

Extraction Efficiency and Chemical Speciation of Iodine in Soil (Technical Paper)

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Abstract

The extraction efficiency of tetra-methyl ammonium hydroxide (TMAH) for soil iodine, and the effects of experimental procedures and conditions on the speciation of extracted iodine were tested. In addition, the possibility of extracting radioactive inorganic iodine forms ($^{129}\text{I}^-$ and $^{129}\text{IO}_3^-$) sorbed on soil metal oxides by competition with PO_4^{3-} was investigated. Results showed that changing TMAH concentration, extraction time, extraction temperature or soil particle size did not generally affect the concentrations of total iodine extracted. The ratio of iodide to total iodine in the TMAH extracts varied with the extraction conditions which led to the conclusion that part, or all, of the measured iodide is possibly produced by hydrolysis of organic iodine forms. This conclusion was confirmed by the detection of high concentrations of iodide in TMAH extracts of a humic acid. Only iodide was observed in the phosphate extracts of soil and it constituted up to 33% of the total iodine in the KH_2PO_4 extracts which indicates that most of the iodine mobilised by KH_2PO_4 is organically bound. When soil / KH_2PO_4 suspensions were spiked with $^{129}\text{I}^-$ and $^{129}\text{IO}_3^-$, at least 50% of $^{129}\text{I}^-$ and 15% of $^{129}\text{IO}_3^-$ was recoverable after 72 hours of reaction. The lowest recoveries were observed at the highest concentration of KH_2PO_4 , which also mobilised the greatest concentrations of DOC, indicating that although KH_2PO_4 is capable of releasing soil-sorbed iodide and iodate, it may also promote iodide and iodate reaction with soil organic matter.

Keywords

Soil iodine, radioiodine (^{129}I) pollution, TMAH extraction, iodine speciation, HPLC-ICP-MS

1. Introduction

Iodine is an essential nutrient for humans and animals; it is used by the thyroid gland to form thyroid hormones that control various physiological processes (Trotter, 1960). Iodine deficiency may lead to a range of clinical abnormalities known collectively as iodine deficiency disorders (IDD) (Underwood, 1977). IDD are a serious worldwide health problem, estimated to affect ~ 35% of the world's population, and a significant social and economic stress on developing countries (Fuge, 2007). Therefore, there has always been a need to increase understanding of iodine behaviour in

soils and the resulting implications for transfer to crops and livestock. Understanding the environmental behaviour of the long lived iodine radioactive isotope ^{129}I is also essential to the safety case for underground nuclear waste disposal.

The main iodine reservoir is the oceans. Iodine may transfer from seawater to the atmosphere via volatilisation of molecular iodine (I_2) or organic iodine forms (Amachi et al., 2005). Iodide and iodate possibly enter the atmosphere within marine aerosols (Fuge, 2007). From the atmosphere iodine is transferred to soils by both wet and dry deposition (Truesdale and Jones, 1996). In soil, inorganic iodine may be retained below pH 6 on positively charged hydrous iron and aluminium oxides and clay mineral edges (Whitehead 1978; Um et al., 2004). Strong correlations between soil iodine and soil organic matter observed in many studies also indicates that an organic iodine pool constitutes a significant portion of total soil iodine (e.g. Steinberg et al., 2008, Shetaya et al., 2012).

Iodine can be a difficult element to assay because of its volatility, especially in acidic conditions (Gilfedder et al., 2007). It is usually found in nature at low concentrations, which necessitates a sensitive analytical tool. Coupling of High Performance Liquid Chromatography (HPLC) (for separation) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (as detector) is now a commonly applied analytical approach for iodine speciation analysis. The flexibility of HPLC arises from the ability to change both mobile and stationary phases and to employ several mobile phases in a single analytical run using gradient-HPLC.

To determine soil concentrations of iodine alkaline extraction (using e.g. NaOH, KOH or TMAH) is required as iodine can readily volatilise from heated acidic media (Whitehead, 1973; Yamada et al., 1996, Watts & Mitchell, 2009). According to Yamada et al. (1996) strongly sorbed inorganic iodine and organically-bound iodine are solubilised by alkaline solutions. It is likely that iodate would also be released from sorption sites on Fe/Al hydrous oxides by increasing the negative charge on the oxide surface at high pH and ligand replacement with hydroxide ions. Humus-bound iodine would be solubilised by a combination of organic-iodine hydrolysis and mobilisation of humic and fulvic acids at high pH. Iodine extraction with tetra methyl ammonium hydroxide (TMAH) and subsequent determination with inductively coupled plasma mass spectrometry (ICP-MS) has become the favoured approach for assaying iodine in solid environmental samples. One advantage of TMAH over inorganic extractants such as NaOH or KOH is that high pH values can be achieved without increasing the salt concentration of the extraction solution and hence reducing the possibility of precipitation in the ICP-MS torch or nebuliser during analysis. Extraction of soil iodine with TMAH has been successfully applied by several studies with good reproducibility and precision (e.g. Watts and Mitchell, 2009). Nonetheless, the effect of the extraction procedure and condition on the reservation or alteration of the original soil iodine speciation has not been investigated. For inorganic iodine, the inverse relationship between the sorption of iodide and/or

iodate and soil pH (Whitehead 1978; Um et al., 2004) resembles that of other anions known to be non-specifically sorbed on soils such as Cl^- , NO_3^- , and SO_4^{2-} . Phosphate (PO_4^{3-}) is strongly adsorbed by aluminum and iron oxides and, therefore, can be used as an extractant for other ions of equal or lower adsorption affinity such as sulphate and iodate (Borggaard et al., 2005).

The main objectives of this work were to:

- 1- Understand the effects of the experimental conditions such as TMAH concentration, extraction temperature and extraction time on the extraction efficiency of total soil iodine and iodine speciation.
- 2- Examine the possibility of using PO_4^{3-} to selectively extract adsorbed iodide and iodate and the effect of the extraction conditions (PO_4^{3-} concentration and extraction time) on iodine speciation.

2. Materials and methods

2.1. Soil sampling and preparation

Soils were sampled from an arable field of University of Nottingham farm, Leicestershire (52°49'48"N-1°14'88"W) and from the adjacent mature woodland and permanent grassland. Two samples were taken from the woodland and arable soils: topsoil (0-20cm depth) and subsoil (30-50 cm depth). Only a topsoil sample was taken from the grassland. Soil samples were collected using a clean stainless steel spade, auger or trowel and sealed in plastic bags for transport. Soils were air dried in aluminium trays, gently disaggregated using a pestle and mortar (where necessary) and sieved to obtain a <2 mm fraction, and to remove stones and plants debris. Samples were homogenized using the 'cone method' (Schumacher et al., 1990) and processed soil samples were stored in clean polyethylene bags. A sub-sample of each soil was ground to a fine powder (clay size) in an agate ball mill (Retsch, Model PM400), and stored in the same way.

