

DETERMINATION OF FLUORINE IN SOME FOOD AND ENVIRONMENTAL SAMPLES

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DOLOČITEV FLUORA V NEKATERIH VZORCIH HRANE IN
OKOLJSKIH VZORCIH

Doktorska disertacija

Supervisor: Prof. Dr. Maja Ponikvar-Svet

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*Don't tell me the sky is the limit
when there are footprints on the moon.*

– Paul Brandt

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Abstract

This thesis deals with the determination of fluorine in various food and environmental samples. A method for fluoride determination is fluoride ion selective electrode (F-ISE). This method relies on the determination of free fluoride ions (F^-), therefore the samples must undergo total sample decomposition prior to F^- measurement. Three methods for total sample decomposition are used in our laboratory. Analyses of certified reference materials with matrix similar to analysed samples proved that alkaline carbonate fusion and pyrohydrolysis assure accurate results within the certified intervals for soil samples and that alkaline carbonate fusion and oxygen bomb combustion assure accurate results for plant samples. However, lower results were observed in the case of oxygen bomb combustion. By calculating Gibbs energies for reactions of some metal fluorides proceeding during these decomposition methods, it was demonstrated that reactions proceeding during alkaline carbonate fusion and pyrohydrolysis are thermodynamically favourable, while reactions proceeding during oxygen bomb combustion are not. Therefore, the results in the case of oxygen bomb combustion are probably lower because only the content of soluble fluorides is determined by F-ISE, while insoluble fluorides are not decomposed during the combustion.

The result of the analysis has to be reported with measurement uncertainty (MU). A procedure for evaluating MU based on the "Guide to the Expression of Uncertainty in Measurement" was developed for fluorine determination in plant samples. The calculated MUs represented 7–15 % of the result. The largest contribution was the uncertainty of bias (76–98 %), the contribution of the uncertainty of precision was 2–23 % and the other uncertainties (weighing, volumetric glassware, cell potential, concentration and density of standard F^- solution) were negligible.

Accurate analytical methods for fluorine determination are of crucial importance because fluorine may be toxic to plants, animals and humans. Fluoride is taken up by plants from the soil via roots or from the air via leaf stomata and is then carried to the margins and tips of the leaves, where it accumulates and can cause necrosis and chlorosis. These symptoms were observed in some plant species in the vicinity of the Rogaška glassworks (Rogaška Slatina, Slovenia), where hydrofluoric acid is used for glass polishing. Fluorine content was determined in five plant species (Norway spruce, peach, hornbeam, bean and grape vine). High values (87–676 $\mu\text{g/g}$) in comparison to the unpolluted area (10 $\mu\text{g/g}$) confirmed the uncontrolled release of gaseous fluorides from the glassworks.

Fluoride is beneficial for humans at low intake as it prevents dental caries, but chronic excessive intake can cause dental fluorosis in children and skeletal fluorosis in children and adults. An important source of fluoride for humans is diet. In recent years, so-called superfoods have become popular due to their supposed extraordinary health benefits. There is practically no information on the fluoride content in superfoods in fluoride databases, in scientific papers or on packaging labels; therefore, it was determined in 18 plant-based superfoods available on the Slovenian market. In eight superfoods (matcha, gotu kola, ginkgo biloba, tulsi, neem, brahmi, gynostemma, moringa), it was surprisingly high (16–373 $\mu\text{g/g}$) in comparison to other commonly consumed foods.

Povzetek

Doktorska disertacija opisuje določanje fluora v različnih vzorcih hrane in okoljskih vzorcih. Vsebnost fluorida določimo s fluoridno ionskoselektivno elektrodo (F-ISE). Meritve s F-ISE temeljijo na določitvi prostih fluoridnih ionov v vodni raztopini, zato je najprej potreben popoln razklop vzorca. V našem laboratoriju uporabljamo tri metode popolnega razklopa. Z analizami certificiranih referenčnih materialov z matrico primerljivo analiziranim vzorcem smo dokazali, da talina z alkalijskimi karbonati in pirohidroliza zagotavljata točne rezultate znotraj certificiranega območja za vzorce zemlje, talina z alkalijskimi karbonati in sežig v kisikovi bombi pa zagotavljata točne rezultate za vzorce rastlin. Vendar pa so rezultati v primeru sežiga v kisikovi bombi nižji. Izračunali smo Gibbsove energije za reakcije nekaterih kovinskih fluoridov, ki potekajo med popolnim razklopom vzorca z omenjenimi metodami in ugotovili, da so reakcije v primeru taline z alkalijskimi karbonati in pirohidrolize termodinamsko ugodne, v primeru sežiga v kisikovi bombi pa ne. Rezultati analiz po sežigu v kisikovi bombi so verjetno nižji, ker razklop netopnih fluoridov med sežigom ne poteče in s F-ISE določimo samo vsebnost topnih fluoridov.

Rezultat analize mora biti vedno podan skupaj z merilno negotovostjo. Za oceno merilne negotovosti pri določitvi fluora v rastlinskih vzorcih smo razvili postopek, ki temelji na priročniku "Guide to the Expression of Uncertainty in Measurement". Izračunane merilne negotovosti so predstavljale 7–15 % vrednosti rezultata. Največji prispevek je bila negotovost pravilnosti analize (76–98 %), prispevek negotovosti natančnosti analize je bil 2–23 %, ostale negotovosti (tehtanje, prostornina steklovine, napetost člena, koncentracija in gostota standardne raztopine fluorida) pa so bile zanemarljive.

Pravilne in natančne analizne metode za določanje fluora so ključnega pomena, ker je fluor lahko strupen za rastline, živali in ljudi. Rastline privzemajo fluorid iz zemlje preko korenin ali iz zraka preko listnih por. Fluorid nato prehaja v listih do robov in konic, kjer se akumulira in lahko povzroči nekrozo in klorozo. Takšni simptomi so se pojavili na nekaterih rastlinah v bližini steklarne Rogaška (Rogaška Slatina, Slovenija), kjer za poliranje stekla uporabljajo fluorovodikovo kislino. Vsebnost fluora smo določili v petih rastlinah (smreka, breskev, gaber, fižol in vinska trta). Visoke vrednosti (87–676 $\mu\text{g/g}$) v primerjavi z neonesnaženim območjem (10 $\mu\text{g/g}$) so potrdile nenadzorovan izpust plinastih fluoridov iz steklarne.

