Review

Iodine Fractions in Soil and Their Determination

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Abstract: Iodine is an essential micronutrient for most living beings, including humans. Despite efforts through various iodine deficiency disorders elimination programs, such as salt iodization, this problem still persists. Sub-clinical deficiencies have often an endemic character, and they are characteristic to certain areas, where iodine is low or inaccessible in the geochemical environment. Thus, the monitoring of iodine speciation and fractionation—especially in soil, drinking water, and food—is needed. Therefore, the aim of this work is to review the iodine distribution in most common soil fractions which relate to its bioavailability; and briefly summarize the available methods for their identification in the environmental matrices as well.

Keywords: iodine; soil; iodine fractionation; sequential extraction; iodine determination

1. Introduction

Iodine is an essential trace element for both humans and animals, and a necessary component for the synthesis of thyroid growth hormones. Therefore, the World Health Organization (WHO) recommends a daily iodine dose for an adult of approximately 150 μg. Insufficient or extensive iodine intake is attributed to various diseases, such as goiter, and hypo- and hyperthyroidism. Iodine deficiency can also cause miscarriages, endemic cretinism, and other diseases [1]. The occurrence of iodine deficiency disorders is often linked to locations where the iodine is deficient in the geochemical environment.

The average iodine content in topsoil of inland areas is estimated at ~2.5 mg·kg^{-1}, comprising of 10% iodine in water-soluble form. Since soils usually contain more iodine than the parent rocks, the atmospheric deposition of marine aerosols, dust, and flying ash particles from volcanic activity is considered a major source of iodine in topsoil [2,3].

Some studies have shown a significant correlation between iodine concentrations in soil and associated vegetation. However, it may not necessarily imply a direct soil-to-plant transfer [4]. Dead plants' biomass with accumulated iodine of atmospheric origin has been suggested as a potential source of iodine in soil [5]. Additionally, Roulier et al. [6] assumed that iodine soil concentration is influenced by litterfall, indicating humus represents its temporary storage. Roulier et al. [7] determined iodine content in various forest compartment. The main iodine reservoir is hummus with more than 1800 μg·kg^{-1} iodine. Trees contain 161 μg·kg^{-1} iodine from which green leaves and stembark accumulate the most of iodine. Lichens and mosses are also considered great reservoirs.

Rainfall can cause slow migration of iodine downwards the soil profile [8]. Still, since iodine shows high upwards mobility due to capillary forces, the water level fluctuations may affect its redistribution in soil layers [9].

Besides its essentiality, the reason why the environmental mobility of iodine is a scientific concern is the potential risks of exposure to radioactive iodine isotopes that are considered dangerous for human health. Shaw et al. [10] reported that the full isotopic equilibrium between the native ^{127}I and ^{129}I has not been achieved even after 29 years

in the Chernobyl exclusion zone, and both isotopes are predominantly associated with extractable soil organic matter.

