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Fractionation and speciation of arsenic in three tea gardens soil profiles and distribution of As in different parts of tea plant (*Camellia sinensis* L.)

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ABSTRACT

The distribution pattern and fractionation of arsenic (As) in three soil profiles from tea (*Camellia sinensis* L.) gardens located in Karbi-Anglong (KA), Cachar (CA) and Karimganj (KG) districts in the state of Assam, India, were investigated depth-wise (0-10, 10-30, 30-60 and 60-100 cm). DTPA-extractable As was primarily restricted to surface horizons. Arsenic speciation study showed the presence of higher As(V) concentrations in the upper horizon and its gradual decrease with the increase in soil depths, following a decrease of Eh. As fractionation by sequential extraction in all the soil profiles showed that arsenic concentrations in the three most labile fractions (i.e., water-soluble, exchangeable and carbonate-bound fractions) were generally low. Most arsenic in soils was nominally associated with the organic and Fe-Mn oxide fractions, being extractable in oxidizing or reducing conditions. DTPA-extractable As (assumed to represent plant-available As) was found to be strongly correlated to the labile pool of As (i.e. the sum of the first three fractions). The statistical comparison of means (two-sample t-test) showed the presence of significant differences between the concentrations of As(III) and As(V) for different soil locations, depths and fractions. The risk assessment code (RAC) was found to be below the pollution level for all soils. The measurement of arsenic uptake by different parts of tea plants corroborated the hypothesis that roots act as a buffer and hold back contamination from the aerial parts.

Keywords:

Soil

Arsenic

Sequential extraction

Speciation

Risk Assessment Code

Tea

1. Introduction

Tea (*Camellia sinensis* L.) plant grows in moderately hot (13 to 32°C) humid climate and in well-drained fertile acidic soils (pH between 4.5 and 5.5). Tea is known as a part of nonalcoholic dietary habits in many countries around the world due to its medicinal values (Higdon and Frei, 2003; Crespy and Williamson, 2004; Cabrera et al., 2006 ; Zaveri, 2006), and therefore it undoubtedly acts as a fillip in the global market. The popularity of tea is also connected to its easy access, therapeutic efficacy, relatively low cost and also for the assumption of the absence of any toxic side effects, which is clear from the fact that about 18-20 billion tea cups are consumed daily in the world (Pedro et al., 2001; Ganguly, 2003).

However, tea has effect in human body; a recent review and research publications discussed and reported cases of heavy metal (e.g. chromium, cobalt, copper, cadmium, zinc, manganese, nickel, lead and mercury) accumulation and contamination in tea (Han et al., 2005; Jin et al., 2005; El-Hadri et al., 2007; Han et al., 2007a; Jin et al., 2008; Ashraf and Mian, 2008; Seenivasan et al., 2008a; Seenivasan et al., 2008b; Karak and Bhagat, 2010). The probable reason behind the accumulation of heavy metals in tea is that this plant is acidophilic, and acidic soils in tea gardens are affected by an increase in heavy metal dissolution, in comparison to neutral and alkaline soils, which increases the uptake of metals by tea leaves (Han et al., 2007a). Besides the above mentioned metals, indigenous soil arsenic might be soluble in tea garden soils and consequently it might be assimilated by tea plants (Karak and Bhagat, 2010). The consumption of arsenic even at low levels through the food chain may lead to carcinogenesis (Mandal and Suzuki, 2002). Among the different oxidation states of As, arsenite [As(III)] and arsenate [As(V)] are the main inorganic forms in most contaminated soils and sediments (Smith et al., 1999). In oxygen-rich environments and well-drained soils, As(V) species dominate, notably in the form of H_2AsO_4^- in acidic soils (Van Herreweghe et al., 2003). Under reducing conditions As (III) is the stable oxidation state. According to the literature, As(III) is ten times more soluble, mobile and toxic than As(V) (Van Herreweghe et al., 2003) and it can react with sulphhydryl groups in enzymes (Faust and Aly, 1981).

After a critical evaluation of the available literature, it was seen that most of the research outcomes were on total soil arsenic, as it reflects the geological origins of soils as well as the anthropogenic inputs. However, the use of arsenic total concentration as a criterion to assess the potential effects of soil contamination

implies that all forms of a given element have an equal impact on the environment; such an assumption is clearly untenable (Tessier et al., 1979). Therefore, fractionation of soil arsenic is an important tool of chemical characterization and can provide useful information on its bioavailability (McLaren et al., 1998). To the best of our knowledge, research on arsenic has mainly been focused on the transfer of As from soil to the major most common plants (or crops), considering highly As-contaminated soils (Ma et al., 2001; Ming et al., 2001; Flynn et al., 2002; Alam et al., 2003; Baroni et al., 2004; Bondada et al., 2004; Hartley et al 2004; Norra et al., 2005 ; Lee, 2006; Chen et al., 2007; Anawar et al., 2008; Ngoc et al., 2009; Lu et al., 2010 and the references therein). Nevertheless, most of the food consumption originates from crops grown in countryside agricultural fields that are not heavily contaminated with arsenic, but may contain meaningful concentrations of this element, which may be harmful if transferred to the food chain.

Assam is the state in North-East India and is characterized by all the favourable conditions for tea plantation. The total tea cultivation area in this state is ~510492 hectares and the total levels of production and exportation of tea in January 2009 were 21.57 MKg and 12.70 MKg respectively (Tea statistics of India, 2009). In India this plant is one of the major cash crops and is one of the major sources of foreign currency from agricultural products. However, no data is available on arsenic in tea garden soils and its uptake by tea plants in Assam, India, although the results of a soil geochemical prospect have revealed arsenic contamination in tea garden soils (Ngoc et al., 2009).

The transfer of arsenic from soils to plants might be a key step in the route of As entry into food stuffs. The typical soil-to-plant transfer factors of As, summarized by Kloke et al. (1984), varied from 0.01 to 0.1. The transfer factors of arsenic for various vegetables, according to Alam et al. (2003) and Warren et al. (2003), ranged from 0.001 to 0.038 and 0.0007 to 0.032 respectively. However, the studies on the dynamics of As in soil and its uptake, translocation and accumulation by tea plants are scanty. Moreover, to the best of our knowledge, the ability of soil extraction methods to distinguish between As(III) and As(V) in the soils of tea gardens has not been reported so far.

In view of the above facts, our studies were aimed to evaluating the ability of previously reported chemical extractants to measure the bioavailable fraction of As(III) and As(V) in soils collected from three tea gardens at different depths in the

state of Assam, India and also the arsenic dynamic from soil to tea plants. In particular, we have applied a sequential extraction scheme mainly based on Tessier's protocol. Arsenic is mainly present as neutral or anionic species in soils, whereas Tessier's protocol was originally designed for cations, like most of the chemical fractionation schemes available in the literature. However, many of such schemes have been adopted by several authors (e.g. Hlavay and Polyák, 1998; Matera et al., 2003; Rodriguez et al., 2003; Anawar et al., 2008) for arsenic fractionation too, and in our opinion they can represent a useful tool for the characterization of the behaviour and mobility of this element. The results of a fractionation study on arsenic also offer the possibility of a classification of the soils, according to element mobility, through the risk assessment code (RAC). The RAC assesses the potential release of elements by the percentages of water-soluble, exchangeable and carbonate-bound fractions (exchangeable and carbonate-bound fractions being obtained following Tessier's sequential extraction scheme) in soils (Singh et al., 2005). RAC is also used as an indicator of ecosystem health (Singh et al., 2005).

In our study, we have also investigated the distribution pattern and uptake of As in the various parts of tea plants. Finally, the differences in the behaviour of As(III) and As(V) in different chemical fractions, soil depths, and places (district) have been statistically examined and the data on soil chemical and physical properties have been processed with multivariate pattern recognition techniques.

2. Materials and methods

2.1. Soil sampling and pretreatment

Three tea gardens from Karbi-Anglong (KA), Cachar (CA) and Karimganj (KG) districts in the state of Assam, India, were selected for the present study (Fig.1).

Soil samples were collected during the tea plucking season from four different depths, viz. 0-10, 10-30, 30-60 and 60-100 cm, to investigate the depth-wise geochemical properties of soil. Another reason for collecting such depth-wise soil samples was the fact that tea plants are deep-rooted plants and roots penetrate to these depths in soil profiles. The investigated soil samples were: KA1 (depth 0-10 cm), KA2 (depth 10-30 cm), KA3 (depth 30-60 cm) and KA4 (depth 60-100 cm) for Karbi-Anglong; CA1 (depth 0-10 cm), CA2 (depth 10-30 cm), CA3 (depth 30-60 cm) and CA4 (depth 60-100 cm) for Cachar and KG1 (depth 0-10 cm), KG2 (depth 10-30 cm), KG3 (depth 30-60 cm) and KG4 (depth 60-100 cm) for Karimganj district. Soil

samples were collected, pretreated for analysis and preserved according to the protocol described by Rubio and Ure (1993).

2.2. Plant sampling and pretreatment

Roots (main roots and feeding roots) from different depths (as per soil sampling depths), stems, old leaves and young shoots comprising of two leaves and a bud were collected. Plant samples were carefully rinsed with tap water and then with deionized water. The young shoots were first treated in a kitchen microwave oven to inactivate enzymes and then dried up in an oven at 80°C. The other plant samples were dried directly in the oven after rinsing with water. The dried samples were grinded, then homogenized using an agate pestle and stored in porcelain airtight stopper jars awaiting analysis.

