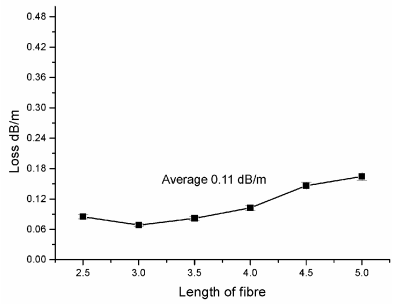

Figure 3. Background loss in optical fibre measured by cut-back method.

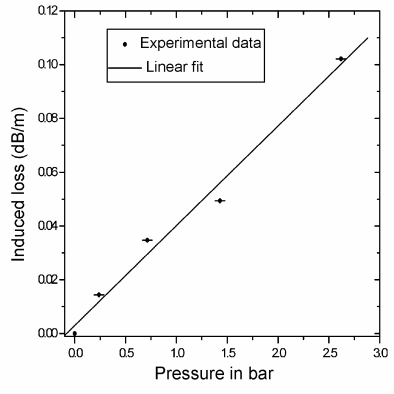

Figure 4. Induced loss in fibre when transverse load is applied.

been tested for single-mode operation, but require detailed characterization.

The wavelength scale features in a holey fibre lead to a strongly wavelength-dependent cladding index. This is responsible for a host of unusual optical properties unique to holey fibres, including endlessly single-mode guidance, whereby only the fundamental mode is guided, regardless of the wavelength. This, together with the flexibility of holey fibre fabrication techniques, which can be used to create extremely large core sizes simply by creating large-scale structures, enable creation of large-mode-area fibres with excellent beam quality.

In this type of fibre the air holes are typically arranged on a hexagonal lattice with a single missing air-hole. However, even larger cores can be created by omitting more than one air-hole from the centre of the fibre. These fibres have applications in high-power beam delivery, where good beam quality, high damage threshold and low nonlinear effects are essential. More complex index structures can also be constructed utilizing arrangements of holes of different sizes in various periodic or non-periodic structures. In addition, highly asymmetric core fibres can be fabricated, thereby creating fibres with high level of birefringence.

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Differential expression of two metallothionein encoding genes during heavy metal stress in the mangrove species, *Avicennia marina* (Forsk.) Vierh.

Industrialization and urban development lead to continuous release of heavy metals into the environment and pose a serious threat to living organisms including human beings[1]. The marine environment in particular is highly polluted, with effluents from industrial sources and urban run-off that contain toxic concentrations of heavy metals such as cadmium, copper, zinc and nickel[2]. Mangroves are plant communities, which form a part of this marine environment and possess great tolerance to high levels of heavy-metal pollution. Mangroves have the capacity to act as a sink or buffer, and remove or immobilize heavy metals[2,3] before they reach the nearby aquatic ecosystems. The mangrove plants have developed various mechanisms like exclusion, chelation, compartmentalization and sequestration for heavy-metal tolerance. In the mangrove plant community, *Avicennia* sp. in particular are considered to be extremely robust to heavy metals and accumulate metals to greater quantities than other mangrove species, before any visible signs of toxicity are evident[4–7]. In a study conducted by Macfarlane[2], *A. marina* was found to be highly tolerant to heavy metals like copper, lead and zinc.

Among all the detoxification mechanisms, chelation by various ligands or proteins like metallothioneins (MTs) or phytochelatins provides greater resistance to the toxic effects of the heavy metals[8]. MTs are low molecular weight, cysteine-rich proteins that bind to heavy metals like Cu, Cd and Zn with greater affinity in a stoichiometric ratio of 7 : 1 for metal and MTs. It has been suggested that the induction of the MT gene is observed primarily at the transcription level[9]. Therefore, studies on the regulation of genes encoding MT in response to heavy-metal stress would be better understood by monitoring the mRNA accumulation of these genes during heavy-metal stress conditions. The present study reports the isolation and differential expression of two MT cDNAs in response to heavy-metal stress in *A. marina* (Forsk.) Vierh.