2. Biogeochemical Cycling of Iodine

The geochemistry of iodine is extensively presented in works of Fuge and Johnson [11,12] who highlighted that the iodine transformation in the environment is a dynamic cycle between "living and non-living" and "organic and inorganic" (Figure 1). There, iodine passes from the main environmental matrices into plants, and then into animals and humans, where it is part of various organic compounds and hormones [13].
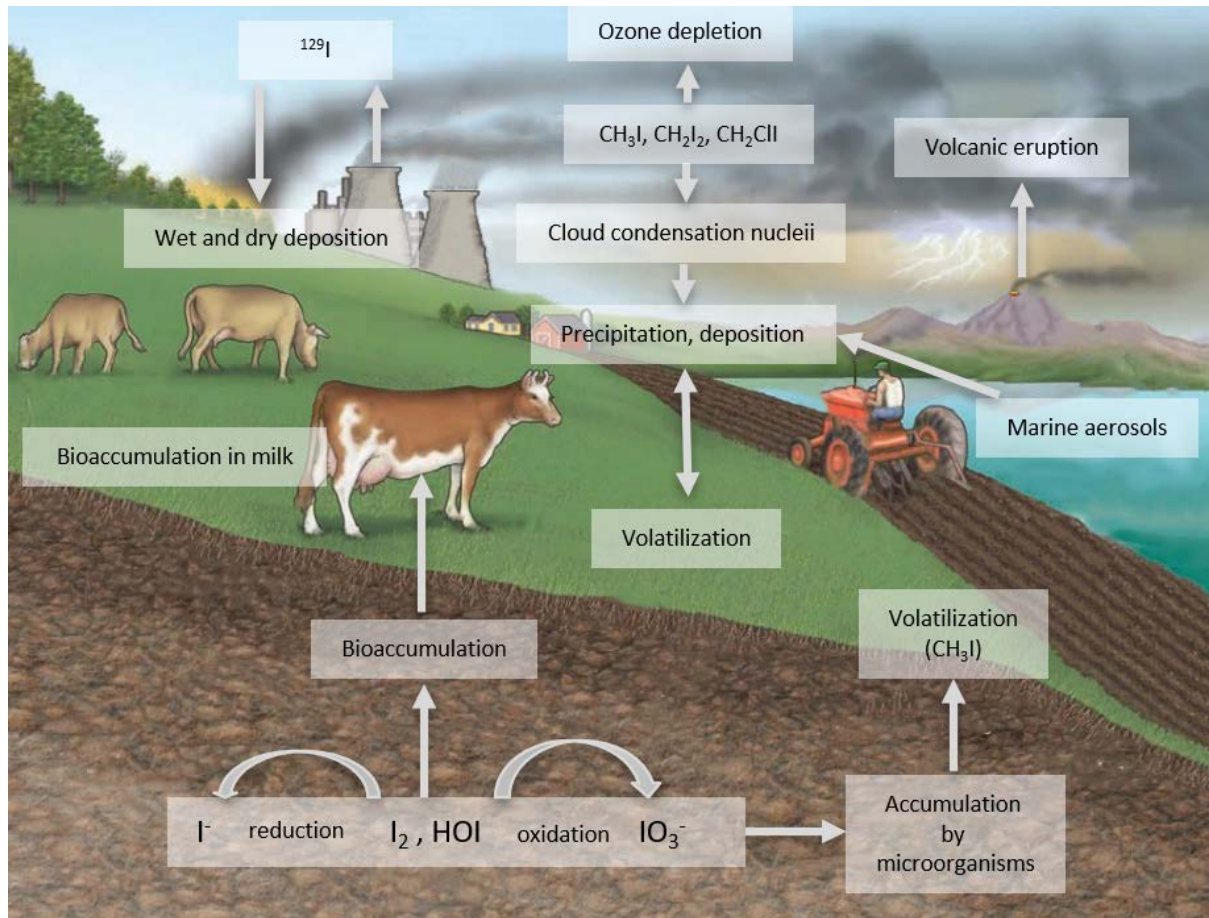

Figure 1. Biogeochemical cycle of iodine.

In water, it most often occurs as iodide and iodate. However, in river and lake sediments, a large proportion of iodine is bound to organic matter. In the atmosphere, both organic (e.g., methyl-iodide) and inorganic (HIO and I_2) iodine gases and vapors can be found [14], which are also involved in the destruction of the ozone layer [15]. In soil, its predominant form is an anion of iodide or iodate [16].

Seawater is considered the iodine's main reservoir. It was calculated that 4.5×10^5 tons of iodine are annually lost from ocean water with 2.4×10^5 tons evaporated in the form of methyl-iodide. The iodine backflow through freshwater streams is estimated to 1.1×10^5 tons per year [17]. From seawater, iodine enters the soil by atmospheric deposition. Another contributor to soil iodine is the parental rock weathering. The main source of iodine in terrestrial systems and forest soils is dry deposition [18]. However Söderlund et al. [19] found that iodine is sorbed weakly in boreal forest soils.