2.3. Reagents

Analytical grade reagents were used throughout the investigation. High quality water (18.2 MΩ/cm resistivity) obtained from a Milli-Q system (Millipore, USA) was used. As(III) stock solution (1000 mg L⁻¹) was prepared by dissolving 0.66 g As₂O₃ in 12.5 mL of 1.0 M NaOH solution, neutralizing with 1.0 M HCl and diluting to 500 mL with deionized water. A 0.01 M As(V) stock solution containing H₃AsO₄ in 0.5 mM HNO₃ was obtained from Merck (Germany). All the standard solutions were prepared by successive dilutions to the required concentration. Soil SRM-2710 (Montana soil), and sediment BCR-144 (sewage sludge) were used as standard reference materials for quality control.

2.4. Soil characterization

The measurement of the soil pH was carried out on soil slurries having soil:water ratios as 1:2.5 using a pH meter (Systronics India Ltd. model 239). Electrical conductivity (EC) was determined with a conductivity meter (Systronics India Ltd. model 507). The soil redox potential (Eh) was measured using a standard Pt electrode (HORIBA redox potential meter, Japan). The moisture was determined gravimetrically in duplicate, by drying 1 g of sample at 105°C until the weight remained constant. Organic carbon was determined according to Nelson and Sommers (1982). Cation exchange capacity (CEC) was analyzed using the silver thiourea method (Van Reeuwijk, 1992). PO₄³⁻ was determined colorimetrically (Varian Cary

50 Bio spectrophotometer, Australia) according to the method described by Peachey et al. (1973).

Assessment of potentially phyto-available elements (Al, As, Ca, Cd, Co, Cu, Fe, Mg, Mn, Ni, Pb, and Zn) was conducted using the DTPA/TEA method developed by Lindsay and Norvell (1978). Briefly, 10 mL of 0.005 M diethylene triamine pentaacetic acid (DTPA), 0.1 M triethanolamine (TEA) and 0.01 M CaCl₂ solution (pH = 7.3) were added to 5 g of soil and the sample was shaken for 2 h. After centrifugation (4000 rpm for 10 min) the supernatant was filtered through 0.2 µm Whatman filter paper in a 25 mL polycarbonate volumetric flask and diluted to 25 mL with deionized water. Samples were stored at 4°C and then analyzed by flame atomic absorption spectroscopy (Varian, Australia).

For the determination of pseudo-total As concentrations, a three-acid mixture was used. 0.25 g of each sample was weighed into a 100 mL Erlenmeyer flask and wetted with a few drops of deionized water. Then concentrated HCl (6 mL), HNO₃ (5 mL) and HClO₄ (2 mL) were added into the flask and carefully mixed with the soil. The mixture was gently heated on a hot plate until half dried and subsequently reattacked with the same three acids and heated until the reaction died down. The residue was redissolved with 20 mL of 2.5 N HCl and filtered (Whatman 45). Finally, the solution was collected in a 25 mL polycarbonate volumetric flask and diluted to 25 mL with deionized water. A blank digest was carried out in the same way. Arsenic was determined using flow injection hydride generation-atomic absorption spectrometry, FI-HG-AAS (Varian VGA 77 spectrophotometer, Australia) according to the method described by Van Herreweghe et al. (2003).

To check the accuracy of analytical results, two standard reference materials (SRM-2710 and BCR-144) were analyzed following the same digestion and analytical procedure as those used for the samples. The obtained values were in good agreement with the certified values.

2.5. Fractionation of As in soils

A sequential fractionation procedure was used to partition As into six fractions operationally defined as water-soluble (F1), exchangeable (F2), bound to carbonates (F3), bound to Fe and Mn (F4), organically bound (F5) and residual (F6). The reagents used in the sequential extraction scheme were selected from those which are

cited in the literature as being relatively selective for fractions of elements bound to different soil components.

It is well known that no fully selective extracting agent exists for soils, i.e. no extractant is able to remove elements from only one soil component without disturbing the other ones; in addition, element redistribution during extraction may occur. Furthermore, Rauret et al. (2000) pointed out the lack of uniformity in the different procedures for metal fractionation described in the literature, which implies that the significance of the results is highly dependent on the extraction protocol performed. The Standard Measurements and Testing Programme (formerly BCR) of the European Community developed (and later revised) a standardized three-step extraction scheme, known as BCR scheme, with the aim of harmonizing measurements of the extractable trace-metal contents in soils and sediments (Quevauviller, 2002); such scheme has been extensively applied in many studies (e.g. Passos et al., 2010; Rauret et al., 2000; Van Herreweghe et al., 2003). In the present work a different scheme was used, which gives rise to the partitioning of the total element contents into a larger number of fractions; such scheme is mainly based on the well known Tessier's protocol, and it enables the comparison with other environmental solid matrices through RAC (see section 3.3).

Although the results obtained with sequential extraction procedures are operationally defined (Bermond and Yousfi, 1997; Quevauviller, 1998; Gómez-Ariza et al., 2000), they can give valuable information on the behaviour and mobility of elements, provided their results are interpreted with full awareness of their limitations (Abollino et al., 2006; Bacon and Davidson, 2007). Bacon and Davidson (2007), in their review on the future of sequential extractions methods, mentioned an IUPAC report which states that, despite some drawbacks, such methods can provide a valuable tool to distinguish among trace element fractions of different solubility related to mineralogical phases (Hlavay et al., 2004). The same report reminds us that the results are operationally defined, so that the understanding of trace element speciation is still unsatisfactory. Indeed, sequential extraction procedures are widely used for the investigation of contaminated soils and for the estimation of the potential harmfulness due to the presence of heavy metals or other elements.

For As fractionation, 1g-aliquots of soil were weighed into 50 mL centrifugation tubes and extraction reagents were added sequentially. The following scheme was adopted: F1) deionized water in ratio 1 : 10 (w/v), 30-min agitation at

room temperature; F2) 40 mL of 1 M MgCl₂, pH 7, 2-h agitation at room temperature; F3) 40 mL of 1 M CH₃COONa (pH 5), 5-h agitation at room temperature; F4) 40 mL of 0.04 M NH₂OH·HCl in 25% CH₃COOH, placed in a water bath at 96 °C for 6 h; F5) 20 mL of 5.3% NaOCl, pH 8.5, placed in a boiling water bath for 30 min; F6) HClO₄ (2 mL) and HF (10 mL) to near dryness, followed by a second addition of HClO₄ (1 mL) and HF (10 mL) and evaporation to near dryness; addition of HClO₄ (1 mL) and evaporation until the appearance of white fumes; dissolution of the residue in 12 N HCl and dilution to 25 mL. Soil suspensions were agitated with a rotary shaker (Model No. Remi RSB-12, India) whenever required. After each extraction step the tubes containing the soil and the extractant were centrifuged for 15 min at 1700×g (Model No. Remi PR-24 centrifuge, India). The solution entrapped in the remaining soil was collected in subsequent wash steps and combined with the corresponding extract. The final solutions were filtered through 0.45 µm cellulose acetate filters and stored in polyethylene bottles. As concentrations were determined as described in section 2.6. The residual soil was used for the subsequent extraction steps. All extractions were performed in triplicate. Extracts which could not be analyzed immediately were stored at 4°C. F2-F4 and F6 were obtained using the protocol described by Tessier et al. (1979); F1 and F5 were extracted following the procedures described by Szakova et al. (2001) and Shuman (1983) respectively.

2.6. Determination of As(III) and As(V) in different chemical fractions

The differentiation between As(III) and As(V) was carried out in the first four fractions obtained by sequential extraction, under the assumption that the application of the extracting reagent does not alter the oxidation state of arsenic present in the soil. Notably, the risk of As(V) reduction in the presence of hydroxylammonium chloride (used in F4) can be ruled out taking into account the results of previous studies: in particular, Georgiadis et al. (2006) found that 0.14 M NH₂OH·HCl did not convert As(V) to As(III) over a 41 h time period; similarly, Montperrus et al. (2002) applied 0.1 M NH₂OH·HCl to standard solutions of various forms of As and found no inter-transformation between As(III) and As(V); other researchers used this reagent, alone or in combination with other extractants, to study arsenic speciation (Gómez-Ariza et al., 1998; Ruiz-Chancho et al., 2005). The speciation study was not performed in fractions F5-F6 because they require the use of extractants based on oxidizing reagents, which cause As(III) oxidation.

The speciation procedure described by Jian-bo et al. (2003) was adopted to distinguish between As(III) and As(V). These authors demonstrated that, since the reduction of As(V) to As(III) and the subsequent generation of arsine by hydride generation is slower at lower acidity, As(III) can be selectively determined by FI-HG-AAS in the presence of As(V) by controlling the reaction pH and the time of hydride generation. They found that 0.1 M citric acid was the most suitable medium for such determination. Furthermore, Jan-bo et al. (2003) investigated the accuracy of the procedure by determining the recoveries of As(III) and As(V) spikes added to extracts: the recoveries were in the range of 89.3–118 and 80.4–111% respectively. The cations most commonly present in soils were found not to interfere with the determination.