The cDNA library was constructed from mRNA isolated from leaf tissue of salt-stressed *A. marina* seedlings[10]. Two MT cDNA clones were isolated from the cDNA library through partial sequencing of ESTs. Both the cDNA clones were se-

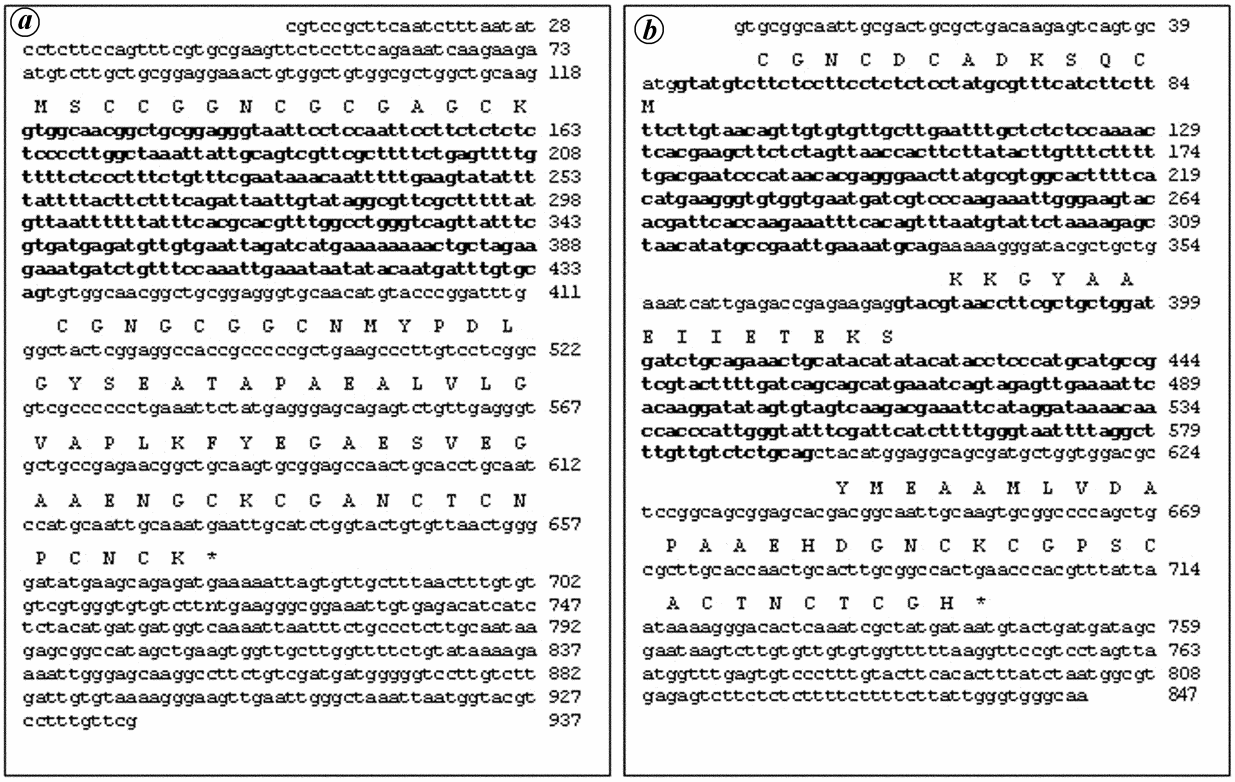

Figure 1. Genomic sequences of *AmMT2* (***a***) and *AmMT3* (***b***). Shown are the nucleotide and deduced amino acid sequences of both genes. Non-coding intronic sequences that start and end with GT and AG are shown in bold letters.

