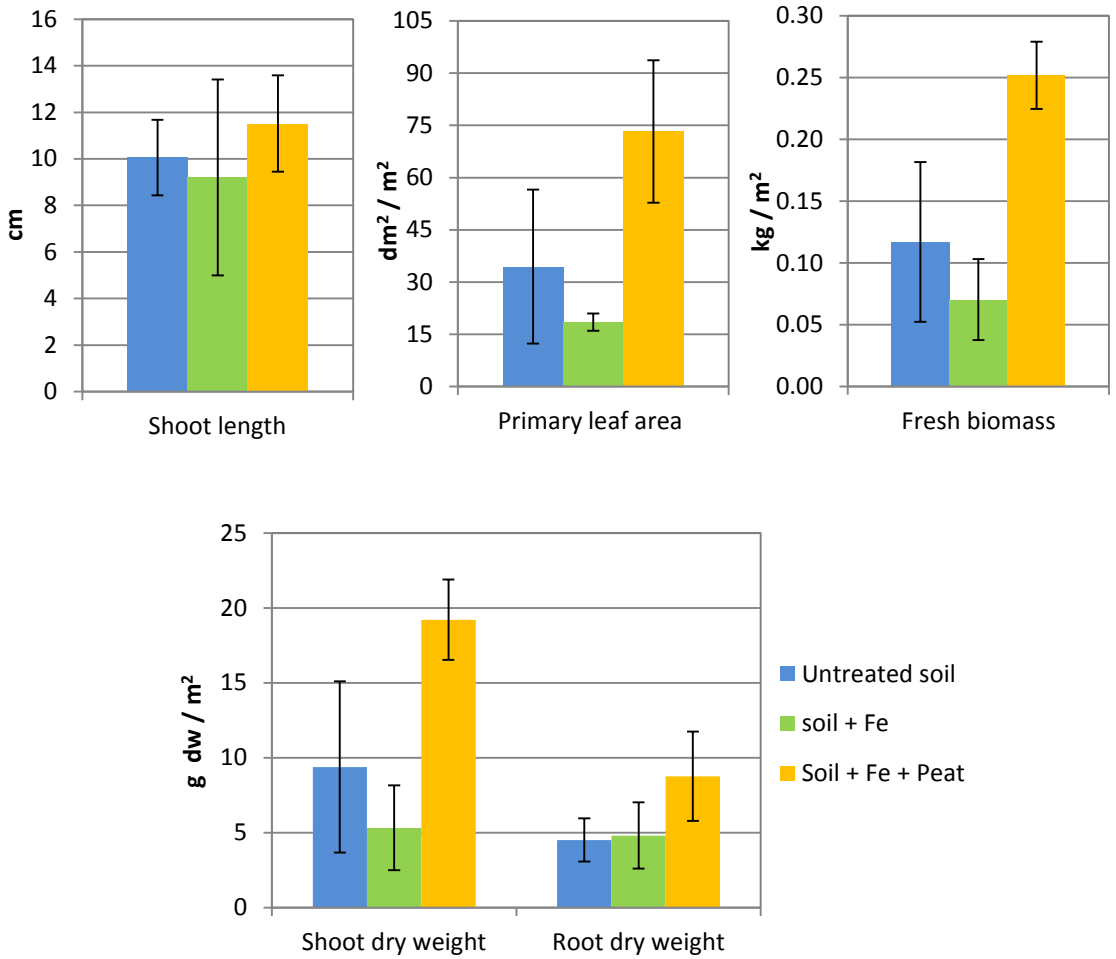

Figure 8 (III). Morphological paramenters: shoot length, primary leaf area, fresh biomass, ahoot and root dry weight measured at the end of the phytotoxicity test (n=4)

III.5.5 Element concentration in plants

Element concentrations of the dried shoot and root samples were measured, the results are presented in Figures 9 and 10.

The average of As concentration evaluate in plant shoots grew on untreated and Fe^0 treated soil are very close (2.16 mg/kg and 2.13 mg/kg respectively). The As concentration is 70% lower for the plant raise on Fe^0 stabilized soil when compared to ones grew on untreated soil.

Fe^0 treated soil showed a quite high SD for both As and Fe concentration, due to the variability of the data.

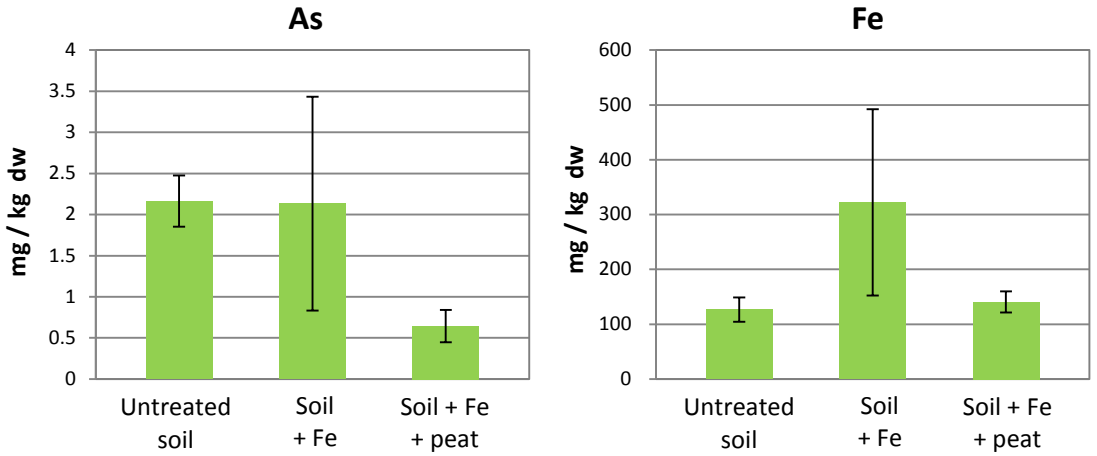

Figure 9 (III). Total Arsenic and Iron concentration measured in plant shoots. The error bars represent the standard deviation of the mean (n=4)

The As concentration measured in plant root is almost one order of magnitude higher than the one determine for plant shoot. The SD is still high, due to the variability of the data, with the exception of concentrations on plant roots from the soil stabilized with Fe^0 and peat.

The differences on As concentration between untreated and Fe^0 treated soil is not statistically significant. While As concentration decreased of 55% in samples grew on Fe^0 and peat stabilized soil with respect to the untreated one (Fig. 10).