Fluorid je pri nizkih vnosih koristen za ljudi, ker preprečuje zobno gnilobo, visoki kronični vnosi pa lahko povzročijo zobno fluorozo pri otrocih in kostno fluorozo pri otrocih in odraslih. Pomemben vir fluorida za ljudi je hrana. V zadnjem času je postala izredno priljubljena tako imenovana superhrana, ki naj bi imela izredno koristne učinke na človekovo zdravje. V fluoridnih podatkovnih bazah, znanstvenih člankih in na etiketah na embalaži ni skoraj nobenih podatkov o vsebnosti fluora v superhrani, zato smo ga določili v 18 rastlinskih superhranah, ki so na voljo v Sloveniji. V osmih (matcha, gotu kola, ginkgo biloba, tulusi, neem, brahmi, gynostemma, moringa) je bila vsebnost presenetljivo visoka (16–373 $\mu\text{g/g}$) v primerjavi z drugo pogosto zaužito hrano.

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Abbreviations

AI	... adequate intake
CFCs	... chlorofluorocarbons
CRM	... certified reference material
DCTA	... diaminocyclohexanetetraacetic acid
EFSA	... European Food Safety Authority
F	... fluorine
F ⁻	... fluoride
F-ISE	... fluoride ion selective electrode
GUM	... Guide to the Expression of Uncertainty in Measurement
HCFCs	... hydrochlorofluorocarbons
HFCs	... hydrofluorocarbons
HFOs	... hydrofluoroolefins
HR-CS AAS	... high resolution continuum source atomic absorption spectrometry
IOM	... Institute of Medicine
IRMM	... Institute of Reference Materials and Measurements
LS AAS	... line source atomic absorption spectroscopy
MU	... measurement uncertainty
NIST	... National Institute of Standards and Measurements
NMR	... nuclear magnetic resonance spectroscopy
PFASs	... perfluoroalkyl substances
TISAB	... total ionic strength adjustment buffer
UL	... upper limit
XPS	... X-ray photoelectron spectroscopy

Chapter 1

Introduction

1.1 Fluorine in the Environment

Fluorine (F)¹ is the most reactive element in the periodic system and is therefore rarely found in nature as elemental fluorine (F_2), but in the form of mainly inorganic and some rare organic compounds. Fluorine is the 13th most abundant element in the earth's crust [1], and it is therefore not surprising that considerable amounts of fluorine are found in the soil, water and air. A schematic view of the fluorine cycle in the environment is illustrated in Fig. 1.1.

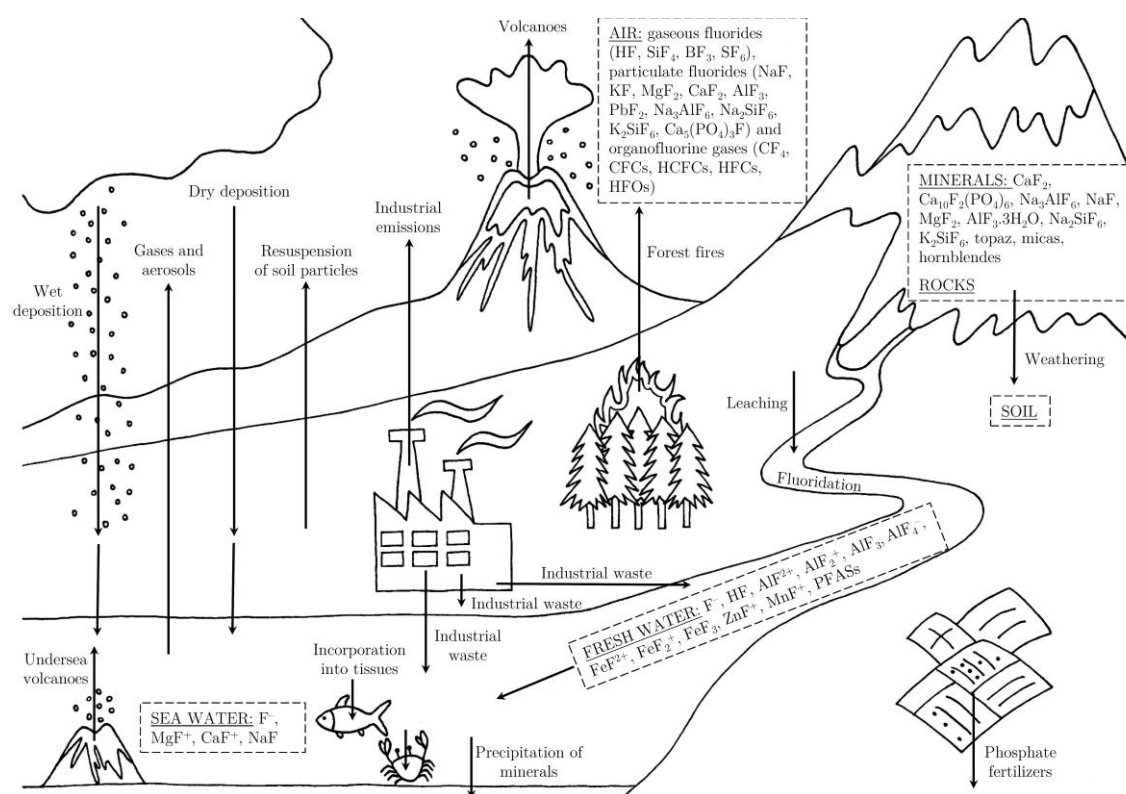


Figure 1.1: Fluorine cycle in the environment.

1.1.1 Fluorine in Minerals and Soil

Fluoride is present in igneous and sedimentary rocks and in the soil in the form of various inorganic minerals. Around 440 minerals are known to contain fluoride [2], with the most important being fluorite (CaF_2), fluorapatite ($Ca_{10}(PO_4)_6F_2$) and cryolite (Na_3AlF_6). Other significant fluoride minerals are villiaumite (NaF), sellaite (MgF_2), rosenbergite ($AlF_3 \cdot 3H_2O$),

¹ The word "fluorine" is used to denote the element in any of its forms and the word "fluoride" is used to denote the inorganic compound or the F^- anion.

malladrite (Na_2SiF_6) and hieratite (K_2SiF_6). Fluoride can occupy the hydroxide sites in topaz ($\text{Al}_2\text{SiO}_4(\text{F},\text{OH})_2$), hornblendes and micas (biotite ($\text{K}(\text{Mg},\text{Fe})_3(\text{AlSi}_3\text{O}_{10})(\text{F},\text{OH})_2$), lepidolite ($\text{K}(\text{Li},\text{Al})_3(\text{Al},\text{Si})_4\text{O}_{10}(\text{F},\text{OH})_2$), muscovite ($\text{KAl}_2(\text{AlSi}_3\text{O}_{10})(\text{F},\text{OH})_2$), phlogopite ($\text{KMg}_3(\text{AlSi}_3\text{O}_{10})(\text{F},\text{OH})_2$)) [3, 4]. Gaseous fluorine (F_2) has been detected in the minerals antozonite [5] and villiaumite [6].