2.2. Soil characterisation

Soil pH was measured using a combined glass electrode after equilibrating 5 g of soil in 12.5 mL of Milli-Q water (18.3 MX) for 30 min. To estimate soil carbon and nitrogen content, finely ground soil, including certified soil reference standards (Sandy and peat standards provided by Elemental Microanalysis; product codes B2180 and B2176, respectively), were weighed into tin capsules and approximately 5 mg of vanadium pentoxide added. Analysis was undertaken using a CNS analyser (Flash EA1112; CE Instruments) and samples were introduced from a MAS200 auto-sampler. Total soil iodine was extracted by suspending approx. 0.25 g of soil in 5 mL of 5% TMAH solution

in polycarbonate centrifuge tubes. Tubes were heated at 70°C, with lids loosened, for 3 hours. A 5 mL aliquot of MQ water was added to each tube before centrifugation (20 min at 3000 g) and filtration (<0.22 µm) (More details in Watts and Mitchell (2009)). Iodine analysis in the filtered extracts was undertaken by ICP-MS.

2.3. ¹²⁷I and ¹²⁹I analysis

Iodine concentrations in the solutions and extracts were measured on a Thermo-Fisher Scientific X-Series^{II} ICP-MS in standard mode. Internal standards included 20 µg L⁻¹ In, 20 µg L⁻¹ Re, and 20 µg L⁻¹ Rh, prepared in a matrix of 2% TMAH and 4% methanol, introduced to the sample stream via a T-piece. TMAH was used prevent the oxidation of iodide ions to I₂ and potential loss by volatilization through the aspiration process in the ICP-MS nebulizer (Liu et al., 2007). Methanol was added to enhance ionization efficiency, sensitivity and stability (Thermo-Electron, 2008) and to eliminate matrix differences between DOC-rich samples and standards. Indium was the internal standard of first choice and normally compensated satisfactorily for instrumental drift during the course of analysis runs for both ¹²⁷I and ¹²⁹I. However, the inclusion of Re and Rh allowed the option of selecting different standards or using interpolation across all three internal standards. The wash solution was 1% TMAH.

Analysis of ¹²⁹I was carried out following the same procedure; however, a correction for ¹²⁹Xe on the ¹²⁹I signal was applied directly in the Plasmalab software. Samples of ¹²⁹I, as sodium iodide solution (SRM 4949C, 0.004 mol L⁻¹ Na¹²⁹I, 3451 Bq mL⁻¹), were obtained from the American National Institute of Standards (NIST), Gaithersburg, Maryland, USA. Iodate (¹²⁹IO₃⁻) was prepared from ¹²⁹I⁻ by oxidation using sodium chlorite as described by Yntema and Fleming (1939), following neutralisation of the preserving NaOH solution by HCl.

Chromatographic separation of ¹²⁷I⁻, ¹²⁷IO₃⁻, ¹²⁹I⁻ and ¹²⁹IO₃⁻ was undertaken using a Dionex ICS-3000 ion chromatography system operated in isocratic mode. Samples were introduced using an autosampler triggered by Chromeleon[®] software. Hamilton PRP-X100 anion exchange columns (250 x 4.6 mm or 50 x 4.6 mm, both with 5 µm particle size) were used for separation. The mobile-phase solution contained 60 mM L⁻¹ NH₄NO₃, 10⁻⁵ mM L⁻¹ Na₂-EDTA, 2% methanol, with pH adjusted to 9.5 with TMAH, at a flow rate of 1.3 mL min⁻¹. The column outflow was connected directly to the nebuliser of the ICP-MS. Sample processing was undertaken using Plasmalab software and peaks for individual species were manually integrated. Mixed ¹²⁷I⁻ and ¹²⁷IO₃⁻ working standards were prepared from stocks, immediately before analysis, using the mobile-phase as diluent. The sensitivity (counts per second per 1 µg L⁻¹; CPS µg L⁻¹) of a standard solution was repeatedly measured at intervals of six samples to correct for instrumental drift by linear interpolation between successive standards. Concentrations of ¹²⁷I⁻ and ¹²⁷IO₃⁻ in samples were then calculated by dividing the corresponding values of CPS by the calculated sensitivity S_(i) for each

sample. Concentrations of $^{129}\text{I}^-$ and $^{129}\text{IO}_3^-$ were calculated by applying a mass discrimination factor (a 'K-factor') to correct for the different sensitivities of the ^{127}I and ^{129}I isotopes (Equation 2):

$$^{129}\text{I}_{\text{conc}} = \frac{^{129}\text{I}_{\text{CPS}}}{S_{127}} \times 1.085 \quad 2.$$

Where, $^{129}\text{I}_{\text{conc}} = ^{129}\text{I}^-$ or $^{129}\text{IO}_3^-$ concentration ($\mu\text{g L}^{-1}$), $^{129}\text{I}_{\text{CPS}} =$ total counts per second of $^{129}\text{I}^-$ or $^{129}\text{IO}_3^-$, 1.085 = measured mass correction factor, $S_{127} = ^{127}\text{I}$ or $^{127}\text{IO}_3^-$ sensitivity.

The limit of detection (LOD) was defined by the reproducibility of integration to be $\sim 0.3 \mu\text{g L}^{-1}$ ($\sim 0.002 \text{ mg kg}^{-1}$).

2.4. Extraction and speciation of soil iodine

2.4.1. Extraction of soil iodine with TMAH

To test whether the efficiency of iodine extraction was related to particle size, simultaneous extractions of sieved and ground soils were undertaken. The standard extraction method described by Watts and Mitchell (2009) was used except that 2 g of sieved soil ($<2\text{mm}$) was extracted with 20 mL of 10% TMAH solution. It was suggested by Yamada et al. (1996) that TMAH extraction methods that include heating may result in thermal decomposition of organically-bound iodine and consequently alter the original speciation. This was examined by HPLC-ICP-MS analysis of TMAH extracts. The efficiency of TMAH extraction at room temperature was also examined over a range of TMAH concentrations. Soil samples (2 g) were suspended in 20 mL of 5%, 10%, 15% and 20% TMAH solutions in polycarbonate centrifuge tubes and shaken reciprocally for 16 h at 200 rpm. The effect of TMAH concentration on ambient extraction of iodine was also tested on the woodland topsoil over a wide range of TMAH concentrations (0, 0.001, 0.01, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5, and 10%).