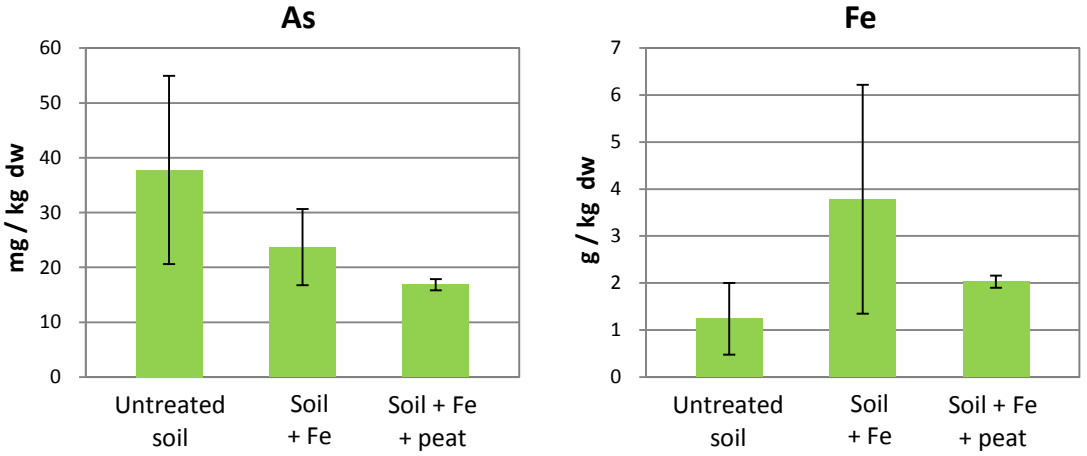

Figure 10 (III). Total Arsenic and Iron concentration measured in plant roots. The error bars represent the standard deviation of the mean (n=4)

III.5.5.1 Pore water analysis

The pore water electrical conductivity was lower in the presence of plants, the samples collected at the end of the phytoxicity test. The values measured are summarized in Table 5.

Table 5 (III). pH and EC of pore water collected from the phytoxicity test pots (n=4, ± SD).

		Unit	Untreated As soil	Soil + Fe	Soil + Fe + peat
1st sampling (130712)	pH	-	6.76 ± 0.08	7.83 ± 0.13	7.71 ± 0.02
	EC	mS cm^{-1}	2.24 ± 0.03	1.27 ± 0.10	4.87 ± 0.40
2nd sampling (130723)	pH	-	6.83 ± 0.08	7.91 ± 0.07	7.59 ± 0.09
	EC	mS cm^{-1}	1.63 ± 0.19	1.05 ± 0.09	4.53 ± 0.17

The concentrations of elements in pore water samples collected on 12th June 2013 are presented in Figure 11. The As concentration is lower in both Fe^0 (38.6% lower concentration than in the untreated soil) and Fe^0-peat treated soils (54% lower concentration than in the untreated soil). The Fe concentration does not show significant differences among the treatments.

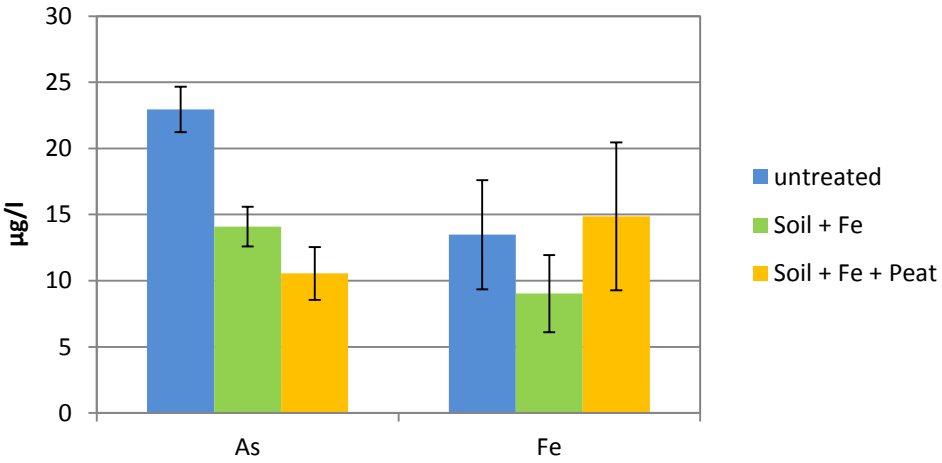

Figure 11 (III). As and Fe concentration in pore water samples collected on 12th July 2013

The second sampling, on 23th June 2013, showed a similar elements concentration trend than the samples collected on 12th June 2013. The As concentration decreased by 34% in Fe treated soil, and it was 51% lower in Fe-peat treated soil (Fig. 12).

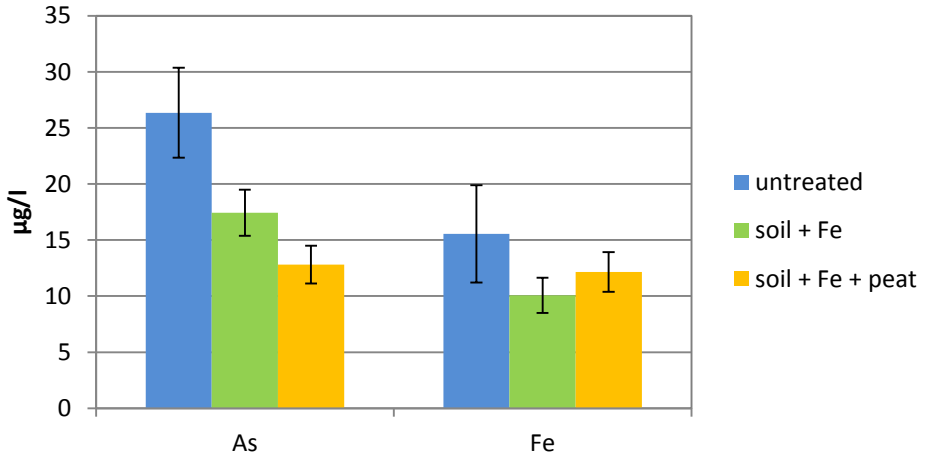

Figure 12 (III). As and Fe concentration in pore water samples collected on 23th July 2013

III.5.6 Bioaccessiblity

Comparison between the bioaccessible As concentration for untreated and treated soils is presented in Figure 13. The As bioaccessible decrease for both treated soils with respect to the untreated one. The decrease in Fe^0 treated soil is of 3%, while for the Fe^0-peat treated soil the reduction is about 23% when compared to untreated soil. The SD for all the soils is low (Fig. 13).

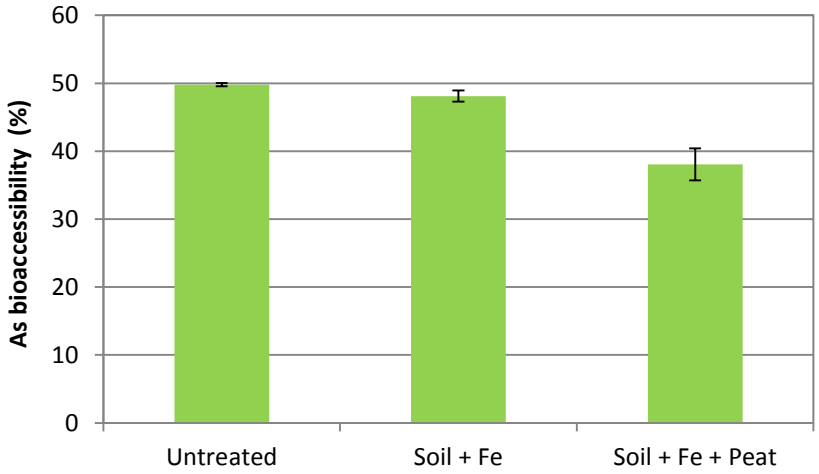

Figure 13 (III). Bioaccessible As fraction measured from untreated and treated soils, % of total As concentration calculated with Aqua Regia test (n=3)

The bioavailability is calculated using the linear regression model, the values obtained are shown in Figure 14. The As bioavailable (%) resulted between 2.5 % and 3.5% greater than the bioaccessible (%). The bioavailable fraction of the Fe^0-peat treated soil was 23% lower than the one evaluate for untreated soil.