quenced full length. Full-length genomic sequences of the type II and type III MT cDNAs were amplified from *A. marina* genomic DNA using primers designed for specific regions in the 5′ and 3′ UTRs of two cDNAs *(AmMT2* 5′ UtrF: CGT CCGCTTCAATCTTTAATATC; 3′UtrR: CATCGACAGAAGGCCTTGCT and *AmMT3* 5 UtrF: TGCGGCAACTGCG ACTGTG; 3′ UtrR: GAGAGAAGACT CTCACGCCATT). PCR amplification conditions were as follows: Pre-amplification denaturation at 94°C for 4 min; cycling conditions (30 cycles) at 94°C for 30 s, 59°C for 45 s, 72°C for 1 min and final amplification at 72°C for 7 min. The products were PCR purified and cloned into pTZ57R/T vector (Fermentas), and sequenced using automated sequencer (ABI 310).

Sixty-day-old *A. marina* seedlings were acclimatized in half-strength MS liquid medium for 24 h and exposed to different concentrations of copper and cadmium: 50 μM, 200 μM, 500 μM and 1 mM of $CuSO_4$ and $CdSO_4$. Visible signs of heavy-metal stress like plant drooping and leaf curling could be seen only at 1 mM concentration. Therefore, this concentration was chosen to study the expression of the two genes under heavy-metal stress. Leaves from *A. marina* seedlings were harvested at 0 h (control), 12, 24, 48 and 72 h after heavy-metal treatment. Total RNA from the harvested leaves was isolated using the GITC method[11]. 15 μg of total RNA was resolved on a 1.3% agarose gel containing 6% (v/v) formaldehyde and transferred to nylon Hybond–N + (Amersham Inc, USA) membrane by capillary transfer[12]. The membrane was UV cross-linked according to the manufacturer's instructions (Hoefer, Germany). The RNA was probed with ^{32}P-labelled 3′ UTR regions amplified from *AmMT2* and *AmMT3,* exposed and detected on an X-ray film. The 3′ UTRs of both the cDNAs were amplified using primers designed for *AmMT2* 3′ UtrF: CATCTGGTACTGTGTTAACTG; 3′ UtrR: CATCGACAGAAGGCCTTGCT and *AmMT3* 3′ UtrF: AATAAAAGGG ACACTCAAATCG; 3′ UtrR: GAGAA GACTCTCACGCCATT. All the treatments and hybridizations were performed in duplicates.

Sequence analysis of 651 bp long *AmMT2* cDNA (GenBank accession no. AF333385) showed the presence of 239 bp open reading frame encoding 79 amino acids. The genomic sequence of *AmMT2* amplified from the genomic DNA was 936 bp long, containing only one intron of 317 bp (Figure 1 *a*). The position of the first intron in *AmMT2* was similar and conserved in comparison to the introns present in different types of MT genes in rice[13]. However, in rice there are two introns in all the three isoforms of type 2 MT, OsMT-I-2a, b, c, in comparison to one intron in *AmMT2*. The deduced amino acid sequence of *AmMT2* possessed CC, CGC, CKC and CGGC motifs at the N-terminal end and CKC, CTC and CNC motifs at the C-terminal end. This comprised of cysteines clustered in the order CCXXXCXCXX XCXCXXXCXXC at the amino-terminus and CXCXXXCXCXXCXC at the carboxyl terminus, typical of plant type-2 MTs[14].