Igneous rocks (andesite, basalt, granite, syenite) contain up to 4700 $\mu\text{g F/g}$. Sedimentary rocks (sandstone, limestone, dolomite, shale, greywacke, loess) contain up to 800 $\mu\text{g F/g}$ [4, 7].

Soil is a complex mixture of mineral fragments and decomposition products due to physical or chemical weathering. Elrashidi et al. [3] collected thermodynamic data for fluoride minerals that may be present in the soil and determined which of them are soluble and which participate in the soil solution. Calcium fluoride (CaF_2) and magnesium fluoride (MgF_2) are stable in acid and neutral soils. Aluminium fluoride (AlF_3), iron(III) fluoride (FeF_3) and cryolite are stable in acid soils. Fluorapatite is stable in neutral and alkaline soils. Sodium fluorosilicate (Na_2SiF_6) and potassium fluorosilicate (K_2SiF_6) are moderately soluble. The solubility of sodium fluoride (NaF), potassium fluoride (KF) and copper(II) fluoride (CuF_2) is very high, and they are not likely to form in soil. The total F content in soil varies within extremely wide limits, from below 10 to up to 7000 $\mu\text{g/g}$ [8-11]. High F content in soil is found in areas where rocks are rich in fluoride minerals and in volcanic or industrial areas due to deposition of airborne fluorides. In addition, phosphate fertilizers, fluoridated water for irrigation and industrial waste contribute to the F content in soil [12].

1.1.2 Fluorine in Water

The speciation of fluorine in fresh water depends on pH and the presence of complexing ions. At neutral and alkaline pH, fluoride ion (F^-) is the predominant species [4]. At lower pH and in the presence of complexing ions, species other than F^- generally dominate. Elrashidi et al. [3] collected thermodynamic data for fluorine species that may be present in water and concluded that the strongest species at pH below 5 are formed with aluminium (AlF^{2+} , AlF_2^+ , AlF_3 , AlF_4^-), iron (FeF^{2+} , FeF_2^+ , FeF_3), manganese (MnF^+) and zinc (ZnF^+). Formation of other species, i.e. with silicon (SiF_4 , SiF_6^{2-}), lead (PbF^+ , PbF_2 , PbF_3^- , PbF_4^{2-}), sodium (NaF), potassium (KF), copper (CuF^+), cadmium (CdF_2) and mercury (HgF^+), is not significant. At strongly acid pH and in the absence of complexing ions, the proportion of F^- decreases due to protonation and the formation of HF and HF_2^- [4].

The speciation of fluorine in seawater is different than that in fresh water because of the presence of magnesium. Fluorine is present mainly as F^- or complexed with magnesium (MgF^+), but small amounts are complexed also with calcium (CaF^+) and sodium (NaF) [7].

The fluoride concentration of surface waters (rivers, lakes) is generally below 1 mg/l. Elevated fluoride concentrations are found in areas where the rock is rich in fluoride minerals, and the highest concentrations are found near industrial outfalls (up to 25 mg/l) or in regions with volcanic activity (up to 2800 mg/l) [13]. Fluoridation of water supplies also contributes to the concentration of fluoride in water [12]. The fluoride concentration of groundwater is usually higher than that in surface waters (up to 8 mg/l) because it is in greater contact with fluoride minerals [12]. Groundwater with high fluoride concentration is often of the sodium bicarbonate (NaHCO_3) type, while in the presence of calcium sulphate (CaSO_4), the fluoride concentration is lower [14].

Seawater contains more fluoride than fresh water, with concentrations ranging from 0.8 to 1.5 mg/l [4]. Fluoride enters the oceans from rivers, undersea volcanoes, atmospheric deposition and industrial discharges. Losses are due to incorporation into the hard tissues of aquatic organisms, precipitation of minerals and release of hydrogen fluoride and fluoride salts into the atmosphere as aerosols [7].

In addition to inorganic fluorides, some organic perfluoroalkyl substances (PFASs) have been found in surface and tap waters. In the past, perfluorooctanoic acid and perfluorooctanesulfonic acid were used as processing aids in the polymerization of fluoropolymers and allowed manufacture of stain- and water- repellents, non-stick pans and waterproof fabric. Since these compounds are very persistent in the environment and tend to bioaccumulate in human and animal tissues they have been replaced by other compounds with fewer carbon atoms in the chain (e.g. perfluorohexanoic acid, perfluorobutanesulfonic acid, perfluorobutanoic acid) or perfluoropolyethers (e.g. ADONA ($\text{CF}_3\text{--O--CF}_2\text{--CF}_2\text{--CF}_2\text{--O--CHF--CF}_2\text{--COO}^-$), GenX ($\text{CF}_3\text{--CF}_2\text{--CF}_2\text{--O--CF}(\text{CF}_3)\text{--COO}^-$)) [14, 15].

1.1.3 Fluorine in the Air

Atmospheric fluorides can be in gaseous or particulate form. The main gaseous fluorides are hydrogen fluoride (HF), silicon tetrafluoride (SiF₄) and boron trifluoride (BF₃), which are all water-soluble, and insoluble sulphur hexafluoride (SF₆). Among particulate fluorides, NaF and KF are water-soluble, while AlF₃, PbF₂, Na₂SiF₆ and K₂SiF₆ are less soluble and MgF₂, CaF₂, cryolite and fluorapatite are insoluble [7, 13, 16].

The concentration of fluoride in the air is usually less than 0.05 µg/m³ [17] but can reach up to 6 µg/m³ in the vicinity of emission sources [13]. The main natural source of atmospheric fluorides are active volcanoes. Other sources are the resuspension of soil particles by the wind, burning wood in forest fires and sea aerosols [7]. Fluorides are also emitted from a number of industrial operations that use fluoride compounds during production [7, 18]. Phosphate rock rich in fluorapatite is used for the production of phosphate fertilizers and elemental phosphorus. Fluoride-containing clays are used for the production of bricks, pottery and cement. Hydrofluoric acid is used in the glass industry for polishing and etching of the glass and in petroleum refining as a catalyst for alkylation. Molten cryolite is used in aluminium smelting for dissolving alumina (Al₂O₃). Gaseous and particulate fluorides are removed from air by wet or dry deposition [12].

In addition to inorganic fluorides, some organofluorine gases are also released into the environment. Naturally occurring gases (CF₄, CF₃Cl, CF₂Cl₂, CFCl₃ and CHF₃) can be trapped and degassed from minerals or emitted from volcanoes. Anthropogenic chlorofluorocarbons (CFCs) (e.g. CCl₂F₂, CCl₃F) were widely used in refrigeration and air-conditioning systems and as propellants. International concern about their effects on ozone depletion through the liberation of chlorine radicals via photolysis led to the signing of the Montreal Protocol in 1987, which phased out the manufacture of CFCs. Hydrochlorofluorocarbons (HCFCs) (e.g. CHClF₂, CHClFCF₃) were introduced as temporary replacements and were later replaced by hydrofluorocarbons (HFCs) (e.g. CH₂FCF₃, CF₃CHFCF₃) and hydrofluoroolefins (HFOs) (e.g. 2,3,3,3-tetrafluoropropene). HFCs and HFOs are chlorine-free and do not contribute to ozone depletion; however, they still act as greenhouse gasses [14, 19].