From soil, it accumulates in plants and microorganisms, which serve as food for animals, and thus supplies the food chain with iodine. Through animal and plant products,

iodine eventually reaches humans [13,20]. After the death of plant and animal bodies, the bioaccumulated iodine is returned to the soil, from where it can be released by evaporation or leaching, and finally returns to the ocean. Plants and microorganisms actively contribute to iodine mobilization and release from soil environment via various mechanisms, as illustrated in Figure 2.
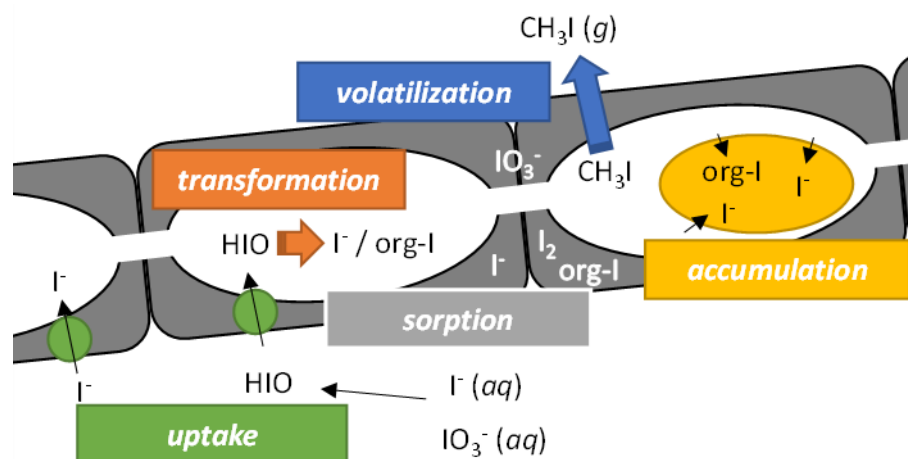

Figure 2. Possible interactions between iodine and microscopic filamentous fungi.

Ban-nai et al. [21] identified eleven filamentous fungal strains, including strains of basidiomycetes, ascomycetes, and fungi imperfecti, which were capable of emitting the methyl-iodide. Ban-nai et al. [21] also suggested that fungi have comparable volatilization potential to soil bacteria. Redeker et al. [22] indicated that the daily iodide emission rate via volatile methyl-iodide significantly varied among species and morphotypes of ectomycorrhizal fungi within the range from 0.15 to 30 $\mu g \cdot g^{-1}$ of fungal dry biomass at 0.2 $mmol \cdot L^{-1}$ iodide concentration. Aqueous and gaseous sampling revealed that methyl-iodide is only produced in presence of active organisms, since, reportedly, no iodide was transformed in sediments under abiotic conditions [23].

Redeker et al. [24,25] concluded that the rice plant alone is capable of producing methyl-iodide, especially during the early seasons, when the plant is rapidly increasing in biomass and height, with minor role of emissions from water or soil column. They also suggested that the methyl-iodide production is an iodide detoxification mechanism, because, compared to other halo-ions, high emission rate of methyl-iodide by rice implies presence of methyl transferase with high affinity to iodide and high catalytic efficiency. Furthermore, it was reported that iodide is the preferred substrate for methylation compared to other halogens [26]. In their early work, Muramatsu and Yoshida [27] suggested that the methyl-iodide is produced in flooded soil with rice plants under anaerobic conditions with possible contribution of both microbial and root activity. They assume that methyl-iodide is emitted into atmosphere through plant shoots, similarly to mechanism of methane release from rice-fields [28]. However, Amachi et al. [29] found out that anaerobic conditions did not facilitate production of methyl-iodide, even when various electron donors were introduced into soil paddies during incubation to stimulate activity of anaerobic soil microorganisms. Furthermore, they suggested that biovolatilization of iodine is not confined to any bacterial group and, thus, biotransformation of iodide into volatile methyl-iodide should be considered a common phenomenon.

The enzyme responsible for iodide methylation in cabbage leaves (*Brassica oleracea* L.) was identified as halide/bisulfide methyltransferase with pH optimum for iodide methylation in the range of 5.5–7. This enzyme catalyzes the S-adenosyl-L-methionine-dependent methylation of the halides (iodide, bromide, and chloride) to monohalogenated methanes [30]. Further assays indicated that the herbaceous plant species from 44 families and 33 orders were capable of producing readily detectable amounts of methyl-iodide [31], thus, it seems that iodide methylation is also widespread among higher plants.