The effect of heated and ambient TMAH extraction on iodide/iodate solutions and humic acid together with a test of the recovery of iodide and iodate spikes added to humic acid and soils was also investigated. Two sets of samples containing various combinations of iodide, iodate, humic acid (prepared by Marshall et al. (1995)), soil and TMAH solution were prepared. The first group of suspensions was placed in an oven at 70°C for 3 hours and tubes were shaken at 1.5 h. The second set of tubes was shaken at room temperature on a reciprocal shaker for 16 hours. After extraction, all tubes were centrifuged (20 min at 3000 g) and the supernatant was filtered ($<0.22 \mu\text{m}$). Solutions were then analysed for total ^{127}I and for ($^{127}\text{I}^-$ and $^{127}\text{IO}_3^-$) using ICP-MS and HPLC-ICP-MS, respectively.

2.4.2. Extraction of soil iodine with potassium di-hydrogen phosphate

To investigate the potential of PO_4^{3-} extraction of adsorbed iodide and/or iodate, soil samples (2 g; $<2\text{mm}$ sieved) of woodland subsoil and arable topsoil were suspended in 20 mL of 0.005, 0.01, 0.05

and 0.1 M KH_2PO_4 solutions and shaken on a reciprocal shaker for 16 h at room temperature. Recovery of spiked $^{129}\text{I}^-$ and $^{129}\text{IO}_3^-$ was also tested using a range of KH_2PO_4 concentrations. Sieved (<2mm) woodland subsoil and arable topsoil samples (2 g) were suspended in 20 mL of 0.01, 0.05 and 0.1 M KH_2PO_4 solutions in polycarbonate centrifuge tubes and then shaken on a reciprocal shaker for 16 h. Suspensions were spiked with $^{129}\text{I}^-$ or $^{129}\text{IO}_3^-$ (0.2 mg kg^{-1} soil) and shaken for another 24 hours. Two further identical sets of tubes were shaken for 48 and 72 hours to test the effect of time on extraction. All samples were prepared in triplicate and after extraction centrifuged (25 min at 3500 g), filtered (0.22 μm) and analysed for total ^{127}I and ^{129}I concentration by ICP-MS and for $^{127}\text{I}^-$, $^{127}\text{IO}_3^-$, $^{129}\text{I}^-$ and $^{129}\text{IO}_3^-$ by HPLC-ICP-MS.

3. Results and Discussion

3.1. Soil properties

Soil properties are given in Table 1. Woodland and grassland topsoils have the highest organic matter content (4.6-5 % C). Lowest carbon contents were recorded in the subsoils. Soil pH was low (~ pH 4.5) in the woodland and grassland soils and ~ pH 6 in the arable soils. Total soil iodine concentrations were mainly in the range 2 - 3 mg kg^{-1} , lower than the worldwide average of 5 mg kg^{-1} (Fleming, 1980) and the UK average of 9.2 mg kg^{-1} (Whitehead, 1979).

Table 1: Soil characteristics

Soil	pH	C (%)	N (%)	I (mg kg^{-1})
Woodland Topsoil	4.8	5.01	0.36	3.11 ± 0.07
Woodland Subsoil	4.3	1.17	0.08	2.05 ± 0.04
Arable Topsoil	6.5	1.98	0.16	2.99 ± 0.04
Arable Subsoil	6.9	0.43	0.04	2.85 ± 0.02
Grassland Topsoil	4.7	4.59	0.35	2.83 ± 0.15

3.2. Extraction and Speciation of Soil Iodine

3.2.1. Extraction with TMAH

3.2.1.1. Heated extraction

Total iodine concentrations determined for the certified reference materials showed good agreement with the certified values (Table 2.). In most of the soils the agreement was comparable to the results obtained by Watts and Mitchell (2009) where the extraction method was developed. However, in

the current work and in Watts and Mitchell (2009) the results obtained for the reference material GSS 8 (loess) were, respectively, 20% and 32% less than the certified concentrations suggesting that for some soils/materials more rigorous extraction procedure may be required.

Table 2: Certified and measured iodine concentrations (mg kg^{-1}) in standard reference materials after extraction with 5% TMAH at 70°C. Results are the average of three replicates.

Reference Material	^{127}I (current work)	^{127}I (Watts and Mitchell, 2008)	^{127}I Certified
GSS 2 (chestnut soil)	1.67 ± 0.01	1.6 ± 0.2	1.8 ± 0.2
GSS 3 (yellow-brown soil)	1.32 ± 0.01	1.3 ± 0.1	1.3 ± 0.4
GSS 5 (yellow-red soil)	3.61 ± 0.03	3.5 ± 0.4	3.8 ± 0.5
GSS 6 (yellow-red soil)	21.4 ± 0.26	20.6 ± 1.5	19.4 ± 1
GSS 7 (laterite)	18.9 ± 0.09	17.3 ± 0.6	19.3 ± 1.1
GSS 8 (loess)	1.27 ± 0.01	1.1 ± 0.1	1.6 ± 0.5

Comparing finely ground and <2mm sieved soil the latter produced approximately 94-98% recovery (Figure 1) suggesting that the degree of surface exposure had a minor but measurable effect. Speciation analysis (HPLC-ICP-MS) indicated that iodide was the only inorganic species present, ranging in ground soils from 0.69 to 1.55 mg kg^{-1} , and in <2mm sieved soils between 0.74 and 1.42 mg kg^{-1} . The concentration ratio of iodide : total iodine varied between soils with the lowest ratios in the arable topsoil and subsoil, respectively. The highest ratios were observed in the highly organic woodland topsoil and grassland topsoil. The results suggest that soil particle size has little influence on the total iodine extracted by 5% TMAH extraction (at 70°C) or the observed speciation of iodine in the extract solution.

3.2.1.2. Room temperature extractions

TMAH concentrations greater than 5% (10, 15 and 20%), at room temperature, also had negligible impact on the total iodine extracted from different soils (Table 3). Typically no change in total iodine content was observed when TMAH concentration was increased with the possible exception of the arable subsoil where an increase in the total ^{127}I extracted was discernable ($P < 0.05$). The maximum total iodine concentrations extracted at ambient temperatures ranged from 95 to 115% of the concentrations measured using 5% TMAH as the extractant at 70°C. This suggests that soil iodine can be effectively extracted with 5% TMAH at room temperature.