1.2 Fluorine in Plants

Plants can uptake fluoride from the soil solution via the roots or from the air via the leaves. In areas free of fluoride emissions, fluoride is taken up through the roots. The F content in most species is less than 10 µg/g [20]. There are some species that can uptake higher amounts of fluoride (up to 250 µg/g) when the fluoride concentration in the soil solution is high (barley grass, clover, cocksfoot, nettle, onion, sorrel, spinach, thistle) [21-24]. Tea plant (*Camellia sinensis* L.) is one of the few plants known to be able to accumulate extremely high F content (up to 1000 µg/g) even when atmospheric and soil solution fluoride concentrations are low [25, 26]. In areas with fluoride emissions, most of the fluoride is taken up through the stomata of the leaves. The F content in some plants (white birch, alder, dogberry) in the vicinity of an emission source may exceed 1000 µg/g [27].

1.2.1 Uptake from the Soil

1.2.1.1 Root Structure and Function

The major regions of the root [28] are presented in Fig. 1.2. The epidermis is the external layer of cells that covers and protects the root. The cells of the cortex are relatively unspecialized. The endodermis is the innermost cell layer of the cortex. The stele is the central part of the root and consists of the pericycle, xylem and phloem. The pericycle consists of relatively undifferentiated cells from which lateral roots arise, xylem conducts water and minerals and phloem conducts carbohydrates.

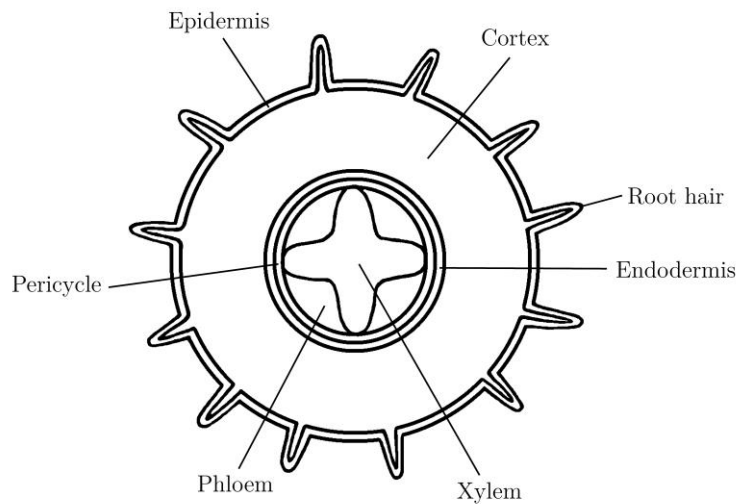


Figure 1.2: Major regions of the root.

Substances can travel from the soil solution through the roots to the xylem via two pathways: the apoplast and the symplast (Fig. 1.3) [28]. Substances access the apoplast by crossing the cell wall and then travel through cell walls and intercellular spaces without crossing a membrane as far as to the Casparian strip of the endodermis. The Casparian strip forms a waxy ring around endodermal cells and prevents substances from moving through endodermal cell walls, and thus they must enter the cytoplasm.

Alternatively, substances access the symplast by crossing the selectively permeable membrane and then travel across the continuous cytoplasm through channels called plasmodesmata. The membrane can be crossed by passive or active transport. Passive transport does not require an input of energy and moves substances down the concentration gradient. Non-polar and small polar uncharged molecules can pass through the membrane by simple diffusion. Large polar uncharged molecules, charged molecules and ions are not soluble in the hydrophobic membrane and cross the membrane via channel proteins by facilitated diffusion. Active transport requires the input of energy to move substances across the membrane against the concentration gradient via membrane proteins.

Once substances have passed the endodermis (through the apoplast or symplast), they remain in the symplast until they reach the xylem, where they are transported into the apoplast, forming the xylem sap.

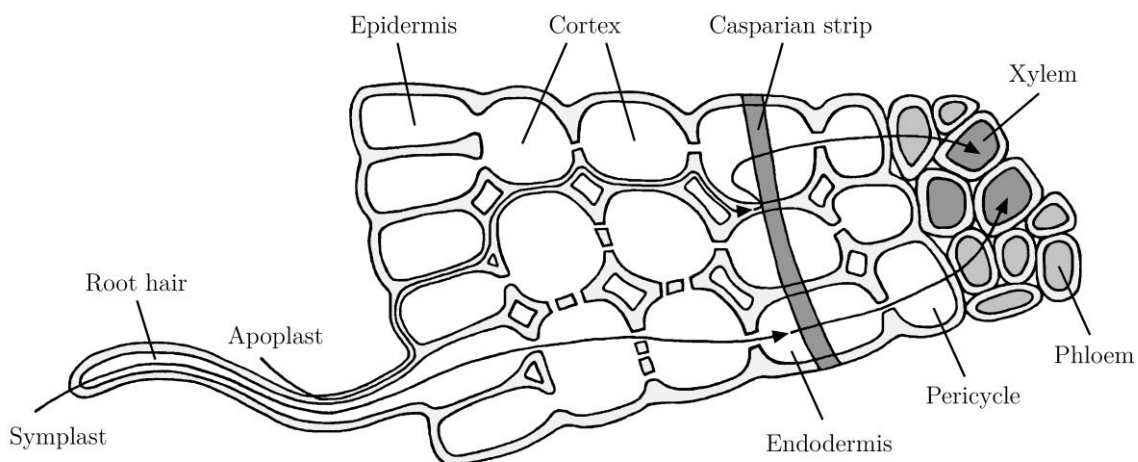


Figure 1.3: Apoplast and symplast. Two pathways of substances traveling from the soil solution to the xylem [28].

1.2.1.2 Mechanisms of Fluoride Uptake from the Soil

The F content in the soil usually does not correlate well with the F content in plants because plants uptake only soluble fluorides from the soil solution [29]. Fluoride binds strongly to soil between pH 5.5–6.5, and solubility increases at both lower and higher pH. In acid soils fluorine exists as the free F^- ion or forms soluble complexes with aluminium (AlF^{2+} , AlF_2^+ , AlF_3 , AlF_4^-), boron (BF_4^-) and hydrogen (HF). In alkaline soils, the F^- anion predominates and the increased solubility can be explained by desorption of F^- due to repulsion by more negatively charged soil surfaces [30, 31].