3. Iodine in Soils and Their Fractions

In soil, iodine is found in association with soil particles and, thus, based on particles' general chemical properties, it may be separated into various fractions. The main identifiable fractions of iodine in soil are: (1) water soluble iodine, (2) exchangeable iodine, (3) iodine bound to carbonates, (4) iodine bound to reducible metal oxides, (5) iodine bound to soil organic matter, and (6) residue.

Some fractions are richer in iodine, especially those which provide abundant sorption sites for iodine anion in the given environment. The factors that affect the mechanism and nature of iodine sorption in soil are very complex, and include (1) chemical properties and form of occurrence (speciation) of iodine; (2) the soil or soil components' chemical and physical properties, e.g., ion exchange capacity and surface net-charge; and (3) specific environmental conditions under which the sorption process is occurring, e.g., redox fluctuations and changes in biological activity [32].

3.1. Water-Extractable Fraction

Water extractable iodine is considered the most mobile and readily bioavailable fraction for bioaccumulation by plants. Thus, this fraction enters the food chain, and its abundance has a key role when evaluating soil iodine content to predict the prevalence of iodine deficiency disorders.

It was reported by several authors that the amount of water-soluble iodine does not exceed 10% of the total soil iodine [16,33,34], and it can be lesser than 0.6% in some cases [35] However, increasing the temperature of water allows labile forms of organic matter to decompose. Thus, the hot water can extract up to 43% of total iodine in the soil [35].

Redox soil properties also affect the abundance of water extractable fraction [36]. Generally, the reduction of iodate leads to the formation of elemental iodine and chemically unstable hypoiodous acid, which are then immobilized in the soil by binding to soil particles, most likely to soil organic matter [37]. This process effectively decreases the water-extractable fraction of iodine.

In wet or flooded soils, where reductive conditions prevail in the surface layers, the soil iodine content increases with the increasing depth and redox potential. Therefore it seems that in anoxic conditions, iodine bound to soil particles can be reduced and released to the soil solution [38]. This is an important factor that can cause iodine leaching from soils after rainfall, or in areas where periodic floods occur. This phenomenon is applicable also to paddy fields. There, the microbial and root activity in the rhizosphere can also significantly contribute to iodine mobilization [39].

3.2. Exchangeable Fraction

Besides water-extractable iodine, the exchangeable fraction is also considered easily mobilizable or bioavailable fraction of iodine in soil. These two fractions are hardly distinguishable in water-saturated soils at flooded conditions that can change the redox state of iodine. There, the iodine species are bound to exchangeable sorption sites weakly and, thus, can be easily mobilized by water.

Since the iodine is in the environment present as an anion (mostly I^- or IO_3^-), the anion exchange capacity (AEC) of soil seems to control its immobilization in exchangeable fraction. Positive charged sites in the soil mostly originate from the rupture of planes of the structural units, or of iron and aluminium oxides that cover some crystalline clays or occupy an interlayer position in lattice layers. The AEC determines the presence of exposed cations on the edges of the crystal structure, the ionization of groups in soil colloids, or the isomorphic exchange in iron oxides. The positive charges induce adsorption of anions [40], including iodine. However, the AEC is rarely studied, mainly because the influence of anions on electrical charge characteristics of the main soil types in temperate zones is weaker than cations.

Generally, the AEC increases with the decreasing of soil pH. Therefore, the soil pH also has a key role in iodine retention since it is linked to AEC of the soil via the point of zero charge (PZC) of solid surfaces, which is generally described as the pH at which the net charge of the total particle surface is zero [41]. Inorganic forms of iodine can be trapped in the soil environment on positively charged surfaces of iron, aluminum, and manganese (oxo)hydroxides, and clay minerals more easily when the pH of the soil solution is below 6 [42]. Under acidic conditions, the surface of these oxides and hydroxides is positively charged, thus, the mobility of inorganic iodine species mostly decreases with decreasing soil pH. This is because iodide and iodate are already completely dissociated at common soil pH, so they can be electrostatically drawn to various positively charged surfaces [43,44]. The optimum pH for iodine binding is estimated to be 3.7 [45]. Still, iodine sorption was also observed under slightly alkaline conditions [46,47].