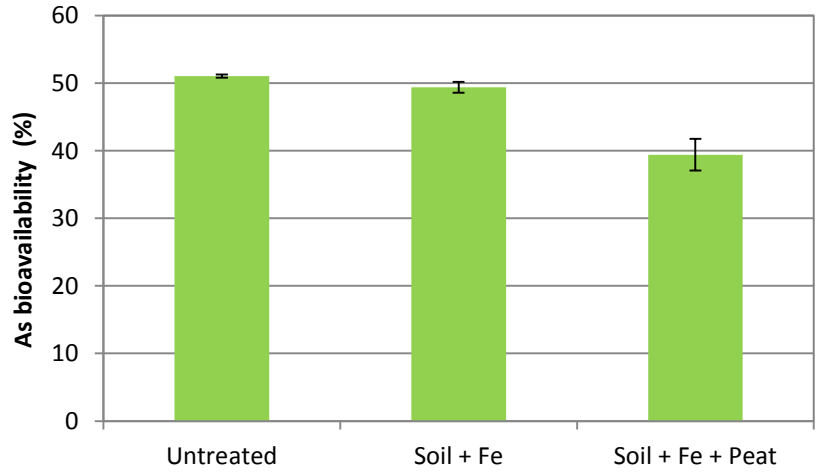

Figure 14 (III). Bioavailable As fraction measured from untreated and treated soils, % of total As concentration calculated with the Aqua Regia test. Calculated with the regression model from bioaccesible (%) for gastric phase (n=3)

III.6 DISCUSSION

Both treated soils increased the WHC compared to the untreated soil, Fe^0 and peat was more effective than only Fe^0. This indicates an improvement of the soil quality, enhancing physical and chemical soil characteristic. Higher WHC means to a greater amount of water available for plants and a smaller amount of water percolates to deeper soil layer. Top layer cover with plants lead to minimize the erosion of the top soil.

The soil treated with Fe^0 and peat showed a decrease of the exchangeable fraction, the most mobile one. Despite of the Fe speciation showed the presence of crystalline Fe oxides in untreated and treated soil, the As fraction bound to crystalline Fe oxides did not increase neither in Fe^0 nor in Fe^0-peat soils. This species is more stable than the fraction bound to the poorly crystalline Fe oxides. Indeed when oxides undergo in the aging process, the poor crystalline compounds develop their crystalline habit and less sorption sites are available, and thus this process can remobilize contaminant previously bonded. Higher the crystallization degree lowers the density of adsorption site. The organic matter and the secondary sulphide fraction were detectable only when Fe^0 and peat was added, but the concentration it was still very low compared to As bound to the other species.

The concentration of contaminant in plants is one of the most significant exposure pathway for human health. It represents the step through which the contaminants enter into the food chain, but it also can be considered a target itself. Moreover, stabilization should be followed by ecosystem recovery and revegetation of the area, aiming to avoid the erosion by wind and surface runoff (Greebelen et al., 2002). The combination of Fe^0 and peat improved the soil quality giving the best result in all the morphological parameters and a decreasing of As uptake by plants shoot and root. This result is consistent with As speciation, indeed root uptake is generally correlated with exchangeable fraction in soil (Kumpiene et al., 2006). The soil treated with Fe^0 did not show any improvement of morphological parameters, only root dry weight was slightly higher than the untreated soil one, while it decreased the As uptake in the plant root.

The analysis of leachate showed that the soil amended with Fe^0 and peat can be effective in the short term application even for quite thick soil (in this study the layer was 2 m deep), keeping the dissolved As equal to the one measured in untreated soil leachate. In the longer term it seemed to reach the leachate As concentration of the Fe^0 treated soil, and thus exceeding the value measured in untreated soil. This behavior could be explained by the occurrence of reducing condition also in Fe^0-peat stabilized soil. In the short term the combination between Fe^0 and peat is effective in promoting the air diffusion, thanks to a low soil density. The leachate needs to be monitored for longer to evaluate the trend of As concentration in the leachate over

time, while the behavior resulting from applying only Fe^0 ameliorant it seems consistent with results obtained in the previous studies.

It was demonstrated by Maurice et al. (2007), Kumpiene et al. (2012) and Kumpiene et al. (2013) that the stabilization can have an adverse effect on As solubility when reducing condition occurs. In this study the leachate is collected below the whole soil layer and thus it is affected by the condition occurring along the whole soil profile. Even though previous studies (Boisson et al., 1999; Mench et al., 2006; Lidelöw et al., 2007; Maurice et al., 2007; Kumpiene et al., 2008) demonstrated the significant decrease of As concentration in the pore water, this results should be considered as a direct consequence of an oxidizing condition occurring in the upper layer.

Leachate samples collected on 4th and 27th June showed that As concentration significantly increase in the Fe^0 amended soil, while for its combination with peat there was the As mobility compared to the untreated soil. Only the Fe^0 amended soil exceeded the limit concentration of As in drinking water, i.e. 0.01 mg L^{-1} (WHO, 2010). In the last sampling, on 21st August, both treated soil had higher As concentration than untreated soil. However, none of the samples exceed the As concentration limit for drinking water given by WHO.

The EC represents the free ion concentration. If it is too high it may represent a limitation for the vegetation development. In this study the highest EC values were detected in the Fe^0-peat treated area, that it was also where the plant diversity was most enhanced. Indeed in the field experiment the difference between the three areas was well outlined by the vegetation. On both untreated and Fe^0 treated top layer the plants did not cover completely the surface, where there was only As contaminated soil one plant species prevailed, while on the soil amended with Fe^0 was growing different plant species. In the area overlaid with Fe^0-peat stabilized soil the plant density was higher than the other ones, it was not possible to see a spot of soil through the vegetation cover, and as mentioned before the plant biodiversity was improved when compared to untreated and Fe^0 treated area. This result was also confirmed in the phytotoxicity test where the pore water collected from the pots with Fe^0-peat stabilized soil had EC greater than the other samples, but it also had the best morphological plant parameters.

The bioaccessibility test showed significant decreasing of As bioaccessible in Fe^0-peat treated (23% lower compared to the value obtained from untreated soil). Using the linear regression model validated by Juhasz et al. (2009), it was evaluated the As bioavailable fraction, that resulted between 2.5% and 3.5% greater than the bioaccessible (%). The bioavailable fraction was estimated equal to 51%, 49% and 40% of the total As concentration for untreated, Fe^0 and Fe^0-peat treated soils respectively. So far the regulation establishes to consider the bioavailable amount equal to the total As concentration.