An aliquot of 5 milliliters of each of the above-mentioned extracts (F1-F4) was transferred into a 10 mL polycarbonate volumetric flask and 2 mL of 0.5 M citric acid solution were added into it. The solution was diluted to 10 mL with distilled water. The total arsenic in the extracts was determined by FI-HG-AAS via on-line reduction of As (V) with L-cysteine prepared in 0.1 M citric acid solution, in order to keep the same acidic reaction conditions for the determination of both total As and As(III) (see below). Jian-bo et al. (2003) pointed out some advantages of using L-cysteine as a pre reducing agent. Pre-reduction of As(V) to its trivalent oxidation state is faster and more efficient with this reagents versus other pre-reductants. Additionally, it also improves sensitivity. After the determination of total arsenic, the reducing agent (i.e. L-cysteine) was replaced by 0.1 M citric acid solution and As(III) was determined. The content of As(V) was calculated by subtracting As(III) from total As concentration. Sodium tetrahydroborate (10 g L^{-1} , prepared in 2 g L^{-1} NaOH) was used for hydride generation.

2.7. Plant sample analysis

For the digestion of tea plant parts, 1.00 g of sample was placed into an Erlenmeyer flask and 3 mL of concentrated HNO_3 and 1 mL of concentrated HCl were added. This mixture was heated for 3 h at 85°C on a hot plate until the solubilization of the sample was complete and then diluted to 25 mL with deionized water in a polycarbonate volumetric flask. A blank digest was carried out in the same way. Arsenic was determined by FI-HG-AAS.

All the results described in the present study are on the basis of dry mass. For arsenic determination, two of the three replicates of all the samples of soils and plants were analyzed. If the data of two replicates were not within an acceptable range of precision (relative error <5% for high concentrations, and <15% for low concentrations), the third sample was analyzed.

2.8. Statistical analysis

Levene's test (Levene, 1960) was applied to test the homogeneity of variances among the three districts with respect to the concentrations of As(III) and As(V). Two samples t-test was applied in order to test the differences between the pairs of samples with respect to As concentrations.

Multivariate chemometric techniques, namely hierarchical cluster analysis (HCA) and principal component analysis (PCA) were applied to the results reported in section 3.1 (Massart et al., 1997). HCA was used to form homogeneous groups of different districts with respect to all the soil parameters as well as to form homogeneous groups of different soil parameters in the three districts. Euclidean distance and Ward's agglomeration method were used; the results were reported in dendrograms. PCA was applied to extract the so-called factors, or Principal Components (linear combinations of the original variables) expressing much of the variability present in the investigated soils. Basically, PCA is based on the diagonalization of the correlation matrix. . The scree plot was observed to determine the number of principal components to be extracted to express reasonable amount of variability in the system. Kaiser-Meyer- Olkin (KMO) measure of sampling adequacy followed by Bartlett's test of sphericity justified the application of factor analysis in the present dataset.

The statistical calculations were carried out using the SPSS 15.0 statistical package (SPSS Inc., Chicago, USA) (Norusis, 2000), with the exception of HCA, which was performed with XLStat 4.4, used as a Microsoft Excel plug-in.

3. Results and discussion

3.1. Physical and chemical properties of soils

Selected physico-chemical properties of the investigated soils are given in Table 1, together with their ranges. All the experimental soils were acidic in nature. With the increase of depth, pH values remarkably increased. The acidity of the soil

could be attributed to the parent material, heavy rainfall, weathering processes, high aluminium, iron and manganese contents. The Eh values of the top soils were 242, 321 and 412 mV for KA1, CA1 and KG1 respectively, indicating that they were oxic soils. The Eh for all the soils were seen to decrease with the increase of depth reflecting the lower oxygen diffusion rate or compactness of soil. These results confirm the findings of Aleksander-Kwaterczak and Helios-Rybicka (2009) that redox potential decreases in the deeper sections of soils and sediment profiles. Moreover, organic carbon ranged from 4.6 to 9.8, 8.7 to 11.5 and 8.1 to 12.1% in soils from Karbi-Anglong, Cachar and Karimganj districts respectively. In all the tea garden samples, organic carbon was higher in top soil, probably due to tea leaf littering, tea branches cutting during pruning (i.e., pruning litter), application of organic matter, shade tree leaf littering etc. Significant variations of depth-wise texture were observed in the soils collected from Karbi-Anglong and Karimganj districts, whereas no variation was observed in textural classes for Cachar district soil. The sand percents in the top soils of Karbi-Anglong and Karimganj districts were higher than the percents in top soils of Cachar district. In all soils, the percent of sand seemed to decrease with the increase of soil depths. The range of CEC values (11.2 to 20.0 cmol kg⁻¹) in the soils was partly within the range of a typical clay loam texture, i.e. from 15 to 30 cmol kg⁻¹ soil (Donahue et al., 1977). This might be due to the presence of montmorillonite (Barua, 2008) and oxides of Fe and Al that have high CEC values (Evangelou, 1998). Linear regression analysis was carried out between CEC and clay contents within soil profiles: a good correlation ($r^2 = 0.903$ to 0.954) was found between these two parameters.

According to several studies, extraction via DTPA solution provides the prediction of trace elements uptake by plants from soils (Lindsay and Norvell, 1978; McLaughlin et al., 2000). In particular, numerous researchers (e.g. Bhattacharyya et al., 2003; Cheng et al., 2004; Nair et al., 2007; Wang et al., 2007) utilized this kind of extraction for evaluating plant-available As, even if arsenic is mainly present in anionic or neutral forms in soils. Other authors adopted different extractants, such as potassium hydrogen phosphate (Cai et al., 2002) or hydrochloric acid (Ahumada et al., 2004). In this work we chose to use DTPA for both As and other elements i.e. Cd, Co, Cr, Pb, Ni, Se and Zn. The results obtained for As, as well as for Se, should be regarded with caution, owing to their anionic nature. A possible development of the present work

can be the comparison of the performances of different extracting agents with regard to arsenic.

The values and ranges of DTPA-extractable element concentrations in the investigated soils are shown in Table 1. DTPA-extractable As in the top soil of Karbi_Anglong district was considerably lower than for Cachar and Karimganj districts. The reduced availability of heavy metals and arsenic with the increase of depth can be explained by the pH-dependent characteristics of element mobility, i.e. formation of more unavailable chemical forms with an increase in pH (Sims and Kline, 1991; Chlopecka et al., 1996). All concentrations of plant-available heavy metals in the soil were within the normal range documented by Kabata-Pendias and Pendias (2000).

An assessment of the overall level of elemental contamination can be made by measuring the pseudo-total element contents in a soil after digestion with mixtures strong acids, e.g. aqua regia, in the absence of HF (Gupta et al., 1996). Pseudo-total soil concentrations give an indication of the maximum potentially soluble or mobile contents of elements and, in the case of environmental contaminants, usually not bound in silicates, a measure of the maximum potential hazard that could occur in the long term or in extreme environmental regimes. Several official methods of analysis, such as the well known ISO 11466 method (ISO, 1995), which involves aqua regia digestion, yield the pseudo-total element contents. When the total concentrations are of interest, HF must be present in the acid mixture, in order to give rise to the release the silicate-bound element fraction.

The pseudo-total amount of arsenic in top soils ranged from 31.26 to 33.97 mg kg⁻¹. The average pseudo-total As was 30.38 mg kg⁻¹ (range: 29.12-31.26 mg kg⁻¹), 34.71 mg kg⁻¹ (range: 32.56-39.56 mg kg⁻¹) and 30.63 mg kg⁻¹ (range: 29.05-33.97 mg kg⁻¹) for soils in KA, CA and KG respectively. According to Kabata-Pendias and Pendias (2000), uncontaminated soils usually contain 1-40 mg kg⁻¹ of arsenic, with lowest concentrations in sandy soils and those derived from granites, and higher concentrations in alluvial and organic soils. Therefore, the investigated soils can be regarded as uncontaminated by arsenic, as the pseudo-total amount of As was within the range 29.05 to 39.56 mg kg⁻¹. Linear regression analysis (n = 12) did not show any fruitful correlation between pseudo-total As and other soil physical and chemical parameters except for pH, organic carbon and clay content. These results agree with

those obtained by Roychowdhury et al. (2002), who reported the same findings on soil from arsenic-affected area of West Bengal, India.

3.2. Fractionation and speciation of arsenic

The concentrations of As(III) and As(V) in F1-F6, obtained by coupling sequential extraction and speciation, are shown in Fig. 2a-f. The total amount of arsenic, i.e. the sum of As(III) and As(V), extracted into each fraction is represented by the height of each bar in the graph. A good agreement was observed between the sum of As concentrations in the six fractions (considering the sum of the two As species in the first four ones) and the pseudo-total contents, nearly all recoveries being in the range 85-115 %, with an average of 97 ± 11 %. These results are in agreement with most literature data on sequential extractions, which typically report recoveries within 10-15% of the total values (e.g. Lu et al., 2003; Zhai et al., 2003) with the exception of samples collected in very heterogeneous sites. In general, slightly lower extraction efficiencies were obtained when the sum of arsenic in different fractions was compared with the pseudo-total arsenic contents. This result suggests that the amount of this element bound to silicates is low.