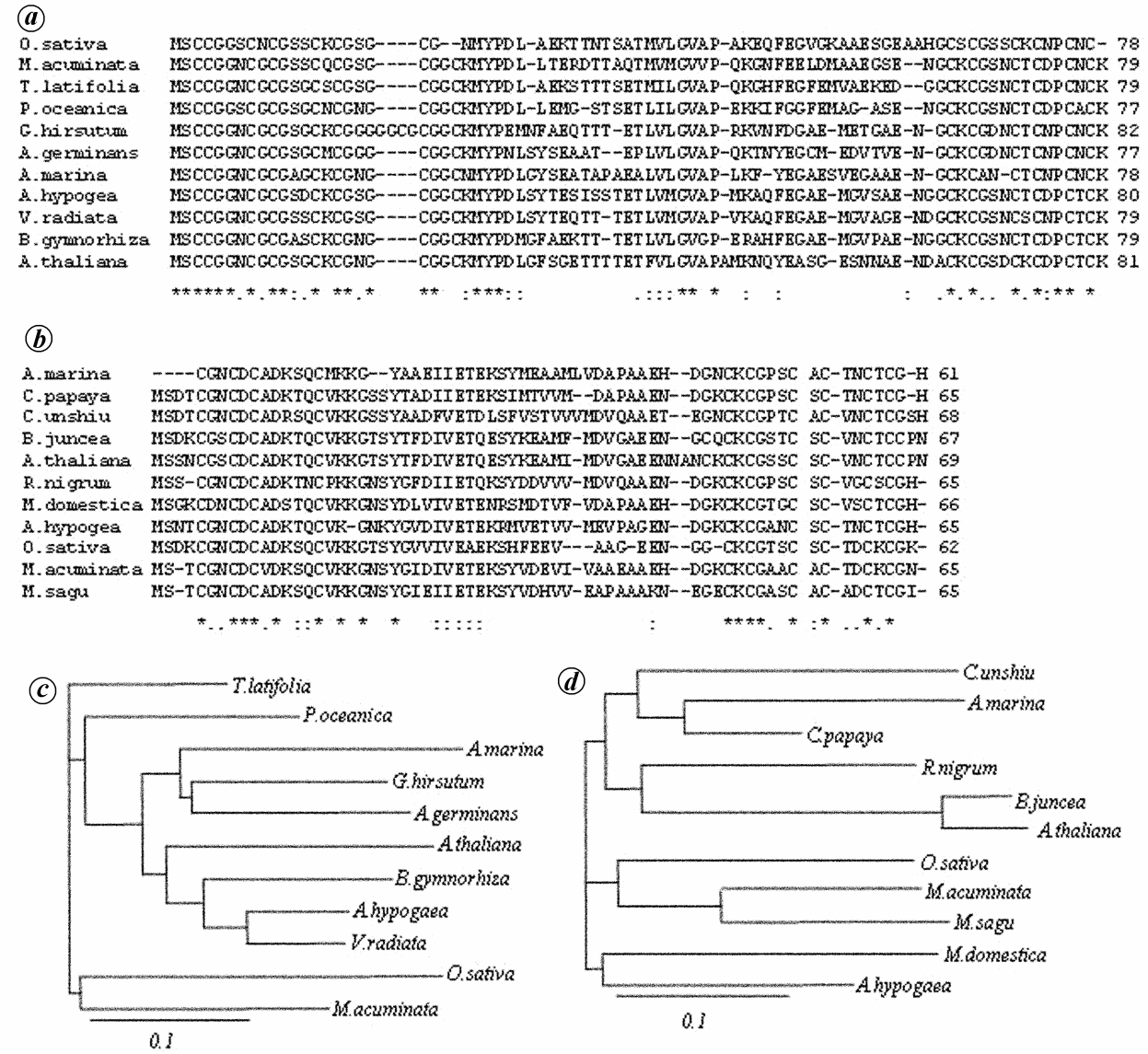

Figure 2. Multiple sequence alignment of *AmMT2* and *AmMT3*-deduced amino acid sequences to (***a***) type-2 plant MTs (*G. hirsutum* AAV74186, *O. sativa* ABR25954, *A. germinans* AAY59706, *B. gymnorhiza* ABF50984, *A. thaliana* NP_187550, *P. oceanica* CAF31410, *A. hypogaea* AAZ20290, *M. acuminata* AAG44757, *V. radiata* BAD18375, *T. latifolia* AAK28022) and (***b***) type-3 MTs (*C. papaya* CAA69624, *R. nigrum* CAA07565, *C. unshiu* AAK08209, *B. juncea* BAB85601, *A. thaliana* NP_566509, *M. acuminate* AAG44759, *M. domestica* AAC23698, *A. hypogaea* AAO92264, *O. sativa* NP_913175, *M. sagu* ABA43635). The CLUSTALW program aligned the amino acid sequences. Phylogram analysis of $AmMT_2$ (***c***) and $AmMT_3$ (***d***) proteins are shown.