The speciation of fluorine in the soil solution is important because plant roots uptake some soluble fluoride complexes more readily than others. Studies by the Stevens group [29, 32, 33] showed that the rate of uptake of fluoride species is in the following order: $F^- = AlF_4^- = AlF_3 < AlF_2^+ = AlF^{2+} < HF < BF_4^-$.

When roots are exposed to F^- in the solution, the absorbed fluoride is almost completely removed by washing with water (89–98 %) [34, 35]. This suggests that the transport of F^- ions across the root is via the apoplast. The rate of uptake of F^- is very low because of two barriers. The first is the cell wall, which has a negative charge and causes the exclusion of negatively charged F^- ions [7]. The second barrier is the Casparian strip of the endodermis. Most of the F^- reaching the xylem leaks past the Casparian strip at the root tips or at sites of developing lateral roots, where the endodermis is not fully formed [36].

When plant roots are exposed to a constant concentration of F^- and an increasing concentration of Al^{3+} in the solution, fluoride uptake is significantly increased, indicating that Al-F complexes are taken up more readily than F^- [29, 35]. Positively charged species (AlF^{2+} , AlF_2^+) are taken up more readily than a complex with no charge (AlF_3) or a negatively charged complex (AlF_4^-). This suggests that the transport of Al-F complexes across the root is via the apoplast and that the formation of positively charged Al-F complexes suppresses the negative charge of fluoride and decreases exclusion by the charge of the cell walls. Higher fluoride concentration in the apoplast leads to a higher amount of fluoride leaking past the endodermis.

When plant roots are exposed to a very acid fluoride solution, where a large portion of fluoride exists as HF, fluoride uptake is significantly increased, indicating that HF is taken up more readily than F^- [33]. The permeability of HF through the membrane is six orders of magnitude higher than that of F^- [37], thus the transport of HF across the root could occur via the symplast by simple diffusion across the cell membrane.

The rate of uptake of BF_4^- is the highest among all fluoride species studied by the Stevens group [33], and the ratio of F content in root- to-shoot is 1:2, while in the case of the other fluoride species studied, it is greater than one. This suggests that the transport of BF_4^- could proceed via the symplast by active transport.

Fluoride uptake is decreased when plant roots are exposed to Ca^{2+} in the solution [38, 39]. The prevention of fluoride entering the transpiration stream is probably related to (1) the precipitation of CaF_2 in the soil solution or on the root surface, (2) effect of calcium on altering the permeability of the cell wall and membrane or (3) change in the speciation of fluorine in the soil solution due to the increase in pH.

All the studies mentioned so far have been performed on non-accumulating plants, with low F content and in which the F content in the roots is usually higher than that in the leaves [32, 35]. By contrast, the tea plant (*Camellia sinensis* L.) uptakes high amounts of fluoride from the soil, and most of it is readily transported to the leaves, excluding the possibility that fluoride travels via the apoplast. Two studies [40, 41] demonstrated that fluoride uptake in the tea plant is energy dependant and inhibited by anion channel and cation channel inhibitors. This suggests that fluoride uptake in the tea plant is an active transport via the symplast and that anion and cation channels may be possible pathways for the entry of fluoride into the root cells.

1.2.2 Uptake from the Air

1.2.2.1 Leaf Structure and Function

Leaves are designed for photosynthesis, the process where light energy is used to convert carbon dioxide (CO_2) and water (H_2O) to oxygen (O_2) and carbohydrates. The major regions of the leaf [28] are presented in Fig. 1.4. The waxy cuticle is impermeable to water and serves to keep the water within the leaf. The epidermis is a layer of cells that covers the entire leaf on

both upper and lower surfaces. The stomata are pores in the leaf epidermis, typically on the lower surface of the leaf, where H_2O and gases (O_2 and CO_2) are exchanged between the interior of the leaf and the environment. Because the abundance of stomata could lead to excessive water loss, guard cells open the stomata during the daytime and close them at night. The leaf has two types of photosynthetic tissue, palisade and spongy mesophyll. Xylem supplies the mesophyll cells with water and phloem transports the carbohydrates produced during photosynthesis to the rest of the plant.

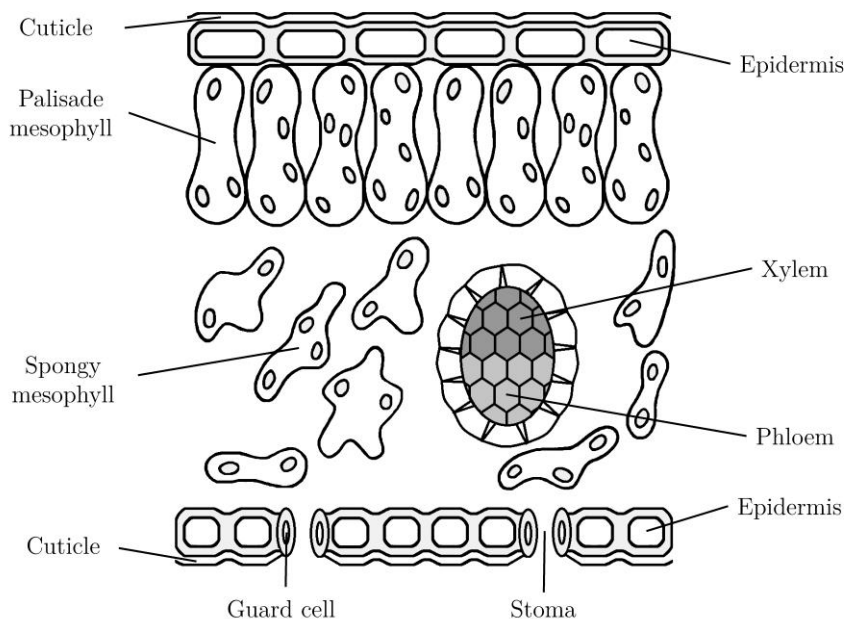


Figure 1.4: Major regions of the leaf.

1.2.2.2 Mechanisms of Uptake of Airborne Fluorides

There are two possible ways for fluorides to penetrate into the leaf, either through the stomata or across the cuticle. Only gaseous fluorides can enter through the stomata because stomatal chambers are filled with gases and do not permit the passage of aqueous solutions of dissolved particulate fluorides [42]. Gaseous HF diffuses into the leaves through the stomata and, due to its high solubility, readily dissolves in the cell wall water, where it dissociates [43].

Particulate fluorides deposited on the leaf surface and gaseous fluorides deposited on the soil particles on the leaf surface can enter across the cuticle [44]. However, the penetration is very slow due to the hydrophobic character of the cuticle [45]. The small hydrophilic pores within the hydrophobic cuticle, where evaporation of water takes place, are lined with negative charges, so small uncharged molecules and cations can permeate, but not anions such as F^- [46].