III.7 CONCLUSION

This study demonstrated that the applicability of As contaminated soil stabilized with Iron amendment or its combination with peat is restricted to a thin layer. Appling it to a thicker layer lead to the occurrence of anaerobic and reductive condition in the deeper soil layers, and so an adverse effect of As solubility. Fe^0-peat stabilization was effective from September to beginning of June, it kept the soil porous and assuring the air diffusion in deeper soil layer. While from the second sampling higher As solubility appeared, probably due to the occurrence of anaerobic conditions.

The stabilization with Fe^0-peat was very effective in reducing the phytotoxicity and plant uptake, and also the morphological parameters were improved with respect to untreated and Fe^0 treated soil. This result is also confirmed in the pilot-scale experiment where there was vegetation cover, with the presence of different plant species.

The As speciation changed with the stabilization. The exchangeable fraction decrease in both treated soil, with better results for the combination Fe^0 and peat.

The chemical stabilization positively affects the As concentration in the pore water. This result was expected because it was demonstrated in previous studies that in oxidizing condition the treatment is effective. When it was analyzed the leachate percolating along the whole soil profile 2 m thick, it was detected adverse effect of the stabilization. The As concentration increased since the first sampling in the Fe^0 treated area, and after the second sampling in the Fe^0-peat treated area, where the soil porosity probably could keep the air diffusion in soil for the first assessment period.

The risk connected to the direct ingestion of soil can be evaluated considering the As bioavailable fraction. The bioaccessibility and the estimated bioavailability were significantly improved for the Fe^0-peat treated soil. The bioavailable fraction was estimated at 49% and 40% for Fe^0 and Fe^0-peat treated soil. So far when a risk assessment is performed for a contaminated site the bioavailable fraction considered is equal to As total concentration, even though from this estimation the value is less than double of the total amount.

III.8 OUTLOOK

Further research on long-term and large-scale applicability of stabilization are needed to investigate the occurrence of anaerobic conditions, and the suitability of Fe^0 combined with others ameliorants. It needs to be assessed at which depth changes in redox condition occur, evaluating the As concentration in pore water sampled at different depth.

Further validation of in vitro bioaccessibility test is also important to evaluate accurately the risk assessment.

III.9 ABBREVIATIONS

WHO	World health organization
Fe^0	Zerovalent Iron
CCA	Chromated copper arsenate (wood impregnation chemical)
BS	Blasting Sand
EC	Electrical conductivity
TS	Total solids
VS	Volatile solids
WHC	Water holding capacity
ICP-OES	Inductively coupled plasma optical emission spectroscopy
SD	Standard deviation

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PART IV

DATA RESULTS

IV. DATA RESULTS

IV.1 ANNEX I: SOIL CARACTERIZATION

	Measured values		Electrical conductivity		pH	
	Electrical Conductivity (µS/cm)	pH	average	SD	average	SD
Untreated soil	411	7.76	403.5*	10.6*	7.85*	0.13*
	396	7.94				
	1076	8.05				
Soil + Fe	256	7.85	297.3	36.5	7.89	0.06
	325	7.85				
	311	7.96				
Soil + Fe + peat	238	7.59	272.3	45.5	7.72	0.15
	324	7.68				
	255	7.88				

Table 1 (IV). pH, EC values of soil samples collected in the field on 17th May 2013, and calculation on average and standard deviation of both values.

	Crucible weight (g)	Crucible weight fill with wet soil (g)	Weight after 24 h at 105°C (g)	Weight after 2 h at 550°C (g)
Untreated soil	32.42	62.49	62.16	61.52
	39.82	75.20	74.91	74.35
	28.18	59.77	59.45	57.85
Soil + Fe	35.12	65.50	64.85	63.41
	36.27	66.94	66.31	65.21
	40.15	72.56	72.19	71.36
Soil + Fe + peat	36.65	69.94	69.24	66.76
	38.34	71.19	70.04	67.90
	37.32	70.33	69.66	67.24

Table 2 (IV). Measured weight of treated and untreated soils, to calculate the TS and VS (19/06/2013).

	Total solids (TS) % (dry weight)			Volatile solids (VS) g/kg [or % of TS]		
		Average	SD		Average	SD
Untreated soil	98.9	99.0	0.2	2.168	1.9	0.4
	99.2			1.588		
	99.0			5.127		
Soil + Fe	97.9	98.2	0.6	4.860	3.7	1.1
	97.9			3.653		
	98.9			2.615		
Soil + Fe + peat	97.9	97.5	0.8	7.637	7.3	0.5
	96.5			6.738		
	98.0			7.482		

Table 3 (IV). Calculations of TS and VS of treated and untreated soils, after the samples have been air dried (19/06/2013).

	Total weight (g)	Seived soil < 2mm diameter (g)	Un-seived > 2mm diameter (g)	% of particles <2mm
Untreated soil	895.84	492.6	402	55.1
	1238.64	618.86	616.6	50.1
	905.92	544.11	359.01	60.2
Soil + Fe	809.41	446.78	361.04	55.3
	783.87	484.1	297.39	61.9
	1091.79	684.2	405.52	62.8
Soil + Fe + peat	1186.81	693.16	491.18	58.5
	1122.39	681.93	438.22	60.9
	1108.79	601.07	499.58	54.6

Table 4 (IV). Total weight of soil samples, sieved and un-seived fraction.

IV.2 ANNEX II: SEQUENTIAL EXTRACTION, AS SPECIATION

As fraction (I)				
	As concentration mg/L	**As concentration mg/kg**	**Average mg/kg**	**SD**
Untreated soil	1.001	25.262	25.22	0.06
	0.997	25.179		
Soil + Fe	0.655	16.674	17.64	1.31
	0.673	17.118		
	0.752	19.130		
Soil + Fe + peat	0.516	13.244	13.31	0.59
	0.543	13.937		
	0.497	12.753		

Table 5 (IV). Arsenic speciation in untreated and stabilized soils. Exchangeable fraction.

As fraction (II)				
	As concentration mg/L	**As concentration mg/kg**	**Average mg/kg**	**SD**
Untreated soil	4.151	157.208	148.76	11.94
	3.705	140.320		
Soil + Fe	4.261	162.699	160.54	1.92
	4.165	159.009		
	4.189	159.923		
Soil + Fe + peat	3.742	143.990	177.56	36.02
	5.604	215.604		
	4.499	173.099		

Table 6 (IV). Arsenic speciation in untreated and stabilized soils. Fraction bound to poorly crystalline Fe(III)-oxyhydroxides.