Speciation of the extracts showed that iodide was the only inorganic species present and this represented 20 to 30% of total iodine, lower percentages than obtained using 70°C extraction (31%-48%). This difference suggests that iodide may be produced by thermally assisted hydrolysis of organically bound iodine during heating. It is not clear however if extraction at ambient temperature conserves the original speciation of solid phase iodine or, if it too releases organically bound iodine as iodide, but to a lesser extent than when heated.

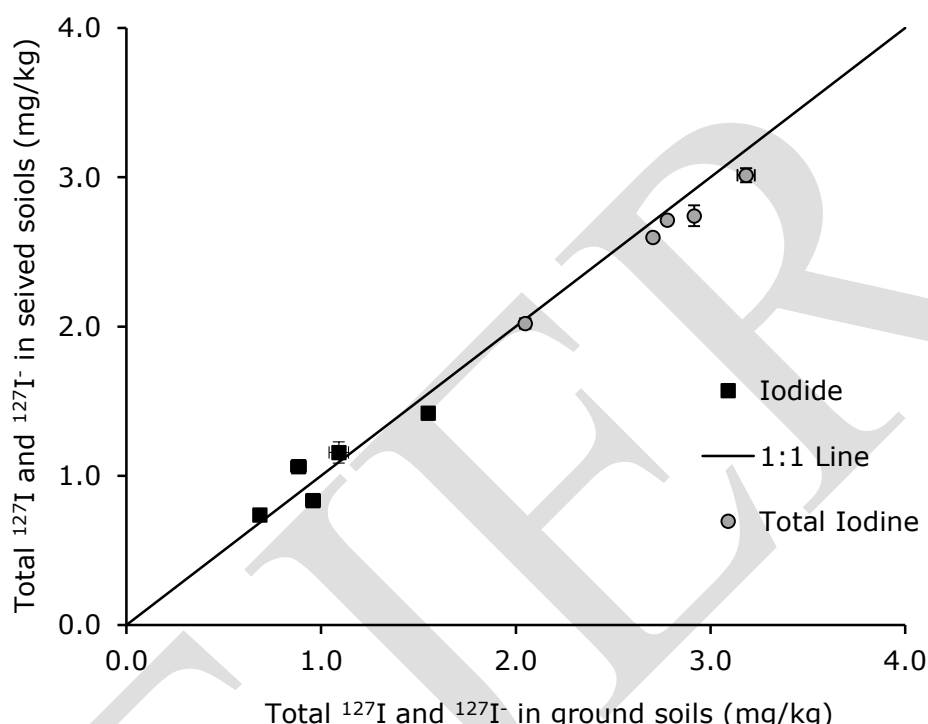


Figure 1: Total extracted iodine and iodide concentrations (mg kg^{-1}) from finely ground (clay size) soils vs. <2 mm sieved soils; the extraction was carried out with 5% TMAH at 70°C

Table 3: Iodide and total iodine concentrations in soil extracts (mg kg^{-1}); extraction was carried out using different concentrations of TMAH by shaking for 16 h at room temperature.

Soil	Woodland Topsoil		Woodland Subsoil		Arable Subsoil		Arable Subsoil		Grassland Topsoil	
	Iodide	Total I	Iodide	Total I	Iodide	Total I	Iodide	Total I	Iodide	Total I
5	0.84 ± 0.04	2.94 ± 0.09	0.49 ± 0.01	2.14 ± 0.04	0.60 ± 0.03	2.50 ± 0.02	0.47 ± 0.00	2.35 ± 0.02	0.72 ± 0.01	2.65 ± 0.03
10	0.92 ± 0.01	3.13 ± 0.04	0.48 ± 0.03	2.19 ± 0.04	0.59 ± 0.01	2.49 ± 0.02	0.52 ± 0.01	2.47 ± 0.01	0.88 ± 0.05	2.80 ± 0.05
15	0.84 ± 0.02	2.95 ± 0.06	0.54 ± 0.01	2.18 ± 0.03	0.65 ± 0.03	2.58 ± 0.02	0.56 ± 0.01	2.61 ± 0.04	0.99 ± 0.04	3.01 ± 0.10
20	0.98 ± 0.06	3.12 ± 0.02	0.59 ± 0.07	2.13 ± 0.07	0.61 ± 0.06	2.63 ± 0.04	0.60 ± 0.03	2.62 ± 0.02	0.83 ± 0.04	2.86 ± 0.06

Applying a wider range of TMAH concentration to the organic woodland topsoil increased the concentration of extracted iodine slightly from 0.14 to 0.2 mg kg⁻¹ at TMAH concentrations of 0-0.01% (pH 4.85-5.35) before increasing substantially to 1.7 mg kg⁻¹ at a TMAH concentration of 0.1% (pH 8.23), the first extraction where pH was alkaline. As TMAH concentration was further increased (0.2-10%) a further slight increase in extracted iodine (2.4-2.75 mg kg⁻¹) was observed (Figure 2.). No iodate was detected in the extracts. Iodide concentrations ranged from 0.006-0.723 mg kg⁻¹ (4 -26% of total iodine) and followed the trend for total iodine with concentrations increasing with pH. The strength of iodide and iodate sorption on hydrous oxides of iron and aluminium is inversely proportional to pH due to the loss of positively charged sites and competition with hydroxyl ions in alkaline medium (Whitehead, 1973; Muramatsu et al., 1990). Consequently, most of the sorbed iodide in soil is expected to be released by alkaline extraction. The continued increase in the ratio of iodide to total iodine extracted, with increasing pH (up to pH 14) suggests that the majority of iodide is produced by hydrolysis of organic iodine rather than desorption from soil metal oxides.