1.2.3 Fluoride Toxicity

Typical symptoms of fluoride toxicity are necrosis and chlorosis (Fig. 1.5) [47]. Fluoride is carried with the transpiration stream to the margins of the leaves, where it accumulates. High fluoride concentration causes cell death and the appearance of necrotic brown patches on the margins and tips of the leaves. When fluoride diffuses into the chloroplasts, it complexes with magnesium and disrupts the chlorophyll molecules, leading to chlorotic yellow areas between the veins.



Figure 1.5: Necrosis (left) and chlorosis (right) [47].

1.2.4 Fluorine Speciation in Plants

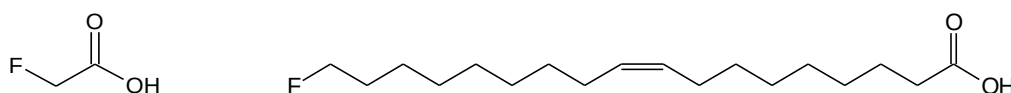
1.2.4.1 Inorganic Fluorides

Most of the early knowledge on the speciation of fluorine in plants was based on assumptions rather than on the results of actual chemical characterizations of compounds. Experiments showed that most of the fluoride can be extracted from the leaf tissues by acid solutions, while only a small amount is soluble in water [44, 48]. These findings support the idea that the fluorine in plant leaves is present mainly in inorganic form and that the greater proportion is not present in the form of soluble fluorides, but in the form of insoluble inorganic salts, possibly CaF_2 and MgF_2 . Takmaz-Nisancioglu et al. [35] analysed the xylem sap of plants grown in NaF or AlF_3 hydroponic culture. In the case of NaF, 95 % of the fluorine was present as F^- , but in the case of AlF_3 , only 59 % was present as F^- , the remaining being complexed with aluminium.

More recently, chemical forms of fluorine in plants have been characterized by nuclear magnetic resonance spectroscopy (^{19}F NMR and ^{27}Al NMR) and X-ray photoelectron spectroscopy (XPS). Nagata et al. observed that in tea leaves aluminium is complexed with catechin, phenolic acids and organic acids [49], while in other parts (root, stem, branches) it is complexed with catechin and fluoride [50], indicating that Al-F complexes are taken up by the roots and transported until they reach the leaves. Morita et al. [51] analysed the xylem sap of the tea plant and observed that aluminium forms complex with citrate and that the main species of fluorine is fluoride and not Al-F complexes. The results of these two studies are not in agreement, probably because of the difference between the pH values of the sample solutions. Cai et al. [52] reported the existence of AlF_3 , MgF_2 and CaF_2 on the surface of tea leaves.

1.2.4.2 Organofluorine Compounds

A unique feature of certain plants is their capacity to metabolize inorganic fluoride and synthesize organofluorine compounds, which act as a defence mechanism as they are extremely toxic to animals. Fluoroacetic acid was the first organofluorine compound discovered in plants in the African shrub *Dichapetalum cymosum* [53] and was later identified in high content (250–8000 $\mu\text{g/g}$) also in some other plant species growing in tropical and subtropical regions in Africa, Australia and South America (*Acacia georginae*, *Gastrolobium grandiflorum*, *Palicourea marcgravii* and *Arrabidaea Bilabiata*) [7, 54]. Another organofluorine compound discovered in these plants was fluorocitrate, which is formed in vivo from fluoroacetic acid. In the seeds of *Dichapetalum toxicarium*, 11 ω -fluoro fatty acids were found; the main two were ω -fluorooleic acid and ω -fluoropalmitic acid, and others were ω -fluorostearic acid, ω -fluorolinoleic acid, ω -fluoropalmitoleic acid, ω -fluoroarachidic acid, ω -fluoroeicosenoic acid, *threo*-18-fluoro-9,10-dihydroxystearic acid, ω -fluorocapric acid, ω -fluoromyristic acid and 18-fluoro-9,10-epoxystearic acid [55–59]. The four major organofluorine compounds found in plants are presented in Fig. 1.6.



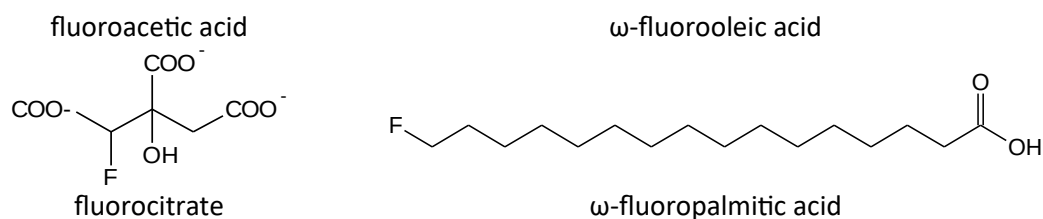


Figure 1.6: Four major organofluorine compounds in plants.

A question arose whether the biosynthesis of organofluorine compounds is limited only to a few tropical plants or is more general but less pronounced also in other plant species. The study from Lovelace et al. [60] was the only study that reported high content of fluoracetic acid in forage crops (179 µg/g). Results from other studies [61, 62] suggested that some plants, when exposed to fluoride, can also biosynthesize fluoracetic acid, but at very low levels.

Surprisingly, the 13th most abundant halogen in the earth's crust has been found in only 13 organic compounds in plants, 11 of which are ω-fluoro fatty acids found in the seeds of the same plant (*Dichapetalum toxicarium*).

1.3 Fluorine in Humans

Fluoride is absorbed mostly through the gastrointestinal tract, in the stomach and small intestine. Under normal conditions, inhalation and absorption through the skin make a negligible contribution. Fluoride is then distributed in the plasma and deposited in calcified tissues, i.e. bones and teeth. Approximately half of the daily fluoride dose is excreted in the urine [63].

Fluoride is both beneficial and harmful to humans. At low intake, it prevents dental caries, but excessive intake over an extended period of time can damage various body tissues and organs. The major clinical manifestations are dental fluorosis in children and skeletal fluorosis in both children and adults.

1.3.1 Prevention of Dental Caries

Dental caries is defined as the breakdown of the tooth enamel due to acids produced by cariogenic bacteria when they metabolize fermentable carbohydrates. Fluoride prevents dental caries via three main mechanisms [64, 65]. (1) F⁻ ions substitute the hydroxide ions (OH⁻) in the hydroxyapatite crystals (Ca₁₀(PO₄)₆(OH)₂) of tooth enamel, forming fluorapatite, which is more resistant to acids, thus inhibiting demineralization. (2) F⁻ ions are absorbed into the enamel surface and attract calcium ions, followed by phosphate ions, leading to the formation of new fluorapatite crystals, thus enhancing remineralisation. (3) Fluoride inhibits enolase, a glycolytic enzyme necessary for cariogenic bacteria to metabolize carbohydrates.