As fraction (III)				
	As concentration mg/L	**As concentration mg/kg**	**Average mg/kg**	**SD**
Untreated soil	0.364	13.794	14.97	1.66
	0.426	16.146		
Soil + Fe	0.493	18.839	12.16	5.79
	0.222	8.487		
	0.240	9.155		
Soil + Fe + peat	0.278	10.685	32.22	25.21
	1.558	59.952		
	0.676	26.018		

Table 7 (IV). Arsenic speciation in untreated and stabilized soils. Fraction bound to crystalline Fe(III)-(oxyhydr)oxides.

As fraction (IV)				
	As concentration mg/L	**As concentration mg/kg**	**Average mg/kg**	**SD**
Untreated soil	0.005	0.101	0.10	0.00
	0.005	0.101		
Soil + Fe	0.005	0.102	0.10	0.00
	0.005	0.102		
	0.005	0.102		
Soil + Fe + peat	0.023	0.466	0.22	0.21
	0.005	0.103		
	0.005	0.103		

Table 8 (IV). Arsenic speciation in untreated and stabilized soils. Fraction bound to Fe-Mn oxides. The grey values are the samples that had As concentration below the detection limit.

As fraction (V)				
	As concentration mg/L	**As concentration mg/kg**	**Average mg/kg**	**SD**
Untreated soil	0.006	0.145	0.16	0.01
	0.007	0.165		
Soil + Fe	0.009	0.240	0.34	0.16
	0.021	0.524		
	0.010	0.246		
Soil + Fe + peat	0.024	0.614	0.73	0.33
	0.043	1.104		
	0.676	26.018		

Table 9 (IV). Arsenic speciation in untreated and stabilized soils. Fraction bound to organic matter and secondary sulphides.

As fraction (VI)				
	As concentration mg/L	**As concentration mg/kg**	**Average mg/kg**	**SD**
Untreated soil	0.005	0.505	0.50	0.00
	0.005	0.505		
Soil + Fe	0.018	1.879	0.97	0.79
	0.005	0.509		
	0.005	0.509		
Soil + Fe + peat	0.005	0.513	0.51	0.00
	0.005	0.513		

Table 10 (IV). Arsenic speciation in untreated and stabilized soils. Residual fraction.

Total As concentration		
	mg/kg	**SD**
Untreated soil	189.715	12.057
Soil + Fe	191.749	6.295
Soil + Fe + peat	224.563	43.969

Table 11 (IV). Arsenic speciation in untreated and stabilized soils. Total concentration calculated as sum of all the fractions.

IV.3 ANNEX III: SEQUENTIAL EXTRACTION, FE SPECIATION

Fe fraction (I)				
	Fe concentration mg/L	**Fe concentration g/kg**	**Average g/kg**	**SD**
Untreated soil	10.352	0.261	0.272	0.015
	11.211	0.283		
Soil + Fe	37.889	0.964	1.377	0.358
	63.404	1.614		
	60.975	1.552		
Soil + Fe + peat	33.094	0.849	0.890	0.048
	34.241	0.878		
	36.783	0.944		

Table 12 (IV). Iron speciation in untreated and stabilized soils. Exchangeable fraction.

Fe fraction (II)				
	Fe concentration mg/L	**Fe concentration g/kg**	**Average g/kg**	**SD**
Untreated soil	146.983	5.566	5.290	0.391
	132.383	5.013		
Soil + Fe	508.199	19.403	20.165	1.322
	568.145	21.691		
	508.128	19.400		
Soil + Fe + peat	533.009	20.508	19.451	1.149
	473.760	18.229		
	509.839	19.617		

Table 13 (IV). Iron speciation in untreated and stabilized soils. Poorly crystalline Fe(III)-oxyhydroxides fraction.

Fe fraction (III)				
	Fe concentration mg/L	**Fe concentration g/kg**	**Average g/kg**	**SD**
Untreated soil	189.606	7.180	7.303	0.174
	196.102	7.426		
Soil + Fe	174.674	6.669	6.547	0.158
	172.987	6.605		
	166.809	6.369		
Soil + Fe + peat	203.923	7.846	7.066	0.726
	166.595	6.410		
	180.416	6.942		

Table 14 (IV). Iron speciation in untreated and stabilized soils. Crystalline Fe(III)-(oxyhydr)oxides fraction.

Fe fraction (IV)				
	Fe concentration mg/L	**Fe concentration g/kg**	**Average g/kg**	**SD**
Untreated soil	114.379	2.310	2.334	0.033
	116.718	2.357		
Soil + Fe	148.722	3.028	3.156	0.275
	145.781	2.968		
	170.545	3.473		
Soil + Fe + peat	159.692	3.277	3.032	0.307
	130.998	2.688		
	152.615	3.132		

Table 15 (IV). Iron speciation in untreated and stabilized soils. Fe-Mn oxides fraction.

Fe fraction (V)				
	Fe concentration mg/L	**Fe concentration g/kg**	**Average g/kg**	**SD**
Untreated soil	1.668	0.042	0.039	0.004
	1.427	0.036		
Soil + Fe	4.126	0.105	0.094	0.011
	3.233	0.082		
	3.691	0.094		
Soil + Fe + peat	13.273	0.340	0.261	0.082
	6.857	0.176		
	10.396	0.267		

Table 16 (IV). Iron speciation in untreated and stabilized soils. Fraction bound to organic matter and secondary sulphides.

Fe fraction (VI)				
	Fe concentration mg/L	**Fe concentration g/kg**	**Average g/kg**	**SD**
Untreated soil	89.621	9.051	9.663	0.866
	101.744	10.275		
Soil + Fe	96.983	9.874	9.941	0.291
	100.766	10.259		
	95.159	9.688		
Soil + Fe + peat	106.695	10.947	10.690	0.364
	101.676	10.432		

Table 17 (IV). Iron speciation in untreated and stabilized soils. Residual fraction.

Total Fe concentration		
	g/kg	**SD**
Untreated soil	24.901	0.966
Soil + Fe	41.280	1.436
Soil + Fe + peat	41.391	1.443

Table 18 (IV). Iron speciation in untreated and stabilized soils. Total concentration calculated as sum of all the fractions.

IV.4 ANNEX IV: PHYTOTOXICITY TEST

Water holding capacity						
	cylindrical beakers + filter paper (g)	cylindrical beakers + filter paper + wet soil (g)	cylindrical beakers + filter paper + dry soil (g)	WHC (%)	Average WHC (%)	SD WHC
Untreated soil	30.22	46.68	43.07	20.31	19.7	0.3
	30.18	45.18	41.81	20.38		
	30.07	47.85	43.97	20.72		
Soil + Fe	30.22	47.41	43.39	22.93	22.2	1.4
	29.82	49.27	44.67	24.24		
	30.32	49.47	45.26	21.49		
Soil + Fe + peat	29.66	49.36	43.61	34.05	33.3	1.1
	30.66	48.12	43.03	33.06		
	30.32	48.13	42.75	35.24		

Table 19 (IV). Water holding capacity calculation (01/07/2013)

Density (g/cm^3 dw)								
	Baker (g)	Baker + soil (g)	Soil (g)	TS (g/kg)	Soil (gTS)		Average	SD
Untreated soil	32.60	132.32	99.71	994.81	99.20	1.33	1.35	0.02
	32.60	134.56	101.96	994.35	101.38	1.36		
	32.60	133.96	101.35	994.05	100.75	1.35		
Soil + Fe	32.61	133.23	100.62	992.63	99.88	1.34	1.33	0.01
	32.61	132.73	100.13	992.73	99.40	1.33		
	32.61	131.62	99.01	992.83	98.30	1.32		
Soil + Fe + peat	32.61	128.53	95.92	979.99	94.00	1.26	1.25	0.01
	32.61	128.38	95.77	979.38	93.80	1.26		
	32.61	127.03	94.42	979.29	92.47	1.24		

Table 20 (IV). Measurement of soil density, the samples were previous air dried and TS has been evaluate. The baker volume is 74.572 cm^3.