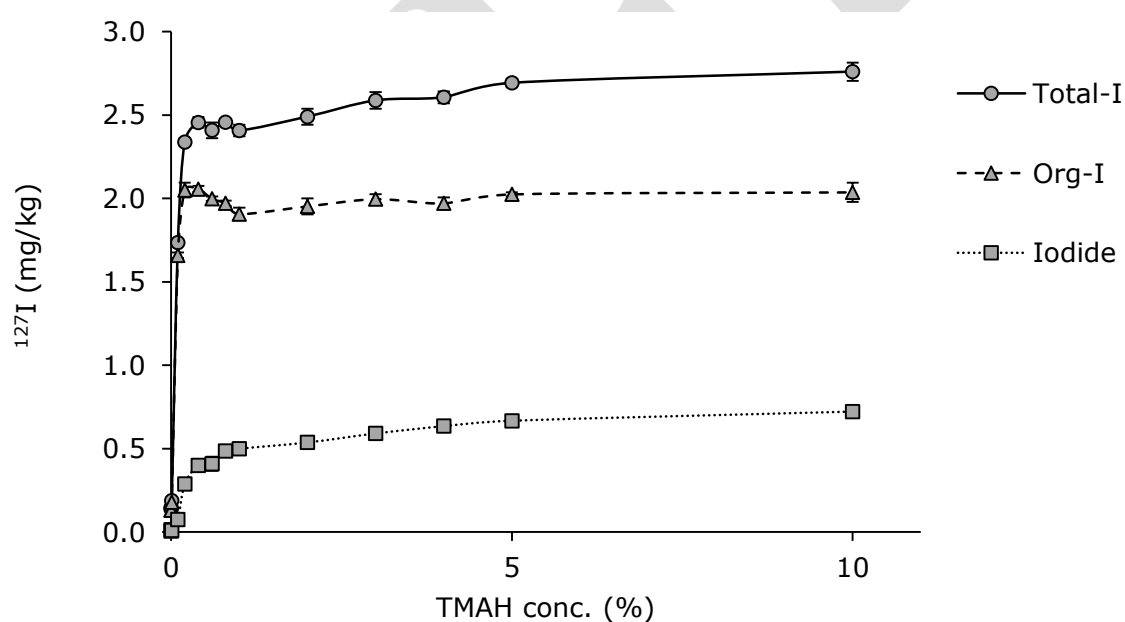


Figure 2: Total iodine, organic iodine (calculated by subtracting iodide from total iodine), and iodide concentrations (mg kg⁻¹) extracted from woodland topsoil over a range of TMAH concentrations; extraction was carried out at room temperature with shaking for 16 h.

3.2.2. Recovery from iodide/iodate/humic acid mixtures

Recoveries of iodide when solutions of iodide or iodide/iodate were mixed with TMAH ranged from 92.8 % to 97.3 % for the 70°C extraction and ~ 103 % for the room temperature extraction (Table 4). Recoveries of iodate were generally less (c. 90%). As the recovery of total iodine was close to 100% this suggests that ~ 10% of iodate was transformed to an unknown form. Good recovery of total iodine was achieved from humic acid samples spiked with iodide or iodate. Recovery was between 97 to 103 % for samples extracted at 70°C, and from 98 to 101 % for samples extracted at room temperature. Recovery of total iodine from soil samples spiked with iodide or iodate was generally less successful (91-93 %). Expected values were taken as the average of three replicate measurements of unspiked humic acid or soil, plus the added iodide or iodate. In unspiked samples of humic acid or soil, no iodate peaks were detected. Extractions of pure humic acid relatively high concentrations of iodide were measured (38% and 24% of total iodine for extraction at 70°C and room temperature, respectively). As the native iodine in humic acid is 100% organically bonded, the detection of appreciable concentrations of iodide is evidence that both ambient and heated extraction with TMAH results in hydrolysis of organically-bound iodine forms, altering the original speciation of the solid phase iodine. In spiked humic acid or soils, recovery of iodide was 90% - 97%. In iodate spiked systems no iodate was detected; almost all of the iodate spiked in humic acid, woodland subsoil and arable topsoil was converted to iodide. This agrees with the findings of Yamada et al. (1996) where 82% of iodate added to a soil solution was reduced to iodide as a result of TMAH extraction (extraction was undertaken using 10mL of 5% TMAH by shaking for 4 h at room temperature).

Table 4: Recovery of iodide, iodate and total iodine (μg) following extraction with 10% TMAH of iodide, iodate, and iodide/iodate solutions, and iodide and iodate spikes added to humic acid (HA) and a range of soils. Hot extraction was at 70°C for 3 h and unheated extractions were shaken for 16 h at room temperature. HA= Humic Acid, WS = Woodland Subsoil, AT= Arable Topsoil, Meas. = Measured, Exp. = Expected, Rec. = Recovery and NA= not applicable.

Extraction	Sample Description						Recovery								
							Iodate			Iodide			Total Iodine		
	I ⁻	IO ₃ ⁻	HA	WS	AT	TMAH	Meas.	Exp.	Rec.	Meas.	Exp.	Rec.	Meas.	Exp.	Rec.
(μg)	(μg)	(μg)	(g)	(g)	(%)	(μg)	(μg)	(%)	(μg)	(μg)	(%)	(μg)	(μg)	(%)	
Hot Extraction	1	0	0	0	0	10	0.00	0.00	NA	0.93	1.00	92.8	0.97	1.00	96.7
	0	1	0	0	0	10	0.89	1.00	89.3	0.00	0.00	NA	1.03	1.00	103
	1	1	0	0	0	10	0.92	1.00	92.3	0.97	1.00	97.3	2.03	2.00	102
	0	0	0.25	0	0	10	0.00	0.00	NA	1.07	1.07	100	2.81	2.82	99.5
	1	0	0.25	0	0	10	0.00	0.00	NA	1.99	2.14	92.7	3.93	4.01	97.9
	0	1	0.25	0	0	10	0.00	1.00	0.00	1.75	1.02	172	3.61	3.68	98
	1	1	0.25	0	0	10	0.00	1.00	0.00	2.82	2.07	136	4.69	4.81	97.4
Unheated Extraction	1	0	0	0	0	10	0.00	0.00	NA	1.03	1.00	103	0.99	1.00	99.5
	0	1	0	0	0	10	0.90	1.00	90.2	0.00	0.00	NA	0.98	1.00	97.6
	1	1	0	0	0	10	0.90	1.00	90.3	1.03	1.00	103	1.97	2.00	98.6
	0	0	0.25	0	0	10	0.00	0.00	NA	0.65	0.65	100	2.71	2.70	100
	1	0	0.25	0	0	10	0.00	0.00	NA	1.67	1.76	94.7	3.65	3.62	101
	0	1	0.25	0	0	10	0.00	1.00	0.00	1.44	0.63	227	3.65	3.62	101
	1	1	0.25	0	0	10	0.00	1.00	0.00	2.46	1.69	146	4.89	4.84	101
	0	0	0	2	0	20	0.00	0.00	NA	0.64	0.64	100	3.10	3.10	100
	2	0	0	2	0	20	0.00	0.00	NA	2.59	2.67	97.3	4.82	5.20	92.7
	0	2	0	2	0	20	0.00	2.00	0.00	2.42	0.67	363	4.84	5.20	93
	0	0	0	0	2	20	0.00	0.00	NA	0.74	0.74	100	3.61	3.61	100
	2	0	0	0	2	20	0.00	0.00	NA	2.50	2.77	90.4	5.29	5.73	92.3
	0	2	0	0	2	20	0.00	2.00	0.00	2.39	0.77	313	5.23	5.73	91.3