It was long assumed that fluoride has to be delivered systemically (with fluoridated water, fluoridated milk, fluoridated salt or fluoride tablets) because it has to be incorporated into the tooth enamel prior to tooth eruption. There is now extensive evidence that the primary preventive mechanism of fluoride relies on its post-eruptive effect from topical contact with the tooth enamel and that even systemic methods act mainly through the topical effect when they are in contact with the teeth. Therefore, topical delivery of fluoride with toothpaste, mouthwash or gel is preferred over systemic delivery [66].

Because of the beneficial effects of fluoride in the prevention of dental caries, the Institute of Medicine (IOM) [67], European Food Safety Authority (EFSA) [68] and many other health authorities worldwide set an adequate intake (AI) of fluoride from all sources (including non-dietary) of 0.05 mg/kg body weight per day for children and adults. This corresponds to 3.0–4.5 mg of fluoride per day for an adult of 60–90 kg body weight.

1.3.2 Adverse Effects

Dental fluorosis develops due to the excessive intake of fluoride during the period of enamel formation (amelogenesis), which usually ends at approximately 6–8 years of age. During the secretory stage of amelogenesis, ameloblasts (enamel-forming cells) secrete matrix proteins, which serve as a temporary scaffold on which hydroxyapatite crystals form. During the

maturation stage of amelogenesis, enamel matrix proteins undergo proteolysis and gradual removal from the enamel to allow final crystal growth and the development of mature hard enamel. F^- disturbs this process because it incorporates into the hydroxyapatite crystals and forms stronger hydrogen bonds with proteins. This results in the retention and incomplete removal of proteins from the enamel and in the formation of impaired, more porous enamel [69, 70]. Dental fluorosis therefore results in opaque, lustreless white patches in the enamel, which can become stained yellow to dark brown, and in severe forms, pitting and brittleness of the teeth can occur (Fig. 1.7) [63].



Figure 1.7: Dental fluorosis [71].

Skeletal fluorosis is caused by high exposure to fluoride over an extended period of time. Fluoride affects the bone in two ways. It has a physicochemical effect on bone crystals [72, 73] as it substitutes OH^- in hydroxyapatite. The formed structure is electrostatically more stable and structurally more compact because F^- ions, in contrast to OH^- ions, reside in the plane of the calcium ions. Increased packing density reduces the diffusion of ions out of the crystal and therefore decreases the solubility. Fluoride also has a biological effect on bone cells [74, 75] as it causes the proliferation of osteoblasts (bone-forming cells). Therefore, at low concentrations, fluoride stimulates the formation of stronger bones; however, at high fluoride concentrations, the newly formed bone may have reduced strength due to structural abnormalities or mineralization defects. Skeletal fluorosis results in pain and stiffness of joints, osteosclerosis, osteoporosis, arthritic symptoms and calcification of ligaments. Severe skeletal fluorosis is characterized by considerable limitation of joint movements and crippling deformities of the spine and major joints (kyphosis, scoliosis, bow legs and knock-knee) (Fig. 1.8) [63, 76].



Figure 1.8: Skeletal fluorosis with a knock-knee deformity [76].

Because of the possible adverse effects of fluoride, the setting of an upper limit (UL) is necessary. The Institute of Medicine [67] concluded that there are no signs of skeletal fluorosis observed unless the fluoride intake is greater than 10 mg per day for many years. The EFSA [77]

set a UL from all sources of 0.12 mg fluoride/kg body weight per day for adults, which corresponds to 7.2–10.8 mg of fluoride per day for an adult of 60–90 kg body weight.

Many other associations between the ingestion of fluoride at high doses and possible adverse effects have been suggested, including gastrointestinal symptoms, abnormalities in glucose tolerance, thyroid hormone derangements, decreased total fertility rate and decreased intelligence in children [78, 79].

1.3.3 Sources of Fluoride

The main sources of fluoride for adults are fluoridated water, fluoridated salt, beverages, tea and food.

In the European Union, the quality of drinking water for human consumption is regulated by the document entitled "Council Directive 98/83/EC", according to which water may not contain more than 1.5 mg F⁻/l [80]. In some countries where the fluoride concentration in drinking water is naturally low, water supplies are fluoridated because of the beneficial effects of fluoride in the prevention of dental caries (e.g. Australia, Chile, Ireland, Malaysia, United Kingdom, United States) [81]. Fluoride is added to water in the form of H₂SiF₆, Na₂SiF₆ or NaF [82] in concentrations ranging from 0.5 to 1.0 mg/l [83]. On the other hand, there are many fluoride endemic countries where the F⁻ concentration in drinking water is naturally very high. F⁻ concentrations between 18–70 mg/l have been reported for Argentina, China and India, and exceptionally high values have been reported for Ethiopia (177 mg/l) and Kenya (2800 mg/l) [84]. The removal of fluoride from water can be achieved by different defluoridation techniques (coagulation-precipitation, adsorption, ion-exchange, reverse osmosis), but unfortunately for many endemic countries, the cost of implementing these techniques is too high [14].

An alternative to water fluoridation is fluoridated salt with an F content between 250–350 µg/g. It is used in countries where water fluoridation cannot be implemented due to technical or ethical reasons (many Latin American countries, France, Germany, Switzerland). In contrast to water fluoridation, fluoridated salt provides an individual choice for consumers [85].

The fluoride content in beverages (juices, carbonated drinks, beer, coffee, chocolate drinks) is usually low. According to the UK fluoride database [86], it is below 0.45 µg/g, and according to the US fluoride database [87], it is below 1.25 µg/g. Higher fluoride content in beverages from the US database can be ascribed to the use of fluoridated water during the production process or in their preparation. The only exceptions that contain higher fluoride content (up to 2.04 µg/g) are some grape juices and wines. Grape products may contain elevated fluoride content due to the use of cryolite as a pesticide in vineyards [88].

Tea (*Camellia sinensis* L.) is one of the few plants known to uptake extremely high amounts of fluoride from the soil. The F content in the leaves usually ranges between 50–820 µg/g. The corresponding F⁻ concentration in the infusion is then 0.33–7.96 mg/l, since 12–100 % of fluorine is released in the form of F⁻ [25, 89].

The F content in food depends on the environment in which the food is grown, the amount of fertilizers and pesticides applied and whether fluoridated water is used during manufacture or in their preparation. The F content of a substantial number of commonly consumed foods is included in two fluoride databases, the UK fluoride database [86] and the US fluoride database [87]. These databases arrange food items in different groups, and the F content is difficult to compare. In our review paper [90], we re-positioned all food items from both databases within the same groups and estimated the ranges of F contents in these groups (Table 1.1).