	Total As concentration (mg/kg)			Total Fe concentration (g/kg)		
		Average	SD		Average	SD
Untreated soil	181.17	135.71	39.84	20.73	23.23	3.75
	124.74			27.54		
	104.22			21.41		
Soil + Fe	153.41	148.75	4.36	40.81	36.92	3.63
	148.08			33.64		
	144.76			36.29		
Soil + Fe + peat	131.43	125.07	6.66	43.86	38.32	4.82
	125.63			35.95		
	118.14			35.14		

Table 21 (IV). Elements total concentration determined with Aqua Regia test

	Crucible weight (g)	Crucible weight fill with wet soil (g)	Weight after 24 h at 105°C (g)	Weight after 2 h at 550°C (g)
Untreated soil	28.86	55.78	53.24	52.90
	50.84	78.69	76.15	75.81
	31.33	53.25	51.25	50.99
Soil + Fe	29.33	56.18	53.25	52.93
	56.01	86.61	83.31	82.95
	30.59	60.55	57.37	57.02
Soil + Fe + peat	51.63	80.05	77.12	75.38
	29.61	50.99	48.93	47.69
	29.87	53.39	51.05	49.67

Table 22 (IV). Measured weight of treated and untreated soils, to calculate the TS and VS (30/06/2013)

	Total solids (TS) % (dry weight)			Volatile solids (VS) g/kg [or % of TS]		
		Average	SD		Average	SD
Untreated soil	90.58	90.8	0.2	1.41	1.4	0.0
	90.90			1.36		
	90.91			1.33		
Soil + Fe	89.08	89.2	0.2	1.33	1.3	0.0
	89.22			1.32		
	89.40			1.31		
Soil + Fe + peat	89.69	90.0	0.3	6.84	6.6	0.2
	90.35			6.40		
	90.07			6.53		

Table 23 (IV). Calculations of TS and VS of treated and untreated soils store in laboratory (30/06/2013)

IV.4.1 ANNEX V: MORPHOLOGICAL PARAMETERS
(Pot area is 0.0113 m^2)

Untreated soil						
	H leaf	**B leaf**	**Leaf area**	**total primary leaf area**	**total primary leaf area**	**Average primary leaf area for each treatment**
Unit	cm	cm	cm^2	$cm^{\wedge 2}$/pot	dm^2/m^2	dm^2/m^2
B1	6.9	4.9	26.55	73.82	65.27	**34.4**
	6.9	4.5	24.39			
	3.3	2.2	5.70			
	3.4	2.7	7.21			
	2.7	2.6	5.51			**SD** 22.1
	2.7	2.1	4.45			
B2	4.7	3.7	13.66	26.66	23.58	
	4.6	3.6	13.01			
			no leaves			
B3	3.9	2.9	8.88	38.79	34.30	
	3.7	3	8.72			
	4.3	2.8	9.46			
	4.2	3.3	10.89			
	0.9	0.6	0.42			
	0.9	0.6	0.42			
B4	3.7	2.8	8.14	16.41	14.51	
	3.9	2.7	8.27			

Table 24 (IV). Calculation of the primary leaf area for plants grew in on utreated soil. The pot area was equal to 0.0113 m^2.

Soil + Fe^0						
	H leaf	**B leaf**	**Leaf area**	**total primary leaf area**	**total primary leaf area**	**Average primary leaf area for each treatment**
Unit	cm	cm	cm^2	cm^^{2}/pot	dm^2/m^2	dm^2/m^2
C1	5.4	3.8	16.12	30.07	26.58	**18.5**
	4.8	3.7	13.95			
C2	3	2.3	5.42	24.67	21.81	**SD 2.5**
	2.8	2.2	4.84			
	3.5	2.6	7.15			
	3.7	2.5	7.26			
C3	5.4	4	16.96	28.97	25.61	
	2	3	4.71			
	2.9	3.2	7.29			
C4	0	0	no leaves	0	0	
	0	0	no leaves			

Table 25 (IV). Calculation of the primary leaf area for plants grew in on soil treated with Fe^0. The pot area was equal to 0.0113 m^2.

Soil + Fe^0 + Peat						
	H leaf	**B leaf**	**Leaf area**	**total primary leaf area**	**total primary leaf area**	**Average primary leaf area for each treatment**
Unit	cm	cm	cm^2	cm^2/pot	dm^2/m^2	dm^2/m^2
D1	6.9	5.4	29.26	102.73	90.83	**73.3**
	6.7	5.2	27.36			
	5.2	4	16.34			
	5.1	4	16.02			
	3.4	2.1	5.61			**SD 20.4**
	3.7	2.8	8.14			
D2	7.2	5.9	33.36	101.30	89.57	
	7.6	5.5	32.83			
	4.6	3	10.84			
	4.5	3.1	10.96			
	2.9	2.4	5.47			
	3.7	2.7	7.85			
D3	5.7	4.3	19.25	72.00	63.66	
	5.9	3.9	18.07			
	5.5	3.7	15.98			
	5.5	4	17.28			
	1	0.9	0.71			
	1	0.9	0.71			
D4	6.9	5.5	29.81	55.44	49.02	
	6.4	5.1	25.64			
	0	0	no leaves			
	0	0	no leaves			
	0	0	no leaves			

Table 26 (IV). Calculation of the primary leaf area for plants grew on soil treated with Fe^0 and peat. The pot area was equal to 0.0113 m^2.

Untreated soil							
	Shoot length	**Average shoot length per pot**	**Average shoot length**	**fresh biomass weight**	**Total fresh biomass per pot**	**Total fresh biomass**	**Average fresh biomass**
Unit	cm	cm	cm	g	g	kg/ m^2	kg/ m^2
B1	14.7	10.9	**10.1**	1.53	2.31	0.204	**0.117**
	9.7			0.36			
	8.3		**SD 1.6**	0.42			**SD 0.065**
B2	12.3	9.4		0.77	1.11	0.098	
	6.5						
B3	11.7	8.13		0.5	1.31	0.116	
	10.5			0.72			
	2.2			0.09			
B4	11.8	11.8		0.56	0.56	0.050	

Table 27 (IV). Calculation of the average shoot length and average fresh biomass for plants grew on untreated soil.