3.3. Extraction with Potassium Di-hydrogen Phosphate

The amount of total iodine extracted increased with increasing concentration of KH_2PO_4 but did not achieve a plateau indicating that further iodine could be extracted with higher concentrations (Table 5). The low solubility of KH_2PO_4 and problems associated with analysis of solutions with high dissolved solid concentrations by ICPMS made it impractical to use higher concentrations. The total amount of iodine extracted with the highest concentration of KH_2PO_4 (0.1 M) was 18% and 6% of total soil iodine content as extracted by TMAH (Table 1) for woodland subsoil and arable topsoil, respectively. No iodate was detected after phosphate extraction. Iodide concentrations were between 0.025 to 0.05 mg kg^{-1} in the woodland subsoil and 0.021 to 0.064 mg kg^{-1} in the arable topsoil. Concentrations of extracted iodide also increased with increasing concentration of KH_2PO_4 but only constituted ~13 % of total iodine measured in woodland subsoil extracts and ~35% in the arable topsoil extracts. This suggests that the majority of iodine mobilised by KH_2PO_4 is organically bound, apparently displaced by HPO_4^{2-} ions through competition with negatively charged soil organic matter for adsorption sites at the surface of hydrated iron and aluminium oxides (Borggaard et al., 2005)

Table 5: Concentrations of iodide and total iodine extracted by a range of KH_2PO_4 concentrations. Extraction was carried out by shaking soil suspensions for 16 h at room temperature.

Soil	KH_2PO_4 (M)	$^{127}\text{I}^-$ (mg kg^{-1})	Total- ^{127}I (mg kg^{-1})	$^{127}\text{I}^- / \text{Total-}^{127}\text{I}$ (%)
Woodland Subsoil	0.1	0.050 ± 0.003	0.376 ± 0.003	13.4
	0.05	0.042 ± 0.005	0.323 ± 0.004	13.1
	0.01	0.035 ± 0.005	0.274 ± 0.007	12.7
	0.005	0.025 ± 0.001	0.271 ± 0.002	9.2
Arable Topsoil	0.1	0.064 ± 0.003	0.179 ± 0.002	36
	0.05	0.053 ± 0.004	0.160 ± 0.002	33.3
	0.01	0.043 ± 0.002	0.122 ± 0.004	35.3
	0.005	0.021 ± 0.001	0.113 ± 0.003	19

In the samples spiked with $^{129}\text{I}^-$, concentrations of $^{129}\text{I}^-$ and total ^{129}I generally decreased with time (Figure 3). Total concentrations of ^{129}I recovered were inversely proportional to the concentrations of KH_2PO_4 and decreased with increasing time. However, similar $^{129}\text{I}^-$ concentrations were extracted regardless of KH_2PO_4 concentration at any given time. Highest total ^{129}I recoveries were achieved with the lowest KH_2PO_4 concentration and shortest shaking times whilst lowest recoveries were associated with longer shaking times and higher KH_2PO_4 concentrations. Similar behaviour was observed in $^{129}\text{IO}_3^-$ spiked samples, with the concentrations of both total ^{129}I and $^{129}\text{IO}_3^-$ decreasing with time (Figure

4). Recovery of $^{129}\text{IO}_3^-$ was more affected by KH_2PO_4 concentration than was $^{129}\text{I}^-$, with the highest recoveries of $^{129}\text{IO}_3^-$ at the lowest KH_2PO_4 concentrations. Total ^{129}I and $^{129}\text{IO}_3^-$ recoveries were lower overall than in $^{129}\text{I}^-$ spiked samples. As $^{129}\text{IO}_3^-$, percentage recoveries were 41% -71% for woodland subsoil and 15% -70% for arable topsoil. Appreciable concentrations of $^{129}\text{I}^-$ were also detected in $^{129}\text{IO}_3^-$ spiked samples indicating reduction of $^{129}\text{IO}_3^-$ to $^{129}\text{I}^-$ was occurring. Reduction in ^{129}I concentrations (as total ^{129}I , $^{129}\text{IO}_3^-$ or $^{129}\text{I}^-$) is unlikely to be due to sorption on sesquioxides because of the large concentrations of PO_4^{3-} ions in the extraction solutions. Phosphate is strongly sorbed onto soil hydrous oxides in preference to other anions (Borggaard et al., 2005). That recovery of ^{129}I was lower at higher KH_2PO_4 concentrations where competition of PO_4^{3-} ions with $^{129}\text{IO}_3^-$ or $^{129}\text{I}^-$ on adsorption sites would be greater also supports this hypothesis. Since, recovery as $^{129}\text{I}^-$ or $^{129}\text{IO}_3^-$ was always less than total ^{129}I there was clear transformation of $^{129}\text{I}^-$ into soluble organic ^{129}I forms.

The overall reduction in total ^{129}I concentrations also suggests that soluble organic ^{129}I forms were gradually fixed as insoluble forms through the cycling of soil organic matter between soluble and insoluble pools (see e.g. Francois, 1987; Fukui et al., 1996; Warner et al., 2000; Schlegel et al., 2006). The lower percentage recoveries of $^{129}\text{I}^-$, $^{129}\text{IO}_3^-$ and total ^{129}I in the arable topsoil which has 60% more C and 100% more N compared to the woodland subsoil (Table 1) could be explained by the incorporation of iodide and iodate into organic matter via an electrophilic substitution mechanism as elaborated earlier. Concentrations of $^{129}\text{I}^-$, which can be produced by reduction of $^{129}\text{IO}_3^-$ (Fukui et al., 1996), measured in $^{129}\text{IO}_3^-$ spiked samples, were higher in the arable topsoil samples than in woodland subsoil samples indicating that the higher organic matter content may result in more $^{129}\text{IO}_3^-$ reduction.