3.3. Carbonate-Bound Fraction

Iodine bound to carbonates is a less abundant iodine fraction which is also considered as easily mobilizable and potentially bioavailable. It has been reported that there is a significant correlation between soil carbonates and iodine content [34]. However, it is most likely linked to soil pH, since the soils rich in carbonates are generally alkaline. It was reported that soils occurring over limestone are generally enriched in iodine compared to those over neighboring non-limestone parented soils [12,48]. Sorption onto calcium compounds has been also reported [49]. Still, the mechanism of iodine retention in carbonates is not completely understood.

3.4. Metal Oxides-Bound Fraction

There is a close relationship between iodine concentration and the iron, aluminium, and manganese content in the soil. According to available studies, iodine bound to metal oxides is between 15–30% from total soil iodine. Its content depends on soil type, and primarily on quantity and quality of organic substances. In soils poor in organic matter, it is the most abundant iodine fraction [13,33,34].

The iodine sorption onto the metal oxides takes place predominantly under oxidative conditions. Under reducing conditions, the dissolution of metal sesquioxides causes the release of the adsorbed iodine into the soil solution [38,44]. Some authors reported that iodate can create chemical bonds with iron oxides by replacing hydroxyl groups on their surfaces [44,46]. Mineral phases, such as hematite (Fe_2O_3) and several aluminium oxides, can directly adsorb iodide from aqueous solutions at pH up to 8 [50].

During the adsorption process, some minerals are capable of changing the redox state of the environment, thereby influencing the speciation and sorption of iodine. For example, in the presence of pyrite (FeS_2), iodate (which is not likely to bind) is reduced to I_2, which is capable of forming the sorption complexes with pyrite's structural components [51]. A similar example is the oxidation of iodide and its subsequent sorption onto MnO_2 surfaces.

The oxidation of iodide to iodate takes place in two steps. Firstly, iodide is oxidized to elemental iodine or hypoiodous acid. These are very reactive species which are subsequently oxidized to iodate (or bound to aromatic moieties of organic matter). The oxidative transformation of iodide to iodate can be catalyzed in the presence of oxidative agents. In natural acidic environments, common oxidizing agents are manganese oxides (e.g., δ-MnO_2). The newly formed iodate is then bound to the manganese oxide surfaces. The oxidation rate of iodide increases with the concentration of manganese oxides, and it decreases with increasing pH of the medium [52]. Positive correlation between the content of metal oxides had been widely reported, some authors even consider aqueous iodine removal via adsorption onto iron ferrous and ferric oxides as a remedial pathway for the vadose zone and groundwater contamination with radioiodine [53].

3.5. Organic Matter-Bound Fraction

It is a common phenomenon that the iodine content decreases with a decreasing amount of organic matter in a vertical soil profile [54,55]. Generally, the iodine bound to organic matter is the most abundant iodine fraction in soils with reported up to 60% prevalence from total soil iodine. All significant soil organic matter components—including humic and fulvic acids, and humin—contribute to the iodine retention in soils [13,33,34,56].

Iodine added to soil appears to be incorporated into solid phase humus via the formation of intermediates such as HOI and I_2. Abiotic reduction of iodate or oxidation of iodide by solid or dissolved organic matter (DOM) are likely to be the main mechanisms [57]. The oxidation of iodide by bacterial and fungal laccases also supports this mechanism in soils [58,59].

Iodine sorption onto organic matter is initiated through the slow diffusion of iodine into micropores and cavities in the structure of organic matter [60]. There, since soil organic matter can act both as electron donor and acceptor in redox transformations, an iodide oxidation or reduction of iodate to I_2 or hypoiodous acid occur. These are extremely reactive intermediates towards organic compounds. The reaction of humic substances with the elemental iodine forms various organoiodine compounds and, thus, limits its oxidation to iodate in soil environment [52,61]. Therefore, the sorption mechanism of iodine comprises of its oxidation/reduction by humic substances to the reactive species of I_2 and HOI, and its subsequent chemisorption to organic matter by the mechanism of electrophilic substitution, which primarily takes place on the aromatic ring of humic components [62]. The iodination of organic matter includes, besides the covalent modification of aromatic-type rings by I_2/HOI, iodine incorporation into newly formed benzoquinone species arising from the oxidation of phenolic species [63].