The order of extractability of arsenic in the fractions is $F1 \approx F2 \approx F3 < F4 < F6 < F5$ in all investigated sites, as discussed in sections 3.2.1-3.2.6. It must be pointed out that the extracting agents used for the fractionation of arsenic were developed for studying the behavior of metals, which are present as cations in soil, whereas arsenic, as recalled in section 3.1, is mainly present in anionic or neutral form. Presently, there is no universally agreed standard method based on single or sequential extraction for the investigation of arsenic partitioning in soil. Several researchers used procedures similar to the one adopted in the present work (Hlavay and Polyák, 1998; Matera et al., 2003; Rodriguez et al., 2003; Anawar et al., 2008); the results are useful in order to assess the mobility of arsenic upon changes of soil conditions (e.g. pH, redox potential, salinity, drainage conditions). Other researchers adopted alternative procedures for arsenic fractionations, generally exploiting its similarities with phosphorus (Gleyzes et al., 2001; Wenzel et al., 2001; Van Herreweghe et al., 2003).

3.2.1. Distribution of As (III) and As(V) in the first fraction (water-soluble elements)

The water-soluble fraction represents the most mobile and toxic portion of arsenic in soil/water systems. The concentrations of As(III) are higher than those of As(V), with one exception (Fig. 2a). A higher amount of As(III) was present in the top soils of KA and KG districts in comparison with CA soils. The concentrations of water-soluble As(III) tend to increase with increasing depth in all investigated soils, whereas the concentrations of As(V) show the opposite trend. This behaviour is presumably related to the decrease of Eh along the soil profiles, which favours the presence of reduced species. A very small percentage of arsenic was extracted by deionized water, contributing only 0.78 to 2.55 % for As(III) and only 0.42 to 1.03% for As(V). This result is not unexpected, since most of this very labile fraction has already been leached over the years by the action of rain waters. Rodriguez et al. (2003) also reported a very small amount of water-extractable arsenic, even though soil samples were collected from a mining and smelter site in the western USA.

3.2.2. Distribution of As(III) and As(V) in the second fraction (exchangeable elements)

Fig. 2b depicts the depth-wise variation of the exchangeable fractions of As(III) and As(V) in soils. The ranges of exchangeable As (III) and As(V) constituted only 0.62 to 1.71% and 0.41 to 2.31% respectively of total arsenic in the investigated soils. This is a disparity to the data of Matera et al. (2003), who found much higher percentages of As in this fraction than those reported here. However, extraction percentages in this study are similar to the results of Garcia-Manyes et al. (2002). The low cation exchange capacities of these soils may partially explain the low amount of exchangeable As in the soils. In all the top soils, the amount of As(V) was always higher than that of As(III). The depth profile of As(III) and As(V) in this fraction differs from that observed for the other fractions, since a general decreasing trend takes place for both species.

3.2.3. Distribution of As(III) and As(V) in the third fraction (carbonate-bound elements)

The concentration of As(V) was significantly higher than that of As(III) in the carbonate-bound fraction, except for KG soils (Fig. 2c). The cause of this different

behaviour is not clear. In all cases, the concentrations of As(III) tend to increase with depth, whereas those of As(V) have the opposite trend. The ranges of As(III) and As(V) in F3 were 0.33-1.49 % and 0.53-1.46 % respectively of total arsenic in the three soils.

3.2.4. Distribution of As(III) and As(V) in the fourth fraction (Fe and Mn oxide-bound elements)

The concentration profiles of As(III) and As(V) nominally bound to Fe and Mn oxides in soils are depicted in Fig. 2d. More arsenic is extracted in this fraction as compared with the first three fractions. This trend is commonly found for elements in soils, and is due to the fact that metal oxides are efficient sorbents. As(III) extracted into this fraction ranged between 4.70 and 6.73 mg kg⁻¹ contributing 14.32 to 23.10% of total arsenic. As(V) ranged between 2.46 and 5.13 mg kg⁻¹, representing 8.40 to 15.29% of total arsenic. Such an association of arsenic in soils had already been pointed out (Voigt et al., 1996; Gleyzes et al., 2001; Wenzel et al., 2001; Matera et al., 2003). In particular, Manful (1992) showed an association of arsenic with iron and aluminium oxides in soils in the vicinity of a gold extraction area (arsenopyrite disposal). The depth profiles of the two forms of arsenic have the same trend as reported for the first and third fraction.

3.2.5. Distribution of As in the fifth fraction (organically-bound elements)

Only the concentration of total arsenic is available for F5 and F6, as pointed out in section 2.5. The extraction percentages of As were in the range of 31.93 to 64.13% of the total arsenic (Fig. 2e). From these results, it is clear that the organically-bound fraction of As(III) and As(V) was the major predominant fraction, probably due to the presence of a relatively high amount of organic matter in the analyzed soil, as demonstrated by the percentages of organic carbon (see Table 1). This result also suggests that the investigated soils show a high percentage of As in the less mobile fractions. However, Taggart et al. (2004) reported that arsenic was present at high percentages in the more mobile fractions in anthropogenic contaminated soils. Tea garden soils are generally rich of organic matter (Table 1) and this could explain the presence of high amounts of As in this fraction. Furthermore, it must be taken into account that organic carbon has a great binding capacity and it could behave as strong As scavenger. The variation of As with depth is different for

the three soils. The concentrations in CA soil are higher in the first two layers and decrease below 30 cm, whereas the concentrations in KA show the opposite behaviour. No clear trend is present in the depth profile of As in KG soils.

3.2.6. Distribution of As in the sixth fraction (residual)

As Fig. 2f shows, the depth profiles of residual arsenic are similar in KA and CA soils, increasing with depth with a maximum between 30 and 60 cm, whereas the trend in KG soil is opposite, and the highest values are found in the first two layers; on the other hand, the concentrations of total arsenic extracted into F4 from these two layers is lower in KG soil than in KA and CA soils, suggesting that the former has a lower amount of amorphous iron oxides (assumed to be extracted into F4) and a higher amount of crystalline iron oxides, decomposed only in drastic conditions, like those applied for F6 (Abollino et al., 2006). The residual fraction of As contributes 12.03 to 14.59%, 7.68 to 12.76% and 16.07 to 20.86% to the total arsenic in CA, KA and KG soils respectively. It is a quite common finding that a significant proportion of the total arsenic in scarcely polluted soils is extracted into the residual fraction only (Kavanagh et al., 1997).

3.3. Risk assessment of arsenic within soil profiles

A statistically significant correlation between DTPA-extractable As and the labile pool of As (i.e. the sum of the first three fractions) in soils was found, indicating a close association between them ($r^2 = 0.908$). Rubio and Ure (1993) and Evanylo and Sukkariyah (2006) also reported a highly significant correlation between the DTPA-extractable contents and the labile fraction of trace elements. This relationship confirms that, assuming that the DTPA-extractable concentrations represent the phytoavailable element portion, plants are mainly able to assimilate the portion of elements weakly bound to the soil structure: hence, such portion can be considered the most hazardous for human health, due to the possibility of entering the food chain. Furthermore, these results suggest that extraction by DTPA might be used to estimate the mobile element fraction, instead of the more costly and time-consuming sequential extractions; on the other hand, sequential extractions give a more informative picture of metal partitioning among different phases, even with the limitations pointed out in section 2.5, and of the risks associated to changes in a particular soil condition (i.e. pH or redox state).

It is evident that As extracted into different fractions is bound to soil with different strengths. The strength values can, therefore, give an indication of soil reactivity, which in turn assesses the risk connected with the presence of As in a soil environment (Passos et al., 2010). In order to assess arsenic accumulation in tea garden soils from a regulatory perspective, the risk assessment code (RAC) was calculated following the method described by Singh et al. (2005), as $\sum_{n=1}^3 F_n$. The fractions addressed in the RAC represent the weakly bound element portion that could become more rapidly bioavailable (Singh et al., 2005). In addition, since elements in such fractions are frequently associated to anthropogenic sources (Abollino et al., 2006; Passos et al., 2010; Zhai et al., 2003), the RAC can be considered an index of pollution due to human activities. The classification of the soils investigated in the present study on the basis of RAC is formulated and depicted in Fig. 3. The overall RAC values in the tea garden soils are between 5.06 and 7.89, reflecting that all soils are below the pollution level, characterized by RAC values higher than 10. Therefore, RAC indicates that soils releasing within 1-10% of the total As into F1, F2 and F3 fractions can be considered at low risk; it can be presumed that the arsenic present in such soil will not easily enter the food chain. RAC was reported here also because it is of use to compare the status of different soils, or of other environmental matrices, of course provided that the same operational procedure is followed in all the investigations. In particular, RAC values have been calculated in several studies (e.g., Singh et al., 2005; Jain et al., 2004; Jain et al., 2007; Karak, 2010; Karak and Bhattacharyya, 2010; Li et al., 2007; Jain et al., 2008; Passos et al., 2010). Our RAC values were similar to those reported by Jain (2004) while studying metal fractionation of Yamuna River sediments (India), Li et al. (2007) studied sediments from lakes Doirani and Kerkini (Greece), which are intensively used for agriculture and fishery purposes, and showed that Zn presented a high risk and could be readily released to the water column. Jain et al. (2007) found that RAC values for Cu, Ni, Cr, Pb, Cd and Zn in sediments from Nainital lake in the state of Uttaranchal (India) indicated low to medium risks; similar results were obtained by Jain et al. (2008) for these six metals in sediments from the Narmada river, mainly sampled in the state of Madhya Pradesh (India). In both regions of India anthropogenic activities have resulted in discharges of domestic and industrial wastes in recent years. The three tea estates investigated in the present paper are located near the neighbouring arsenic-affected region of

Bangladesh. As contamination in groundwater of Bangladesh is a serious concern (Roychowdhury et al., 2002); however, the nearby tea garden soils are not yet affected by As contamination and, in the light of RAC, it can be presumed that the release of As from soil profile is restricted. Han et al. (2007) used a different index, based on the comparison with soil environmental standard, to assess the pollution status of tea garden soils from Shandong province of China. A contamination by Cd was identified, whereas Cr, Cu, Pb and Zn were below the pollution level; the overall classification of the soils was “slightly polluted” (Han et al., 2007B).