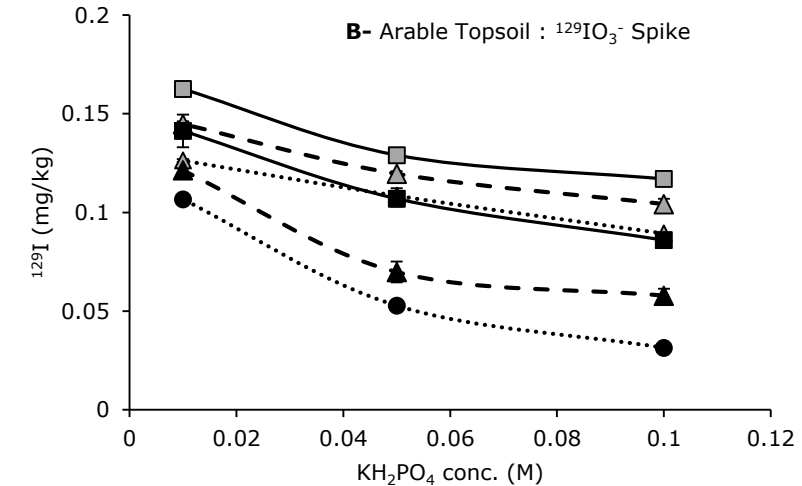
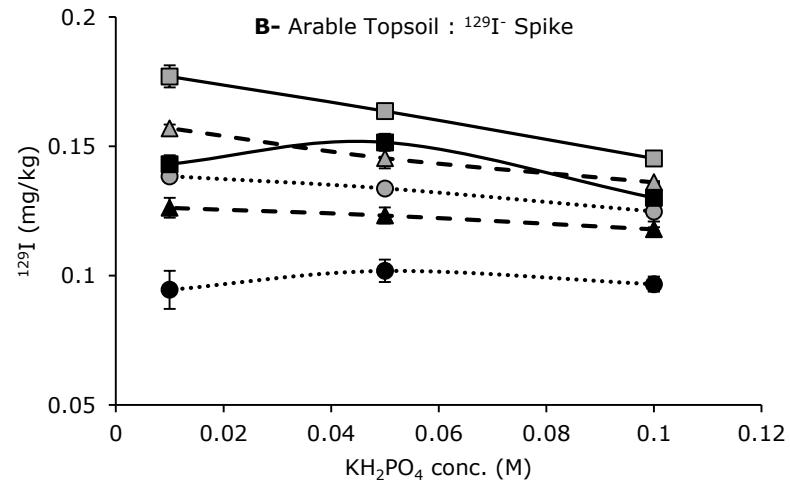
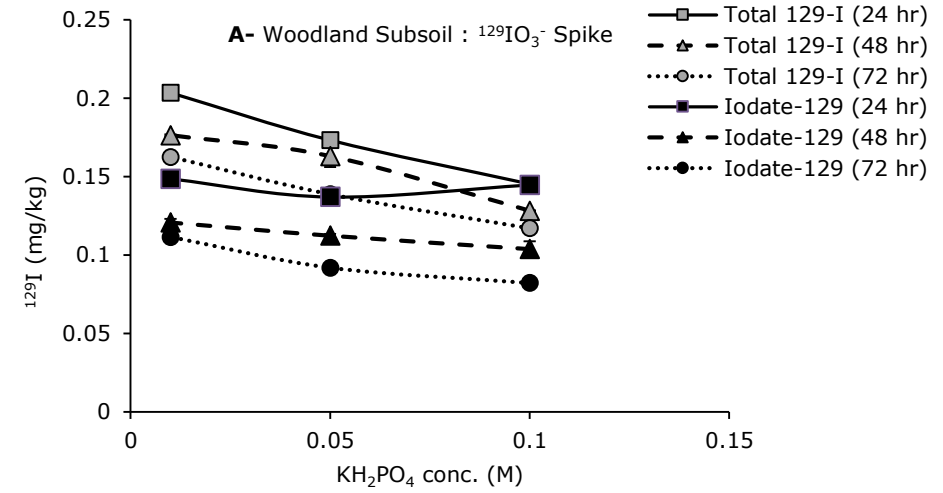
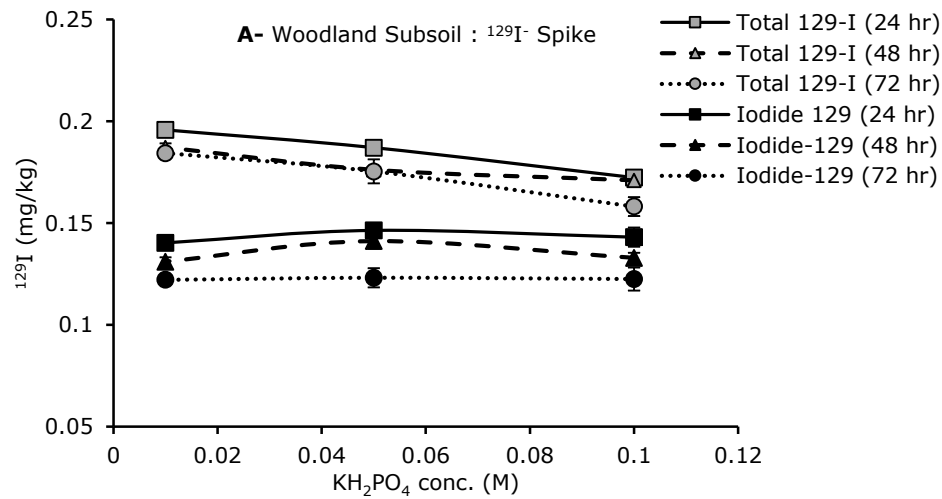


Figure 3: Changes in total- ^{129}I and $^{129}\text{I}^-$ concentrations in woodland subsoil (A) and arable topsoil (B) at different equilibration times and KH_2PO_4 concentrations after spiking suspensions with $0.2 \text{ mg kg}^{-1} \text{ }^{129}\text{I}^-$.

Figure 4: Changes in total- ^{129}I and $^{129}\text{IO}_3^-$ concentrations in woodland subsoil (A) and arable topsoil (B) at different equilibration times and KH_2PO_4 concentrations after spiking suspensions with $0.2 \text{ mg kg}^{-1} \text{ }^{129}\text{IO}_3^-$.

4. Conclusions

The conditions of extraction of soil iodine with TMAH, including TMAH concentration (5% - 20 %), extraction time (3 – 16 h), extraction temperature (room temperature or 70°C) and soil particle size (finely ground or <2mm sieved) was found to have little influence on the concentrations of total iodine extracted. However, the proportion of iodide to total iodine in the TMAH extracts varied depending on the extraction conditions. This suggests that part, or all, of the measured iodide may have been originally organically-bound and was hydrolysed as iodide as a result of the extraction. Evidence that extraction with TMAH alters the original speciation of soil iodine was also suggested by the detection of appreciable concentrations of iodide in TMAH extracts of humic acid where 100% organic iodine would be expected.