Soil + Fe^0							
	Shoot length	**Average shoot length per pot**	**Average shoot length**	**fresh biomass weight**	**Total fresh biomass per pot**	**Total fresh biomass**	**Average fresh biomass**
Unit	cm	cm	cm	g	g	kg/ m^2	kg/ m^2
C1	12.8	12.8	**9.2**	1.09	1.09	0.096	**0.070**
C2	8.8	9.9		0.3	0.66	0.058	
	10.9			0.36			
C3	13	11	**SD 4.2**	0.68	1.1	0.097	**SD 0.033**
	9			0.42			
C4	4.1	3.2		0.27	0.33	0.029	
	2.2			0.06			

Table 28 (IV). Calculation of the average shoot length and average fresh biomass for plants grew on soil treated with Fe^0.

Soil + Fe^0 + Peat							
	Shoot length	**Average shoot length**	**Average shoot length**	**fresh biomass weight**	**Total fresh biomass**	**Total fresh biomass**	**Average fresh biomass**
Unit	cm	cm/pot	cm	g	g/pot	kg/ m^2	kg/ m^2
D1	14.6	13.3	**11.5**	1.64	3.13	0.277	**0.252**
	14.1			0.92			
	11.3			0.57			
D2	17.2	12.9		1.85	2.9	0.256	
	11.5		**SD 2.1**	0.59			**SD 0.027**
	10			0.46			
D3	14.6	11.0		1.06	2.41	0.213	
	14.5			1.11			
	4			0.24			
D4	16.4	8.8		1.77	2.95	0.261	
	6.4			0.42			
	8.6			0.43			
	3.8			0.33			

Table 29 (IV). Calculation of the average shoot length and average fresh biomass for plants grew on soil treated with Fe^0 and peat.

Untreated soil						
	Shoot dry weight	**Shoot dry weight**	**Average shoot dry weight**	**Root dry weight**	**Root dry weight**	**Average root dry weight**
Unit	g dw	g dw/m^2	g dw/m^2	g	g dw/m^2	g dw/m^2
B1	0.197	17.419	**9.39**	0.053	4.651	**4.51**
B2	0.081	7.118		0.032	2.812	
B3	0.101	8.913	**SD 5.71**	0.072	6.322	**SD 1.44**
B4	0.046	4.094		0.048	4.253	

Table 30 (IV). Calculation of the average shoot dry weight and average root dry average for plants grew on untreated soil.

Soil + Fe^0						
	Shoot dry weight	**Shoot dry weight**	**Average shoot dry weight**	**Root dry weight**	**Root dry weight**	**Average root dry weight**
Unit	g dw	g dw/m^2	g dw/m^2	g	g dw/m^2	g dw/m^2
C1	0.085	7.542	**5.32**	0.091	8.020	**4.81**
C2	0.055	4.819		0.049	4.297	
C3	0.084	7.410	**SD 2.83**	0.045	3.944	**SD 2.21**
C4	0.017	1.521		0.034	2.997	

Table 31 (IV). Calculation of the average shoot dry weight and average root dry average for plants grew on soil treated with Fe^0.

Soil + Fe^0 + Peat						
	Shoot dry weight	**Shoot dry weight**	**Average shoot dry weight**	**Root dry weight**	**Root dry weight**	**Average root dry weight**
Unit	g dw	g dw/m^2	g dw/m^2	g	g dw/m^2	g dw/m^2
D1	0.241	21.283	**19.21**	0.118	10.451	**8.77**
D2	0.246	21.760		0.081	7.188	
D3	0.188	16.605	**SD 2.69**	0.062	5.455	**SD 2.98**
D4	0.194	17.189		0.135	11.972	

Table 32 (IV). Calculation of the average shoot dry weight and average root dry average for plants grew on soil treated with Fe^0 and peat.

	Unit	**Untreated As soil**	**Soil + Fe**	**Soil + Fe + peat**
Shoot length	cm	10.1 ± 1.6	9.2 ± 4.2	11.5 ± 2.1
Primary leaf area	dm^2/m^2	34.4 ± 22.1	18.5 ± 2.5	73.3 ± 20.4
Fresh biomass	kg/ m^2	0.117 ± 0.065	0.07 ± 0.033	0.252 ± 0.027
Shoot dry weight	g dw/m^2	9.39 ± 5.71	5.32 ± 2.83	19.21 ± 2.69
Root dry weight	g dw/m^2	4.51 ± 1.44	4.81 ± 2.21	8.77 ± 2.98

Table 33 (IV). Morphological parameters of untreated and treated soils

	Shoot As concentration (mg/kg)			Shoot Fe concentration (mg/kg)		
		Average	SD		Average	SD
Untreated soil	2.53	2.16	0.31	154	126.5	22.2
	1.88			114		
	2.31			134		
	1.93			104		
Soil + Fe	0.92	2.13	1.30	203	322.2	170.1
	1.31			183		
	2.50			352		
	3.80			551		
Soil + Fe + peat	0.45	0.64	0.20	119	140.7	19.3
	0.77			130		
	0.85			158		
	0.51			156		

Table 34 (IV). Total As and Fe concentration measured in plant shoots from the phytotoxixity test.

	Root As concentration (mg/kg)			Root Fe concentration (g/kg)		
		Average	SD		Average	SD
Untreated soil	50.3	37.8	17.2	1.15	1.24	0.76
	52.7			2.32		
	32.1			0.96		
	16			0.53		
Soil + Fe	18	23.7	6.9	2.11	3.78	2.43
	19.9			2.66		
	33.6			7.40		
	23.3			2.97		
Soil + Fe + peat	16.9	16.8	1.0	2.08	2.03	0.13
	17.6			2.14		
	17.5			2.06		
	15.4			1.84		

Table 35 (IV). Total As and Fe concentration measured in plant roots from the phytotoxixity test.

	Element	Untreated As soil	Soil + Fe	Soil + Fe + peat
Shoot	As	2.16 ± 0.31	2.13 ± 1.3	0.64 ± 0.20
	Fe	127 ± 22	322 ± 170	141 ± 19
Root	As	37.8 ± 17.2	23.7 ± 7.0	16.9 ± 1.0
	Fe	1241 ± 764	3785 ± 2436	2030 ± 131

Table 36 (IV). Summaizing table of element concentrations measured in shoot and root samples.

IV.5 ANNEX V: PORE WATER DATA

	Measured values		Electrical conductivity (mS/cm)		pH	
	Electrical Conductivity (mS/cm)	pH	average	SD	average	SD
Untreated soil	2.255	6.78	2.239	0.029	6.76	0.08
	2.198	6.64				
	2.264	6.84				
	2.238	6.76				
Soil + Fe	1.346	7.77	1.3	0.1	7.83	0.13
	1.133	8.03				
	1.343	7.74				
	1.276	7.78				
Soil + Fe + peat	4.443	7.74	4.9	0.4	7.71	0.02
	4.622	7.70				
	5.250	7.69				
	5.156	7.72				

Table 37 (IV). pH, EC values of pore water samples collected from the pots of the phytotoxicity test on 12th June 2013, and calculation on average and standard deviation of both values.