The *AmMT3* EST isolated from the library was a partial gene sequence lacking four amino acids at the N-terminus. Genomic sequence showed the presence of two introns of size 293 and 218 bp (Figure 1 *b*), which are similar to that of type-3 MT, OsMT-I-3a of rice[13]. The amino acid sequence predicted for *AmMT3* consisted of conserved CGNCDC residues at the N-terminal end and CKC, CAC and CTC motifs at the C-terminal end. This is typical of the plant type-3 MTs that possess the characteristic CXXCXC residues at the N-terminal domain and CXC motifs in the C-terminal domain[9]. This arrangement of cysteine residues separated by a non-cysteine spacer revealed that *AmMT2* and *AmMT3* belonged to class-1 MTs[15]. Alignment of deduced amino acid sequences of type-2 and type-3 MTs from *A. marina* respectively, with other plant MTs using CLUSTALW shows that the N- and C-terminal cysteine-rich domains are highly conserved across different plant species (Figure 2 *a* and *b*).

Analysis of the expression pattern of the two genes in root, shoot and leaf revealed that both the genes had highly expressed in the leaves compared to shoots and roots (Figure 3 *a*). This suggests a more specific role for these two MTs in the leaves than in the roots. Under heavy-metal stress, no alteration in the mRNA accumulation pattern of *AmMT2* in *A. marina* was observed in the leaves