3.4. Arsenic in different parts of tea plant

Table 2 summarizes the concentrations of arsenic in different parts of tea plants. The amount of arsenic in feeding roots ranged from 1.33 to 1.61, 1.15 to 1.82 and 0.81 to 2.80 mg kg⁻¹ for Cachar, Karbi-Anglong and Karimganj district respectively. Main roots always accumulated higher amount of As than feeding roots. These results suggest that, under acidic conditions, As is accumulated in tea plant roots and the accumulation mainly depends on its availability. The concentrations of As in stems ranged from 1.2 to 1.9 mg kg⁻¹. Arsenic concentrations in mature leaves were 16 to 40, 14 to 54 and 26 to 77 times lower than in stem, feeding roots and main roots respectively. No arsenic was detected in young shoots (i.e. two and a bud; the detection limit was 5 µg kg⁻¹). These results also show that the mobility of As in tea plants was low: most As appeared to be fixed in roots and only a limited amount was translocated above the ground portion. This finding also corroborates the hypothesis that tea roots possibly act as a buffer and hold back the contaminations from the aerial parts: thus tea plants play an important role in sequestering arsenic in their roots. This finding is in line with the behaviour of tea plants observed in an As-contaminated site (Shi et al., 2007).

3.5. Statistical analysis

We studied the trends of As(III) and As(V) concentrations separately over different soil locations (i.e. CA, KA and KG districts), depths and fractions. On application of Levene’s test (Levene, 1960) it was found that the different districts were having equal variances with respect to As(III) and As (V) concentrations. From pairwise comparisons (two sample t test assuming equal variances) between districts, it was found that the concentrations of As(III) in the soil of KA district were

significantly greater than in CA and KG soils at 1% levels of significance; on the other hand, the concentration of As(V) in the soil of KA district was significantly greater than in the soils of KG district but not significantly different from that present in the soils of CA district.

It was observed from the pairwise comparisons of depth profiles that the concentration of As(III) was significantly lower in the depth 0-10 cm in comparison to the depths of 10-20, 30-60 and 60-100 cm at 1% of significance. Again the pairwise comparison of depth profiles showed that the concentration of As(V) was significantly greater in the depth 0-10 cm in comparison to the depths of 30-60 and 60-100cm at 1% level of significance.

Pairwise comparisons of the concentrations in soil fractions showed that F4 was significantly higher than F1, F2, F3 at 1% and 5% level of significance. Thus, it was seen that the soil location, depth and fraction have a significant effect on both As(III) and As(V) concentrations.

PCA and HCA were applied to the dataset reported in Table 1 in order to gain insight into the characteristics of the investigated soils with a multivariate approach, taking into account the effects of all variables simultaneously (Helena et al., 2000; Abollino et al., 2011; Giacomino et al., 2011). HCA revealed that with respect to the investigated soil parameters, CA and KG districts are similar and are distinctly different from KA district, with the exception of sample KG4 (Fig. 4a). The dendrogram reported in Fig. 4b shows that the soil parameters are divided into two main groups, further divided into sub-groups. The interpretation of the meaning of variable associations is not straightforward; one interesting finding is that DTPA-extractable As is clustered with organic carbon and Eh, in agreement with the relatively high concentrations of As extracted into F4 and F5.

PCA extracted two components expressing more than 99 % of the combined variability present in the soil. The first component (56%) had higher loadings for the variables pH, Eh, OrgC, silt, clay, Al, Cd, Co, Cr, Fe, Mn, Ni, Se and Zn and the second principal component (43%) had a higher loading for the variables EC, P, Sand, CEC, Ca, Pb, Mg and total arsenic. The component plot (Fig. 5) in the rotated space showed four distinct groups of different soil parameters in the four quadrants, indicating that there is strong relationship between the soil parameters within a group.

4. Conclusions

The fractionation and depth-wise variation of As(III) and As(V) in tea garden soils and arsenic distribution in the different parts of tea plant have been investigated in this study. The statistical data treatment showed that a significant difference in the distribution of As(III) and As(V) contents exists between the districts Karbi-Anglong and Karimganj, as well as between Karbi-Anglong and Cachar: in particular, the concentrations of As(III) in the soil of KA district were significantly greater than in CA and KG. Again depthwise variation of the concentrations of As(III) and As(V) has been seen in the tea gardens. Significant variations of the contents of As(III) and As(V) in fractions obtained by sequential extraction have also been seen. Regarding As fractionation, large portions of this element were extracted in oxidising (F5) and in reducing (F4) conditions from tea garden soil. Arsenic was also considerably bound to the residual fraction. Therefore, it might be hypothesized that organic matter, amorphous Fe and Mn oxyhydroxides as well as oxides and silicates play a role in As retention in tea garden soils. This conclusion must be regarded with caution since i) the fractions obtained in sequential extractions are operationally defined and ii) as pointed out in sections 1 and 3.3, the fractionation scheme adopted was originally designed for cations. However, the results can be of use for the characterization of arsenic behaviour in soils, for the comparison with literature data (since other studies used similar fractionation schemes for arsenic) and for the prediction of the amount of arsenic released upon a change in pH, redox conditions, salinity or drainage conditions.

A very high correlation between DTPA-extractable As and the labile pool of As (i.e. the sum of the first three fractions) was found, suggesting that the latter is the portion of As most hazardous for human health, due to the possibility of entering the food chain. As for sequential extraction, also this conclusion must be regarded bearing in mind that DPTA extraction had originally been developed for metal cations, even if it has been extensively applied for arsenic.

Low amounts of As extractable into the first three fractions gave rise to low RAC values and corroborated the scarce release of As through DTPA extraction. Arsenic uptake by tea was mainly confined in roots, which act as a buffer and hold back the contaminations from the aerial parts of the plants.

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Captions to figures

Fig. 1. The map showing the tea gardens from where soil samples and tea plants were collected in the state of Assam, India.

Fig. 2. Distribution pattern of As(III) and As(V) in the first four fractions (F1-F4) and of pseudo-total As in the last two fractions (F5, F6) obtained by sequential extraction. a) F1; b) F2; c) F3; d) F4; e) F5; f) F6. CA1, CA2, CA3 and CA4 (and similarly for KA and KG) indicate the sample depths 0-10, 10-30, 30-60 and 60-100 cm respectively.

Fig. 3. Classification of the investigated soils according to the risk assessment code (RAC). The standard RAC values are adopted from Singh et al., 2005.

Fig. 4. Dendrograms obtained by HCA: a) clustering of samples; b) clustering of soil parameters.

Fig. 5. Factor loadings pattern of the physicochemical parameters of the three tea garden soils obtained by PCA followed by Varimax rotation.

Table 1
Geochemical properties of the investigated soils.

Parameter	Locations												Range
	Cachar district				Karbi-Anglong district				Karimganj district				
Sample ID*	CA1	CA2	CA3	CA4	KA1	KA2	KA3	KA4	KG1	KG2	KG3	KG4	
pH	4.8 (0.03) [#]	4.9 (0.01)	5.1 (0.06)	4.5 (0.01)	5.2 (0.02)	5.8 (0.02)	5.9 (0.01)	6.1 (0.03)	4.0 (0.02)	4.5 (0.01)	4.9 (0.04)	5.1 (0.01)	4.0-6.1
EC (1:5) (mS/cm)	0.21 (0.001)	0.22 (0.001)	0.19 (0.001)	0.18 (0.001)	0.11 (0.001)	0.23 (0.001)	0.31 (0.002)	0.32 (0.002)	0.32 (0.002)	0.22 (0.001)	0.28 (0.001)	0.29 (0.001)	0.11- 0.32
Eh (1:1) (mV)	321 (21)	228 (18)	156 (7)	-25 (2)	242 (15)	189 (10)	125 (9)	-50 (4)	412 (20)	316 (28)	109 (8)	-75 (3)	-75-412
Org. C (%)	11.5 (0.1)	10.3 (0.4)	9.8 (0.6)	8.7 (0.2)	9.8 (0.3)	8.6 (0.1)	8.9 (0.1)	4.6 (0.1)	12.1 (0.4)	12.1 (0.3)	10.9 (0.2)	8.1 (0.4)	4.6- 12.1
PO ₄ ³⁻ (mg kg ⁻¹)	769 (27)	662 (17)	536 (10)	512 (20)	829 (29)	748 (28)	265 (12)	236 (8)	897 (32)	219 (19)	128 (16)	253 (8)	128- 897
Sand (%)	39.6 (2.1)	38 (1.8)	32.5 (1.8)	34.5 (1.9)	47.7 (2.1)	39.6 (1.8)	31.4 (1.1)	40.7 (1.3)	52.1 (1.6)	48.4 (1.9)	42.1 (1.5)	22.1 (0.9)	22.1- 52.1
Silt (%)	19.6 (0.8)	24.8 (0.4)	25.5 (0.3)	23.6 (0.1)	5.6 (0.01)	22.3 (0.4)	31.2 (0.7)	18.8 (0.4)	21.8 (1.1)	21.1 (1.0)	29.5 (1.6)	22.3 (0.7)	5.6- 31.2
Clay (%)	40.8 (1.7)	37.2 (1.1)	42.0 (1.5)	41.9 (1.4)	46.7 (1.3)	38.1 (1.3)	37.4 (1.1)	40.5 (1.4)	26.1 (0.9)	30.5 (1.4)	28.4 (0.9)	55.6 (1.8)	26.1- 55.6