	Total As concentration (µg/l)			Total Fe concentration (µg/l)		
		Average	SD		Average	SD
Untreated soil	22	22.95	1.72	15.1	13.47	4.13
	25.1			15.8		
	23.5			7.3		
	21.2			15.7		
Soil + Fe	16.1	14.1	1.50	5	9.02	2.92
	12.9			10.3		
	13			9		
	14.4			11.8		
Soil + Fe + peat	8.52	10.55	2.00	17.8	14.87	5.59
	9.98			8.8		
	10.4			11.8		
	13.3			21.1		

Table 38 (IV). Calculations of As and Fe total concentrations of treated and untreated pore water samples from the pots of the phytotoxicity test on 12th June 2013.

	Measured values		Electrical conductivity (mS/cm)		pH	
	Electrical Conductivity (mS/cm)	pH	average	SD	average	SD
Untreated soil	1.428	6.94	1.631	0.195	6.83	0.08
	1.654	6.83				
	1.552	6.80				
	1.888	6.75				
Soil + Fe	1.043	7.87	1.049	0.087	7.91	0.07
	1.092	7.95				
	0.930	7.98				
	1.130	7.83				
Soil + Fe + peat	4.338	7.48	4.533	0.166	7.59	0.09
	4.490	7.68				
	4.736	7.55				
	4.568	7.65				

Table 39 (IV). pH, EC values of pore water samples collected from the pots of the phytotoxicity test on 23rd June 2013, and calculation of average and standard deviation of both values

	Total As concentration (µg/l)			Total Fe concentration (µg/l)		
		Average	SD		Average	SD
Untreated soil	30.7	26.35	4.00	11.8	15.50	4.34
	26.9			19.6		
	26.8			19		
	21			11.8		
Soil + Fe	19.9	17.425	2.05	7.9	10.07	1.57
	15.5			10.9		
	18.3			10		
	16			11.5		
Soil + Fe + peat	11	12.8	1.69	13.1	12.10	1.77
	11.9			14.1		
	14.8			10.2		
	13.5			11.2		

Table 40 (IV). Calculations of As and Fe total concentrations of treated and untreated pore water samples from the pots of the phytotoxicity test on 23rd June 2013.

IV.6 ANNEX VI: LEACHATE DATA

IV.6.1 04/06/2013 samples

	Measured values		Electrical conductivity (mS/cm)		pH	
	Electrical Conductivity (mS/cm)	pH	average	SD	average	SD
Untreated soil	0.1374	7.85	0.280	0.249	7.60	0.21
	0.1357	7.49				
	0.567	7.47				
Soil + Fe	4.88	7.06	7.513	2.757	7.06	0.03
	10.38	7.03				
	7.28	7.08				
Soil + Fe + peat	12.9	7.31	13.050*	0.226*	7.15*	0.23*
	-	-				
	13.2	6.99				

Table 41 (IV). pH, EC values of leachate samples collected from the field experiment on 4th June 2013, and calculation of average and standard deviation of both values. * marks average and SD calculated from two values, because one lysimeter below the Fe^0 and peat treated soil was empty.

	Total As concentration (µg/l)			Total Fe concentration (mg/l)		
		Average	SD		Average	SD
Untreated soil	3.84	4.39	1.17	0.158	0.211	0.046
	5.73			0.237		
	3.59			0.237		
Soil + Fe	20.3	12.79	6.79	5.39	3.444	2.510
	11			4.33		
	7.08			0.611		
Soil + Fe + peat	3.71	4.37*	0.93*	0.236	0.162*	0.105*
	-			-		
	5.02			0.0872		

Table 42 (IV). Calculations of As and Fe total concentrations of leachate samples collected from the field experiment on 4th June 2013, and calculation of average and standard deviation of both values. * marks average and SD calculated from two values, because one lysimeter below the Fe^0 and peat treated soil was empty.

IV.6.2 27/06/2013 samples

	Measured values		Electrical conductivity (mS/cm)		pH	
	Electrical Conductivity (mS/cm)	pH	average	SD	average	SD
Untreated soil	2.055	7.76	2.285	0.694	7.83	0.18
	1.735	7.70				
	3.064	8.03				
Soil + Fe	4.539	7.35	7.630	3.212	7.27	0.09
	10.95	7.18				
	7.400	7.27				
Soil + Fe + peat	1.663	7.01	1.467	0.285	7.03	0.29
	1.301	7.32				
	1.437	6.75				

Table 43 (IV). pH, EC values of leachate samples collected from the field experiment on 27th June 2013, and calculation of average and standard deviation of both variables.

	Total As concentration (µg/l)			Total Fe concentration (mg/l)		
		Average	SD		Average	SD
Untreated soil	7.69	6.24	1.53	0.054	0.490	0.727
	4.64			0.087		
	6.39			1.330		
Soil + Fe	18.5	10.17	7.23	2.410	3.090	2.587
	6.47			5.950		
	5.54			0.912		
Soil + Fe + peat	2.74	4.83	2.96	2.95	2.890	0.085
	23.3			4.53		
	6.93			2.83		

Table 44 (IV). Calculations of As and Fe total concentrations of leachate samples collected from the field experiment on 27th June 2013, and calculation of average and standard deviation of both variables.

IV.7 BIOACCESSIBILITY TEST

Bioaccessible As concentration				
	mg/L	mg/kg	Avarage mg/kg	SD
Untreated soil	0.683	68.004	68.062	0.316
	0.674	67.779		
	0.687	68.402		
Soil + Fe	0.668	66.623	65.777	1.104
	0.657	66.181		
	0.647	64.528		
Soil + Fe + peat	0.522	51.737	52.016	3.217
	0.537	48.947		
	0.564	55.363		

Table 45 (IV). Bioaccessible As concentration measured with in vitro SBRC method, only gastric phase.

	Bioaccessible fraction			Bioavailable fraction		
	%	Avarage %	SD	%	Avarage %	SD
Untreated soil	49.74	49.78	0.23	51.00	51.04	0.23
	49.58			50.84		
	50.03			51.29		
Soil + Fe	48.73	48.11	0.81	50.00	49.38	0.80
	48.41			49.68		
	47.20			48.48		
Soil + Fe + peat	37.84	38.05	2.35	39.20	39.40	2.33
	35.80			37.17		
	40.50			41.83		

Table 46 (IV). Bioaccessible and bioavailable As % calculated with in vitro SBRC method.

Bioaccessible Fe concentration				
	mg/L	(mg/kg)	Avarage mg/kg	SD
Untreated soil	8.964	892.518	886.214	9.492
	8.704	875.297		
	8.947	890.826		
Soil + Fe	24.279	2421.461	2370.921	166.132
	24.877	2505.914		
	21.912	2185.389		
Soil + Fe + peat	25.075	2485.275	2541.522	136.135
	26.797	2442.524		
	27.473	2696.767		

Table 47 (IV). Bioaccessible Fe concentration measured with in vitro SBRC method.

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