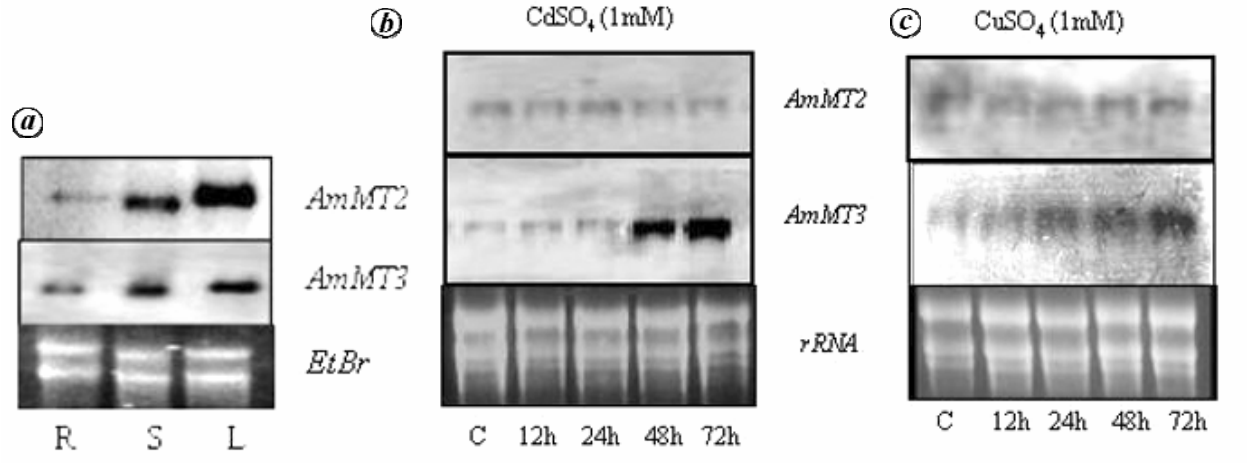

Figure 3. Northern analysis of *AmMT2* and *AmMT3* gene expression. ***a***, Tissue-specific expression of *AmMT2* and *AmMT3* in root (R), shoot (S) and leaf (L). Quantity One software was used to obtain [CNT∗mm^2] data for radiolabelled RNA bands which is as follows: *AmMT2*: R: 358; S: 477; L: 1082 and *AmMT3*: R: 135; S: 175; L: 251. ***b***, One-month-old *A. marina* seedlings were stressed with 1 mM $CdSO_4$ and 1 mM $CuSO_4$. Total RNA was isolated from leaf tissues harvested at 0 (control), 12, 24, 48 and 72 h and probed with α-^{32}P labelled 3′ UTR's of *AmMT2* and *AmMT3* cDNA. Quantity One software was used to obtain [CNT∗mm^2] data for radiolabelled RNA bands which is as follows: *AmMT2* – $CdSO_4$ stress: time points 0–72 h: 140, 134, 146, 153, 146 for each lane respectively. $CuSO_4$ stress: time points 0–72 h: 174, 151, 145, 159, 168 for each lane respectively. *AmMT3:* $CdSO_4$ stress: time points 0–72 h: 496, 535, 627, 1542, 2109 for each lane respectively. $CuSO_4$ stress: time points 0–72 h: 434.63, 692.9, 727, 815.07, 1154.03 for each lane respectively.

even after 72 h of exposure to 1 mM $CuSO_4$ and $CdSO_4$, compared to the untreated control. Whereas mRNA of *AmMT3* gene was induced in response to both copper (threefold increase) and cadmium (fourfold increase) stress and attained maximum expression level at 72 h (Figure 3 *b*). This revealed that the two MT genes in *A. marina* are differentially regulated during heavy-metal stress. Our results are in agreement with those of a previous report in *Arabidopsis*, where type-3 MT (*AtMt3*) was more strongly induced by cadmium than type-2 MT (*AtMt2*)[16]. Multiple sequence alignment of *AmMT2* and *AmMT3* with *AtMt2* and *AtMt3* respectively, revealed identical arrangement of cysteine residues between the two sequences (data not shown). In *A. thaliana* induction of both type-2 and type-3 MT transcripts was observed, with induction being stronger in *AtMt3* than in *AtMt2*. However, in *A. marina*, no induction was observed in *AmMT2* and only *AmMT3* was induced during heavy-metal stress. Results from the present study and those of the previous studies suggest that both type-2 and type-3 MTs[16] are differentially regulated during heavy metal stress conditions, with type-3 showing more specific response during heavy metal stress in plants.

Type-3 MT mRNA in *A. marina* is induced in the presence of cadmium and copper, both belonging to different classes of metals. Metals like cadmium, zinc, nickel and lead have low redox potential and hence cannot participate in biological redox reactions[1]. These metals cannot produce reactive oxygen species directly by autooxidation and Fenton reaction like other transition metals (Cu, Fe, Hg, Ag), but block essential functional groups in biomolecules and displace essential metal ions from organic molecules. It has been suggested that plant MTs have a high affinity for copper and are induced less by cadmium[17]. Further, MTs prevent oxidative stress in plants by sequestering heavy metals like copper, which generate free radicals by participating in the Fenton reaction[17]. Therefore, the response of type-3 MT in *A. marina* to heavy metal stress is proportional to increased heavy-metal concentration, irrespective of the nature of the heavy metal and its participation in generating oxidative stress. The induction of type-3 MT suggests a more specific role for it during heavy-metal stress in *A. marina*.