4. Methods for Iodine Fractionation

To evaluate the mobility and phytotoxicity of iodine in soil, it is necessary to identify and characterize its present chemical forms or their fractions. There are several methods for fractionating total iodine in soil. One of these methods is sequential extraction, by means of which individual fractions of the element are successively obtained from the soil matrix using various extracting agents [64].

The majority of iodine is generally obtained from the organic fraction, but a significant proportion of the total iodine is also bound in oxides or leachable by water [13,33,65]. By suitable extraction procedures, it is even possible to determine the amount of iodine bound to humic acids and fulvic acids in the organically bound iodine fraction. This is also illustrated in Figure 3, which shows the determinable iodine fractions in soil and suitable extractants for their extraction. Similar extraction methods had been used to fractionate other essential elements which have anionic character such as selenium [66,67]. The relative distribution of iodine among these fractions acquired by these methods from various studies is presented in Table 1.

Table 1. Distribution of iodine in each soil fraction.

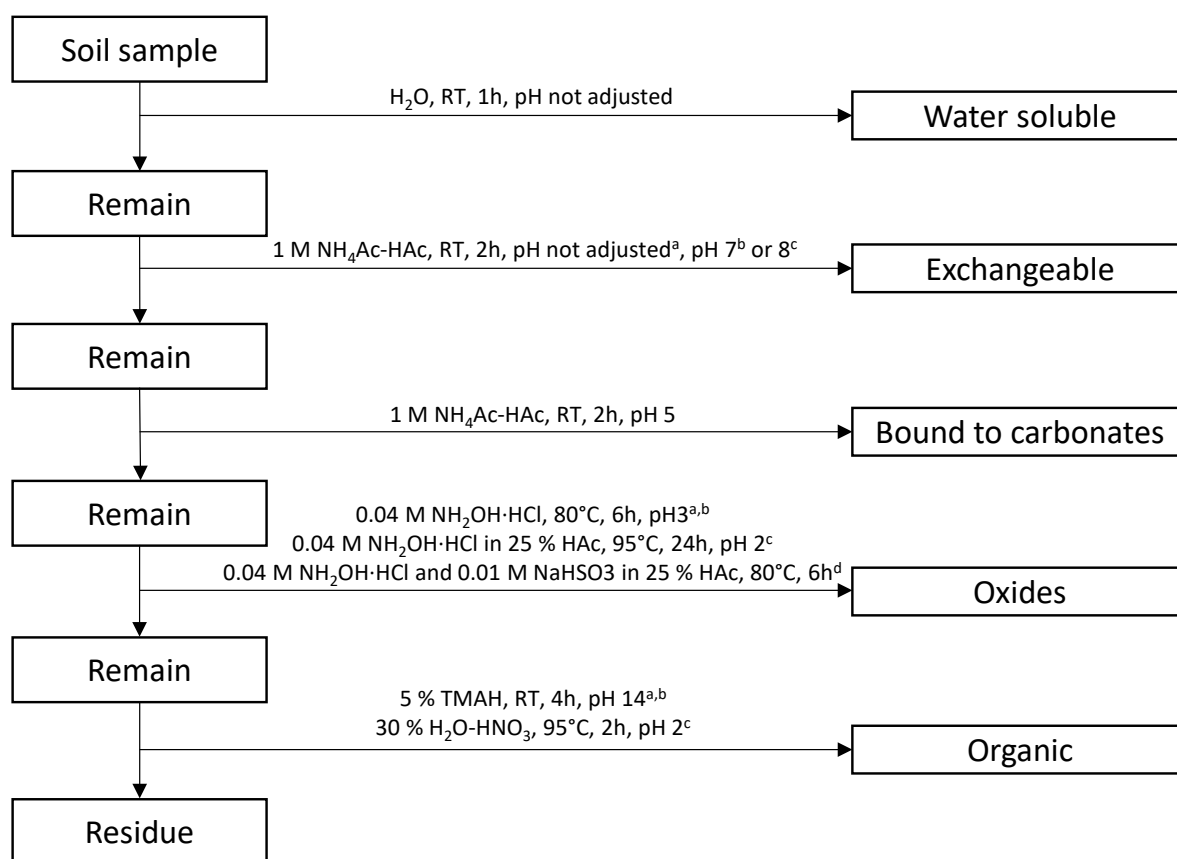| Fraction	Hou et al. [68]	Hansen et al. [33]	Duborská et al. [34]
Water soluble	13%	5–7%	2–7%
Exchangeable	8%	4–17%	0–4%
Bound to carbonates	4%	4–5%	1–8%
Bound to oxides	31%	16–18%	24–85%
Bound to humic acids		38–40%	1–39%
Bound to fulvic acids	39%	11–17%	1–12%
Residual	7%	not determined	2–26%