The total iodine extracted with 0.1 M KH_2PO_4 was 6 - 18% of total soil iodine. No iodate was measured and iodide constituted only 13% and 33% of total iodine (in KH_2PO_4 extracts) in the woodland subsoil and arable topsoil, respectively, which indicates that most of the iodine mobilized by KH_2PO_4 is organic in nature. When soil suspended in KH_2PO_4 solution was spiked with ^{129}I or $^{129}\text{IO}_3^-$, at least 50% of ^{129}I and 15% of $^{129}\text{IO}_3^-$ were recoverable after 72 hours of reaction. However, the lowest recoveries were observed at the highest KH_2PO_4 concentrations suggesting that, although KH_2PO_4 could be useful in releasing any inorganic iodine forms in soils, it also facilitates inorganic iodine incorporation in organic matter by mobilising soluble forms of soil organic matter. The information obtained from KH_2PO_4 extraction was generally not conclusive about iodine speciation in soil because of the possible inter-conversion between soil iodine forms.

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References

- AMACHI, S., FUJII, T., SHINOYAMA, H. & MURAMATSU, Y. (2005) Microbial Influences on the Mobility and Transformation of Radioactive Iodine in the Environment. *Journal of Nuclear and Radiochemical Sciences*, 6, 21-24.
- BORGGAARD, O. K., RABEN-LANGE, B., GIMSING, A. L. & STROBEL, B. W. (2005) Influence of humic substances on phosphate adsorption by aluminium and iron oxides. *Geoderma*, 127, 270-279.

- FLEMING, G. A. (1980) Essential micronutrients II: Iodine and selenium. in Applied Soil Trace Elements, B.E. Davies, ed., pp. 199-234, John Wiley & Sons, Chichester.
- FRANCOIS, R. (1987) The influence of humic substances on the geochemistry of iodine nearshore and Hemipelagic marine sediments. *Geochimica Et Cosmochimica Acta*, 51, 2417-2427.
- FUGE, R. (2007) Iodine deficiency: An ancient problem in a modern world. *Ambio*, 36, 70-72.
- FUKUI, M., FUJIKAWA, Y. & SATTA, N. (1996) Factors affecting interaction of radioiodide and iodate species with soil. *Journal of Environmental Radioactivity*, 31, 199-216.
- GILFEDDER, B. S., ALTHOFF, F., PETRI, M. & BIESTER, H. (2007) A thermo extraction-UV/Vis spectrophotometric method for total iodine quantification in soils and sediments. *Analytical and Bioanalytical Chemistry*, 389, 2323-2329.
- LIU, W., YANG, H. X., LI, B., CHEN, D. Y. & ZHANG, H. J. (2007) Study on speciation stabilities of iodine in underground water by high performance liquid chromatography-inductively coupled plasma mass spectrometry. *Chinese Journal of Analytical Chemistry*, 35, 571-574.
- MARSHALL, S. J., YOUNG, S. D. & GREGSON, K. (1995) Humic acid proton equilibria - A comparison of 2 models assessment of titration error. *European Journal of Soil Science*, 46, 471-480.
- MURAMATSU, Y., UCHIDA, S., SRIYOTHA, P. & SRIYOTHA, K. (1990) Some Considerations on the Sorption and Desorption Phenomena of Iodide and Iodate on Soil. *Water Air and Soil Pollution*, 49, 125-138.
- SCHLEGEL, M. L., REILLER, P., MERCIER-BION, F., BARRE, N. & MOULIN, V. (2006) Molecular environment of iodine in naturally iodinated humic substances: Insight from X-ray absorption spectroscopy. *Geochimica Et Cosmochimica Acta*, 70, 5536-5551.
- SCHUMACHER, B. A., SHINES, K. C., BURTON, J. V. & PAPP, M. L. (1990) A comparison of soil sample homogenisation Techniques, Environmental monitoring system laboratory, Office of research and development, U.S. Environmental protection agency Las Vegas, <http://www.epa.gov/esd/cmb/research/papers/bs120.pdf>, Date Accessed: 13 September 2010.
- SHETAYA, W. H., YOUNG, S. D., WATTS, M. J., ANDER, E. L. & BAILEY, E. H. (2012) Iodine dynamics in soils. *Geochimica et Cosmochimica Acta*, 77, 457-473.
- STEINBERG, S. M., BUCK, B., MORTON, J. & DORMAN, J. (2008) The speciation of iodine in the salt impacted Black Butte soil series along the Virgin river, Nevada, USA. *Applied Geochemistry*, 23, 3589-3596.
- THERMO-ELECTRON (2008) X Series ICP-MS Clinical Applications Note 2: Determination of Cu, Zn and Se in human serum samples. Thermo Electron Co., Application Note: AN_EO604, http://www.thermo.com/eThermo/CMA/PDFs/Articles/articlesFile_24876.pdf, Date Accessed: September, 3, 2008.
- TROTTER, W. R. (1960) The Association of Deafness with Thyroid Dysfunction. *British Medical Bulletin*, 16, 92-98.

- TRUESDALE, V. W. & JONES, S. D. (1996) The variation of iodate and total iodine in some UK rainwaters during 1980-1981. *Journal of Hydrology*, 179, 67-86.
- UM, W., SERNE, R. J. & KRUPKA, K. M. (2004) Linearity and reversibility of iodide adsorption on sediments from Hanford, Washington under water saturated conditions. *Water Research*, 38, 2009-2016.
- UNDERWOOD, E. J. (1977) Iodine, Trace elements in human and animals. 4th ed. Nueva York, Academic Press, p:271-301.
- WARNER, J. A., CASEY, W. H. & DAHLGREN, R. A. (2000) Interaction kinetics of I-2(aq) with substituted phenols and humic substances. *Environmental Science & Technology*, 34, 3180-3185.
- WATTS, M. J. & MITCHELL, C. J. (2009) A pilot study on iodine in soils of Greater Kabul and Nangarhar provinces of Afghanistan. *Environmental Geochemistry and Health*, 31, 503-509.
- WHITEHEAD, D. C. (1973) Sorption of Iodide by Soils as Influenced by Equilibrium Conditions and Soil Properties. *Journal of the Science of Food and Agriculture*, 24, 547-556.
- WHITEHEAD, D. C. (1978) Iodine in Soil Profiles in Relation to Iron and Aluminum-Oxides and Organic-Matter. *Journal of Soil Science*, 29, 88-94.
- WHITEHEAD, D. C. (1979) Iodine in the Uk Environment with Particular Reference to Agriculture. *Journal of Applied Ecology*, 16, 269-279.
- YAMADA, H., KIRIYAMA, T. & YONEBAYASHI, K. (1996) Determination of total iodine in soils by inductively coupled plasma mass spectrometry. *Soil Science and Plant Nutrition*, 42, 859-866.
- YNTEMA, L. F. & FLEMING, T. (1939) Volumetric oxidation of iodide to iodate by sodium chlorite. *Industrial and Engineering Chemistry-Analytical Edition*, 11, 0375-0377.