It has been reported that *A. marina* has the capacity to excrete heavy metals such as zinc through its glandular trichomes in the leaves[18]. Halophytes other than *A. marina* also excrete heavy metals through salt excretory glands[19]. For example, the halophytic shrub, *Tamarix aphylla* was found to excrete toxic heavy metals like cadmium and lithium through its salt glands[20]. Such physical adaptations in these plants could play a key role in heavy metal tolerance, in addition to the heavy-metal sequestering proteins such as MTs and phytochelatins. The possibility of MTs acting in conjunction with the physical adaptations to ameliorate heavy-metal stress could exist in *A. marina* like in *Vicia faba*, in which MT expression is confined predominantly to the trichomes in the leaves acting as a sink to remove heavy metals from the plant[21]. This is also supported by the presence of higher expression of MT 2 and 3 in the leaves than in the other tissues in *A. marina* (Figure 3 *a*). However, more studies in this regard will have to be carried out to establish the presence of such interactions between the roles of MT and physical adaptations in response to heavy-metal stress in mangroves. Studies on the effect of heavy metal on mangrove plants have revealed a biochemical response to heavy-metal stress. An increase in the peroxidase activity concomitant with the heavy-metal stress has been observed in *A. marina*[3]. The present study reports on MT gene expression under heavy-metal stress in *A. marina*, a mangrove species growing in a harsh climate and under edaphic conditions. Further studies are underway to identify, isolate and study other MT

and MT-like proteins from *A. marina* that would help understand the role of MTs in heavy metal tolerant plants.

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Rapid determination of iodine in soils using inductively coupled plasma mass spectrometry

Iodine plays a pivotal role in many of the geological, chemical and biological processes because of its litho-, bio-, atmo-, hydro- and chalcophilic nature. Speciation of iodine in the form of inorganic iodine (iodides and iodates), molecular iodine and organically bound iodine provides its chemical signature in diverse forms. The secondary environment (soil) has high iodine content compared to the primary environment (parent rocks) from which it is derived as a result of weathering. On an average, the igneous rocks contain an average of 0.25 mg per kg of iodine, the sedimentary rocks, including shales, have 2.3 mg per kg iodine and the metamorphic rocks have 0.81 mg per kg iodine[1,2]. Organic matter is the major concentrator of iodine in sedimentary basins. Hence increase in iodine concentration is considered as an anomaly, indicating the presence of hydrocarbons in an area[3]. Oil-field brines, especially those of the Anadarko basin, Oklahoma, have been found to contain more than 500 ppm of iodine and the origin is considered to be organic[4].

Iodine was also known to be the first element essential to humans and is a major constituent of the thyroid hormone, the deficiency of which leads to endemic goitre[5]. The WHO has recommended a daily intake of 100–150 μg iodine in the diet. Similarly, for the prevention of health hazards due to radioactive forms of iodine (I^{129} and I^{131}), potential sorbents of iodine like organic matter are under study[6]. Iodine plays an important role in biogeochemical cycles of nature and the main source of iodine supply is from the oceans in the form of I_2 and CH_3I. Iodine is transferred back to the land surface through wet and dry precipitation[7].

To study all these natural and anthropogenic phenomena involving iodine in any of its bio-geochemical forms on land surface, a suitable method is required for the extraction and accurate determination of iodine in different kind of soils. Different analytical techniques have been developed to extract and measure iodine concentration from the soil. Pyrohydrolysis sample preparation followed by analysis using inductively coupled plasma mass spectrometry (ICP–MS)[8], hexane extraction of the iodo-derivatized sample and subsequent analysis by gas chromatography–mass spectrometry (GC–MS)[9], spectrophotometric determinations based on Sandell–Kolthoff reaction[10], isotope dilution mass spectrometric determination (IDMS)[11], neutron activation analysis (NAA)[12], accelerator mass spectrometry (AMS)[13], etc. have been described earlier. All these methods have their own advantages and disadvantages. Extraction of iodine using tetra methyl ammonium iodide (TMAH) as a chemical modifier in iodine determination is now being widely utilized[8,14–17]. However, the sample preparation requires specialized combustion apparatus and trapping systems for iodine. For plant samples and biological materials, halogen extraction using TMAH under mild conditions has proved to be effective[17]. For the determination of iodine in soil samples by ICP–MS, we have adopted and modified this procedure[17]. The iodine extraction efficiency in soils has been improved by optimization of reaction conditions and reagents, the details of which are presented here.

Ten per cent TMAH solution was prepared by diluting 25% TMAH solution (in water from Qualigens Fine Chemicals, India). Internal standard, antimony, of 250 ppb concentration, was prepared