Table 1. Continued

Parameter	Locations												Range
	Cachar district				Karbi-Anglong district				Karimganj district				
Sample ID*	CA1	CA2	CA3	CA4	KA1	KA2	KA3	KA4	KG1	KG2	KG3	KG4	
Texture	Clay	Clay	Clay	Clay	Sandy clay	Clay loam	Clay loam	Clay	Sandy clay loam	Clay loam	Clay loam	Clay	
CEC (cmol kg ⁻¹)	12.5 (0.1)	13.6 (0.3)	11.2 (0.1)	11.2 (0.1)	20.0 (0.2)	15.2 (0.1)	12.3 (0.1)	15.9 (0.4)	15.8 (0.2)	15.3 (0.6)	15.2 (0.3)	13.9 (0.4)	11.2- 20.0
DTPA-extractable elements (mg kg ⁻¹ , unless otherwise stated)													
Aluminium (%)	2.31 (0.01)	2.42 (0.02)	1.27 (0.01)	1.85 (0.01)	2.72 (0.01)	1.56 (0.01)	2.91 (0.03)	1.70 (0.01)	1.24 (0.01)	2.56 (0.02)	0.99 (0.001)	0.21 (0.001)	0.21- 2.91
Arsenic	2.54 (0.01)	2.12 (0.01)	2.02 (0.01)	2.11 (0.01)	1.71 (0.01)	1.41 (0.008)	1.43 (0.003)	1.27 (0.001)	2.45 (0.003)	1.79 (0.001)	1.94 (0.004)	1.64 (0.01)	1.27- 2.54
Cadmium	0.42 (0.002)	0.43 (0.001)	0.21 (0.001)	0.29 (0.001)	0.44 (0.003)	0.34 (0.002)	0.31 (0.002)	0.24 (0.001)	0.15 (0.001)	2.12 (0.004)	2.12 (0.001)	1.02 (0.007)	0.15- 2.12
Calcium (%)	0.88 (0.001)	0.56 (0.001)	0.78 (0.003)	0.25 (0.002)	0.31 (0.001)	0.28 (0.001)	0.89 (0.004)	0.25 (0.001)	0.25 (0.001)	0.21 (0.001)	0.18 (0.001)	0.07 (0.001)	0.07- 0.89
Cobalt	12.38 (1.1)	15.32 (1.2)	12.85 (1.1)	4.25 (1.4)	21.32 (1.4)	11.21 (0.09)	11.23 (0.08)	5.65 (0.01)	2.35 (0.01)	6.78 (0.37)	4.98 (0.56)	3.65 (0.29)	2.35- 21.32
Chromium	8.98 (0.91)	15.25 (0.58)	21.25 (0.62)	15.36 (0.09)	23.45 (0.91)	28.52 (0.56)	2.56 (0.08)	4.56 (0.03)	0.25 (0.01)	6.39 (0.41)	3.56 (0.23)	1.85 (0.01)	0.25- 28.52
Iron	2005 (129)	3569 (167)	3145 (130)	7825 (198)	2356 (192)	2534 (115)	2514 (192)	2012 (204)	6958 (292)	5236 (228)	1287 (176)	2547 (109)	1287- 7825
Lead	52.63 (2.16)	58.94 (1.17)	14.25 (0.92)	25.36 (0.02)	20.12 (0.09)	32.25 (1.02)	8.96 (0.01)	18.56 (0.03)	31.45 (0.07)	32.85 (0.09)	16.96 (0.01)	26.38 (0.24)	8.96- 58.94

Table 1. Continued

Parameter	Locations												Range
	Cachar district				Karbi-Anglong district				Karimganj district				
Sample ID*	CA1	CA2	CA3	CA4	KA1	KA2	KA3	KA4	KG1	KG2	KG3	KG4	
Manganese	28.96 (0.06)	32.56 (0.28)	26.38 (0.18)	13.65 (0.08)	58.12 (1.13)	66.32 (1.45)	62.53 (1.07)	51.25 (1.16)	28.94 (0.84)	45.12 (1.45)	27.89 (1.03)	62.12 (1.98)	13.65- 66.32
Magnesium (%)	0.59 (0.001)	1.23 (0.060)	1.23 (0.020)	2.15 (0.01)	0.04 (0.001)	0.23 (0.001)	0.23 (0.001)	0.25 (0.001)	0.98 (0.021)	0.25 (0.001)	0.52 (0.002)	0.65 (0.002)	0.04- 2.15
Nickel	51.21 (1.12)	22.31 (1.04)	20.13 (1.12)	27.85 (1.04)	35.23 (1.01)	23.85 (1.03)	25.69 (1.05)	26.35 (1.01)	51.42 (1.92)	39.25 (1.00)	34.56 (0.92)	37.82 (1.04)	20.13- 51.42
Selenium	0.01 (0.000)	0.12 (0.001)	0.11 (0.001)	0.08 (0.001)	0.60 (0.001)	0.52 (0.001)	0.23 (0.001)	0.21 (0.001)	0.07 (0.001)	0.10 (0.001)	0.11 (0.001)	0.09 (0.001)	0.01- 0.60
Zinc	224.5 (12.36)	351.2 (32.87)	324.1 (19.02)	301.2 (17.02)	398.2 (12.98)	315.2 (12.09)	124.1 (8.95)	123.2 (12.08)	203.5 (10.98)	412.2 (9.97)	401.2 (43.76)	331.2 (23.09)	123.2- 412.2
Total As (mg kg ⁻¹)	33.56 (0.23)	32.56 (0.98)	39.56 (0.28)	33.16 (0.24)	31.26 (0.18)	31.26 (0.11)	29.86 (0.09)	29.12 (0.18)	33.97 (0.18)	30.26 (0.09)	29.05 (0.01)	29.25 (0.01)	29.05- 39.56

*In sample ID, 1, 2, 3 and 4 indicate the sample depths 0-10, 10-30, 30-60 and 60-100 cm respectively

Values in parenthesis indicate \pm standard deviations

Table 2
Concentration of As in tea plants.

District	Sample ID	Arsenic concentration (mg kg ⁻¹)				
		Below the ground		Above the ground		
		Feeding root	Main root	Stem	Mature leaves	Young shoot
Cachar	CA1	1.61	2.32			
	CA2	1.47	2.14	1.2	0.03	ND*
	CA3	1.33	1.93			
	CA4	1.61	2.05			
Karbi-Anglong	KA1	1.36	2.92			
	KA2	1.17	2.14	1.3	0.08	ND
	KA3	1.15	2.36			
	KA4	1.82	2.94			
Karimganj	KG1	2.80	3.12			
	KG2	1.03	1.67	1.9	0.06	ND
	KG3	0.81	1.87			
	KG4	0.81	1.56			

*ND = not detectable.