Figure 3. Extracting agents and conditions used for iodine fractionation from soil in selected studies. RT—room temperature; TMAH—tetramethylammoniumhydroxide, [a] [33], [b] [34], [c] [68], [d] [65].

To evaluate iodine content from the residue, the total extraction methods or decomposition of the sample is usually applied. Those are the same methods which are used prior total iodine determination in environmental samples. Destructive methods include alkaline melting with KOH or K_2CO_3. The mixture is often supplemented with oxidizing additives, such as KNO_3 and $KClO_3$. Melting takes place in a muffle furnace at a temperature of 420–650 °C in thermally resistant porcelain containers [69]. Alkaline dry ashing is also used, which is performed at 800 °C with the addition of NaOH and $Na_2S_2O_5$ [70]. For determination in biomass, pyrolytic decomposition at 900 °C has been used [71].

Pressurized microwave assisted digestion is usually used with HNO_3 and H_2O_2 [72], or a mixture of HNO_3 and $HClO_3$ [73]. However, with this method iodine losses were observed during the decomposition. It was due to the formation of iodine volatile derivatives in an acidic environment; thus, an alkaline environment is more suitable.

Tetramethylammonium hydroxide (TMAH) is most often used to non-destructively extract total iodine from solid phases. Single extraction of iodine with TMAH is most efficient at 70 °C using its 5% (*w/v*) solution [34,74–76] or at 90 °C [77] while mixing samples in closed glass or plastic containers for at least 4 h. Extraction with TMAH at 80 °C was also applied to extract iodine from biomass and foodstuff [78–80]. During extraction at elevated temperatures, iodine can be sorbed and diffused onto the walls of plastic containers, so their reuse is not recommended [77].

To eliminate volatilization and decomposition of thermally labile iodine species during extraction, TMAH method can also be performed at room temperature. This so-called "cold" extraction can recover up to 96% of the total iodine contained in the soil [74].

The instrumental technique used to determine iodine in the decomposed sample or extract must be verified by certified reference materials. There are various types of certified reference materials (CRM) currently available on the market, including soils, and river

sediments [75]. Hay powder is the most often used CRM from available plant materials, while powdered milk, eggs, and meat are frequently used as a reference material for other biological matrices [77].

Currently, the most widely used method for iodine determination is ICP-MS, which is reliable, and reaches the preferred limit of determination (0.05 µg·L^{-1}). Furthermore, it has a very wide application range [81] with ^{125}Te, ^{126}Te, ^{128}Te, ^{103}Rh, or ^{121}Sb as internal standards [82–84]. In addition to the total iodine content analysis, ICP-MS is also used to determine the ratio of different iodine isotopes, most commonly ^{127}I/^{129}I [85]. Aside from soils, this method is suitable for the determination of iodine in water samples [84] and biomasses [86].

Using ICP-MS to reliable determine iodine, great care must be taken during preparation of solutions of the analyzed samples and calibration of standards. It is most suitable to use alkaline solutions of TMAH, ammonium hydroxide (NH_4OH) or sodium hydroxide (NaOH) in a concentration range of 0.5% to 1% (*w/v*) to stabilize iodine before analysis. Acidic solutions are not suitable, since they can cause iodine to evaporate and affect the sensitivity of analytical instrument. Therefore, direct determination of iodine in acidic solutions and rainwater is not recommended [87]. Among the alkaline additives, the most suitable is the TMAH, which does not affect the sensitivity of ICP-MS and, compared to NaOH and NH_4OH, shows a less significant memory effect of the instrument in the analysis [88].