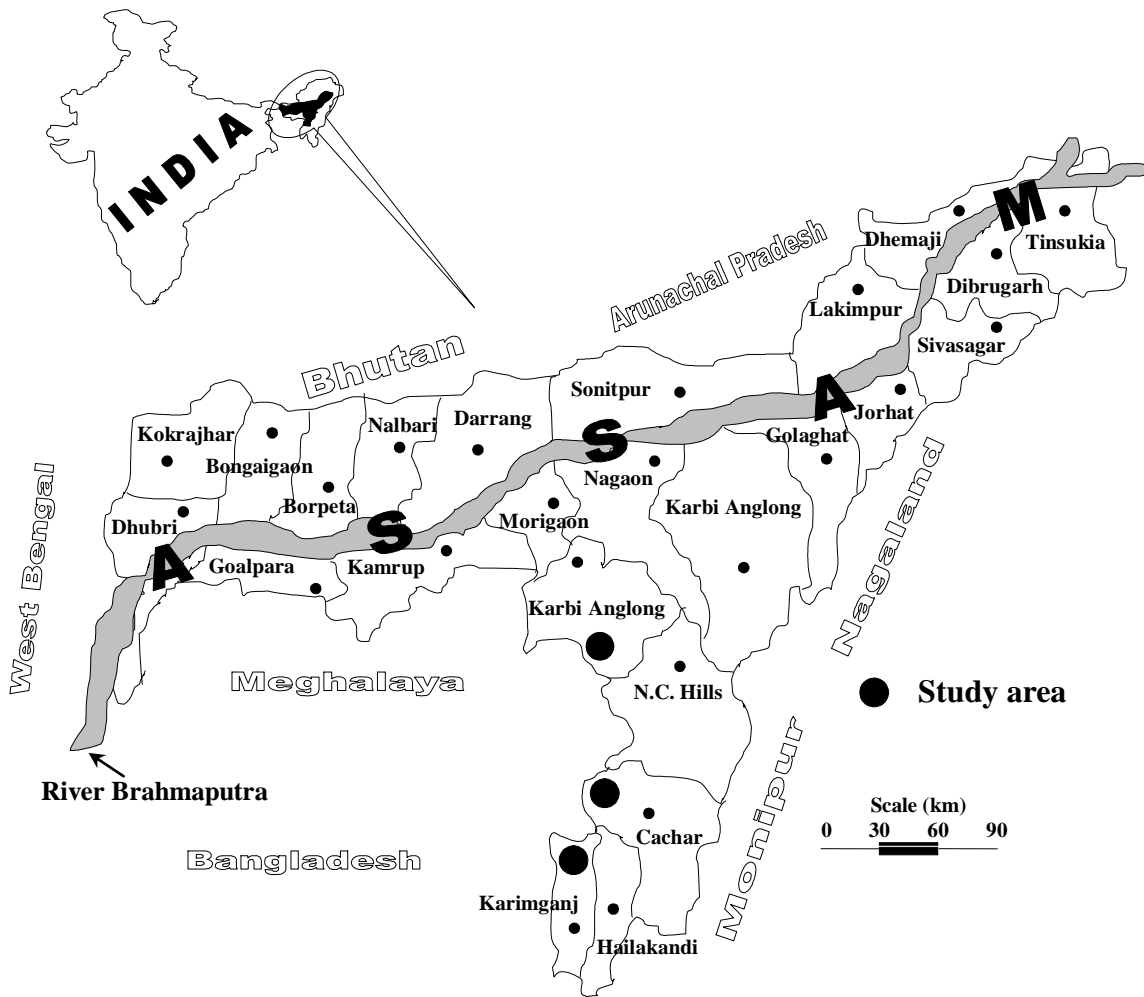


Fig. 1

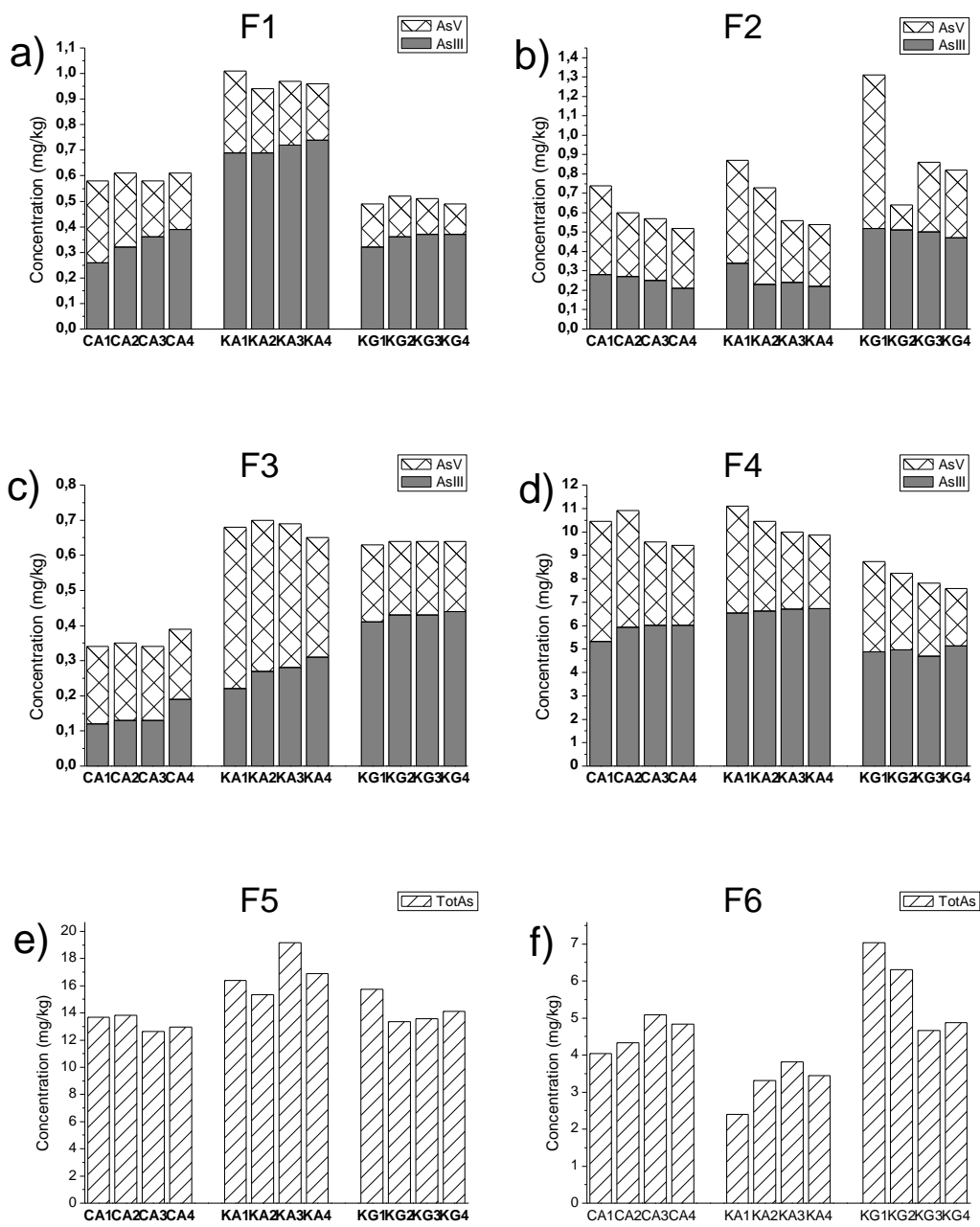


Fig. 2

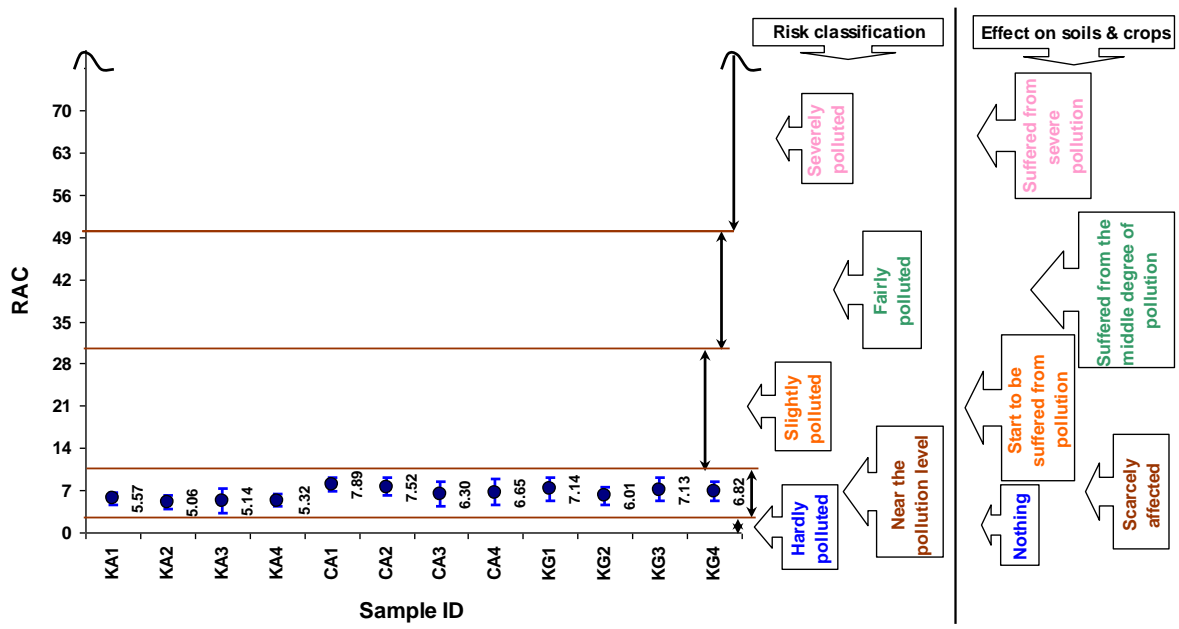


Fig. 3.

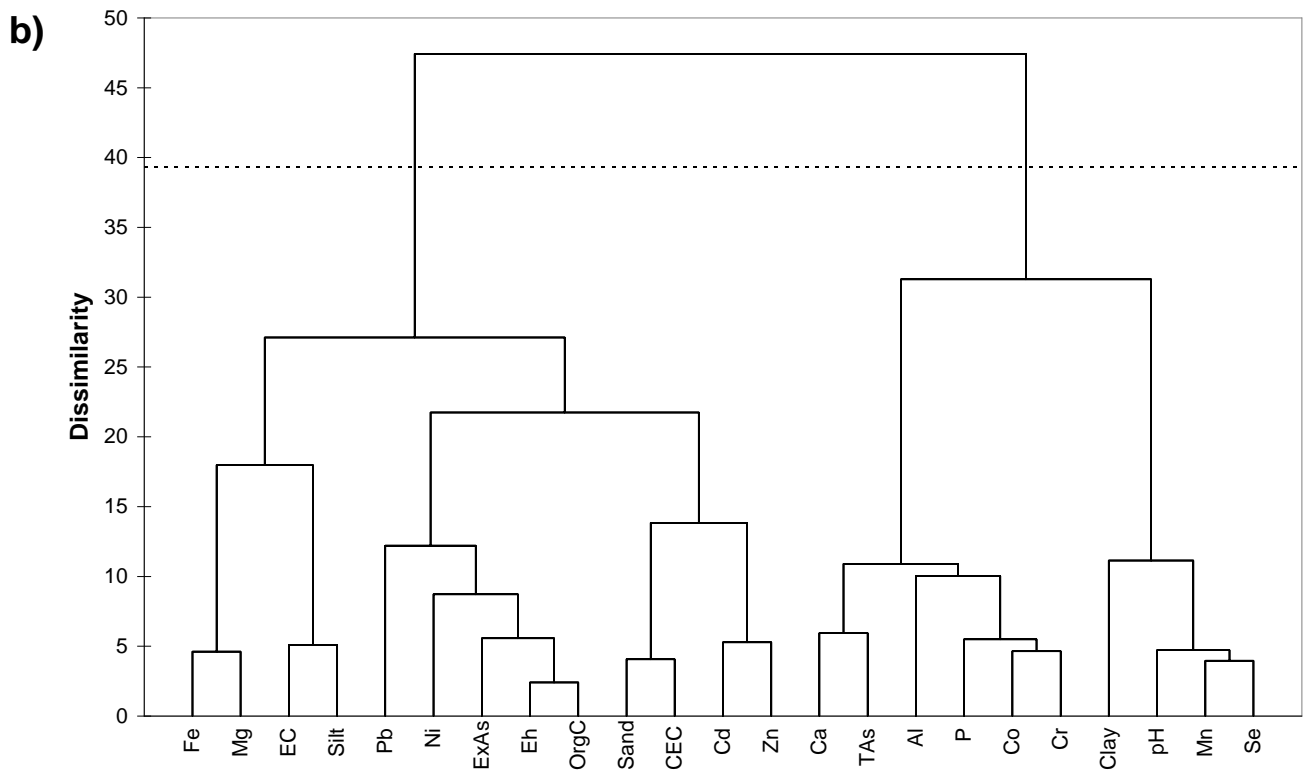
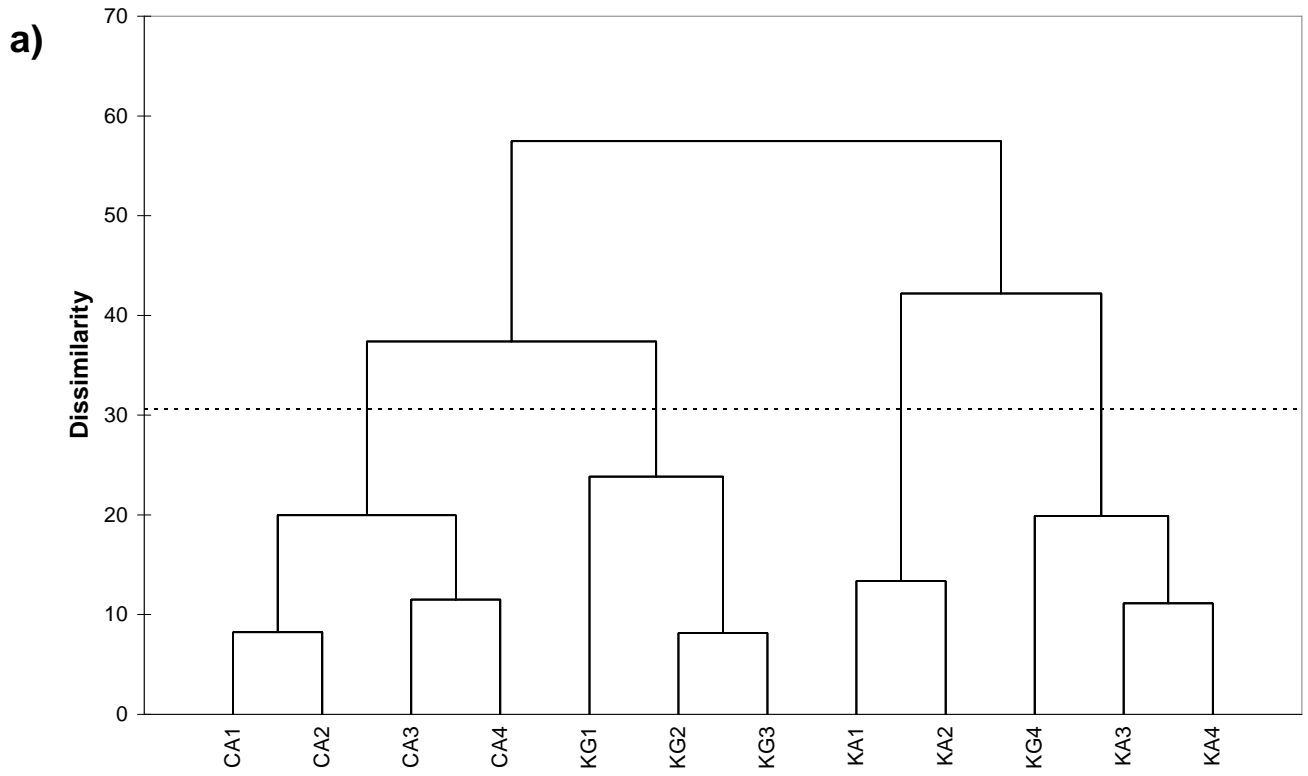


Fig. 4

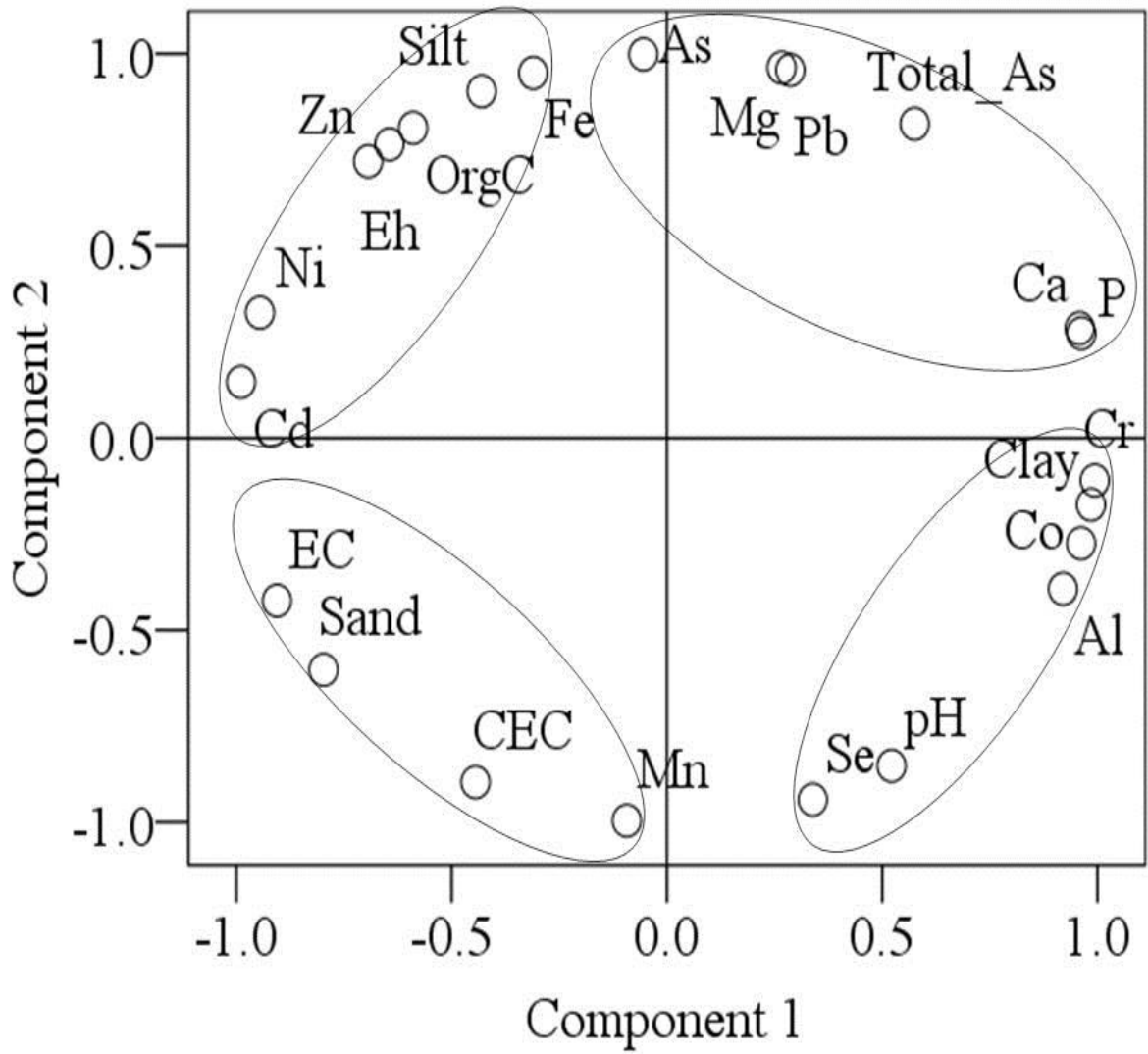


Fig. 5.