Inductively coupled plasma optical emission spectrometry in the vacuum ultraviolet region of the spectrum (VUV ICP-OES) is also widely used, but this method has a higher limit of determination (30 µg·L^{-1}) than ICP-MS [89,90].

Non-destructive speciation analysis by X-ray absorption near edge structure spectroscopy (XANES) is also used to determine iodine in soils [91]. Techniques as XANES and EXAFS (Extended X-ray absorption fine structure) provide information on the oxidation state and its chemical surrounding without using extraction procedures [92].

These methods reliably determine the content of iodine bound in mineral phases, but due to the relatively high limit of detection, it is not suitable for the determination of iodine in environmental materials [93].

5. Recent Studies on Iodine Soil Fractionation and Extraction

All aforementioned or slightly modified extraction methods and analytical techniques have been successfully employed in various recent experimental studies examining the dynamics of iodine's transformation and mobility in the soil environment. For example, Ahmad et al. [94] applied a three-stage sequential extraction on soil samples collected at various districts of Gilgit-Baltistan (Pakistan) to assess the status of iodine mobility and (bio)availability in this region. Extractions with 0.01 M KNO_3, 0.016 M KH_2PO_4, and 10% TMAH were used to determine soluble, adsorbed, and organic fractions of iodine, respectively. The iodine in the soluble and adsorbed fractions accounted for lesser than 3% and 4% of the total iodine in soil samples, respectively. Furthermore, the most abundant iodine species in both fractions were organoiodine compounds (up to 98%). The same fractionation method was utilized by Wang et al. [95] to study the radioidine ^{129}I mobility and bioavailability in various soil types, including the samples collected at uncontaminated locations within the Chernobyl Exclusion Zone (Ukraine).

The prevalence of the organoiodine species in soil solutions was reported by Humphrey et al. [96] who studied the dynamics of iodine in soils collected at Nottinghamshire (UK) to critically evaluate the radioiodine mobility. While the 5% TMAH was used to extract total iodine in soil samples prior analysis using the triple quadrupole ICP-MS (ICP-QQQ), no sequential extraction techniques were applied since the extracting of the soil solution was carried out by microdialysis method.

The one-step extraction using 25% TMAH (60 °C for 12 h) was successfully utilized by Takeda et al. [97] to determine the total iodine in soil samples collected at the vicinity of the nuclear fuel reprocessing plant in Rokkasho (Japan). Similar extraction method (25%

TMAH, 90 °C for 3 h) was also applied by Jensen et al. [98] who analyzed the total iodine in soils sampled from the intensively grazed pastures at numerous locations throughout the New Zealand.

The iodine fractionation in conjunction with XANES and EXAFS analysis was performed to identify the binding behavior of iodine in the typical agricultural soil representatives collected in Germany [99]. Authors concluded that the soil organic matter seemed to have the highest impact on the sorption of iodide and iodate, both in short-term and long-term experiments. On the other hand, Balzer et al. [100] suggested that the strong retention of iodine in weathered tropical soils, collected at Alberto Manuel Brenes Biological Reserve (Costa Rica), was primarily caused by the sorption of organoiodine onto ferric oxides and oxohydroxides. This was revealed by the solid phase sequential extraction, where on average 79% of the total iodine was associated with the reducible fraction extracted from soil samples using 0.04 M $NH_2OH \cdot HCl$ dissolved in 25% AcOH solution.

6. Conclusions

The determination of iodine distribution in soil fractions enables us to identify and predict its mobility and bioavailability, as well as its chemical transformation, more accurately (e.g., iodide to iodine oxidation). It also helps to predict the differences in behavior of naturally and anthropogenically introduced iodine in the environment. By generalizing the available findings in literature about iodine mobility and soil distribution, we can better understand and explain the prevalence of iodine deficiency in certain areas as a factor of soil characteristics, or on the other hand, use these findings to produce biofortified crops which could eliminate iodine deficiency disorders.

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