Table 1.1: The ranges of F contents (w_F) in different food groups listed in the US fluoride database and the UK fluoride database.

	w_F (µg/g)	
	USA database	UK database
Fruits, nuts ^a	0.01–0.12	0.01–0.19
Fats, oils	0.01–0.25	0.00–0.17
Vegetables, spices, herbs ^b	0.01–0.49	0.01–0.19
Meat and products	0.04–0.48	0.02–0.24
Milk, egg and products ^c	0.01–0.35	0.01–0.59
Cereal products	0.06–0.51	0.04–0.57
Breakfast cereals	0.17–0.72	0.04–0.75

Soups, sauces, gravies	0.01–1.32	0.01–0.49
Sweets, snacks	0.01–1.06	0.01–0.90
Fish, shellfish and products	0.18–2.10	0.08–10.5
Dishes	0.05–0.84	0.01–0.51

^a The highest F content is not considered (2.34 µg/g in raisins)

^b The highest F content is not considered (1.15 µg/g in McDonald's French fries)

^c The highest F content is not considered (1.12 µg/g in powdered cream substitute)

The F content in food is usually low, below 1 µg/g. Processed food (soups, sauces, gravies, sweets and snacks) can contain higher F content if fluoridated water is used during the production process or in their preparation. Fish and shellfish may contain considerable amounts of F if analysed samples include the remains of bones and exoskeleton, where fluoride is accumulated from the sea.

1.4 Fluorine Analysis

High F content in soil, water or air represents a threat to plants, animals and humans. Thus, accurate analytical methods for fluorine determination are of crucial importance.

Numerous analytical methods have been developed for the determination of fluorine. The first method for total fluorine determination was proposed by Willard and Winter in 1933. In this method, the sample is decomposed by a strong acid (sulphuric(VI) acid (H₂SO₄) or phosphoric(V) acid (H₃PO₄)), the released fluoride is distilled in the form of hexafluorosilicic acid (H₂SiF₆) and F[−] is determined by titration with thorium nitrate solution (Th(NO₃)₄) [91]. This method has been largely replaced by the fluoride ion selective electrode introduced by Frant and Ross in 1966, a breakthrough method in the analytical chemistry of fluorine. Alongside potentiometry, other frequently used methods for fluorine determination nowadays include spectrophotometry, atomic absorption spectrometry and ion chromatography. Less frequently employed methods include polarography [92, 93], fluorimetry [94, 95], sensors [96, 97] and proton-induced gamma emission [98, 99].

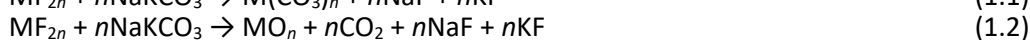
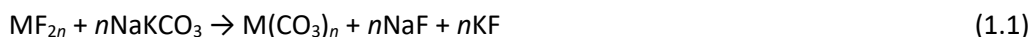
Most of these methods rely on the determination of fluorine in the form of the F[−] ion in the aqueous solution. Therefore, samples have to be decomposed before F[−] determination to ensure the release of fluorine from inorganic and organic compounds and the formation of F[−]. Since water, acids and alkaline solutions may dissolve the fluoride in the samples only to varying degrees, more powerful reagents and harsh conditions are required for total sample decomposition. Most published methods nowadays for total sample decomposition for subsequent fluoride determination involve alkaline fusion, oxygen combustion, pyrohydrolysis and diffusion as trimethylfluorosilane.

1.4.1 Sample Decomposition Methods

1.4.1.1 Alkaline Fusion

The sample is mixed with the flux in a crucible and heated with a modified Bunsen burner or muffle furnace until a transparent melt without any solid remains is obtained (Fig. 1.9). The most frequently used fluxes are sodium hydroxide (NaOH) [100, 101] and sodium potassium carbonate (NaKCO₃) [102, 103].

The potassium carbonate (K₂CO₃) (*T*_{m.p.} = 899 °C [104]) and sodium carbonate (Na₂CO₃) (*T*_{m.p.} = 856 °C [104]) in equimolar mixture form eutectic mixture with a melting point of 712 °C, which is lower than the melting point of either of the single compounds. Therefore, NaKCO₃ represents the most convenient flux [105]. During the fusion of metal fluorides (MF_{2n}) with NaKCO₃, cationic constituents are converted to carbonates and oxides and fluoride to NaF and KF [106]. The general reaction of the decomposition of MF_{2n} (*n* = 1/2, 1, 3/2) can be expressed by Eqs. 1.1 and 1.2.



Fusions with NaOH can be carried out at much lower temperatures because of the lower melting point of NaOH (*T*_{m.p.} = 323 °C [104]) [105]. During the fusion of MF_{2n} with NaOH, cationic

constituents are converted to hydroxides and fluoride to NaF. The general reaction of the decomposition of MF_n ($n = 1, 2, 3$) can be expressed by Eq. 1.3.

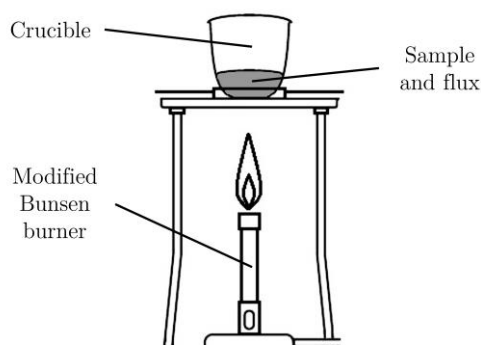


Figure 1.9: Alkaline fusion using a modified Bunsen burner.

1.4.1.2 Oxygen Combustion

A combustible sample is burned in an oxygen atmosphere within a closed vessel containing absorption solution, where combustion products are absorbed. Combustion in oxygen can be performed in three different ways. Classical oxygen flask combustion proposed by Schöniger involves igniting the filter paper in which the sample is wrapped and inserting it into a quartz flask filled with oxygen [107, 108]. Oxygen bomb combustion involves placing the sample in an oxygen combustion bomb, pressurizing the bomb with oxygen and initiating ignition by electric current (Fig. 1.10) [109, 110]. Microwave-induced oxygen combustion involves placing the sample and filter paper soaked with explosive ammonium nitrate solution (NH_4NO_3) into a quartz vessel, pressurizing the vessel with oxygen and initiating ignition with microwave radiation [111, 112].

Reactions of fluoride compounds proceeding during oxygen combustion have to the best of our knowledge not been described in the literature. In our paper [113] we have calculated that oxygen combustion is thermodynamically unfavourable process for decomposition of MF_{2n} and that fluorine remains bound in the form of metal fluorides (Eq. 1.4) (Chapter 4.1).

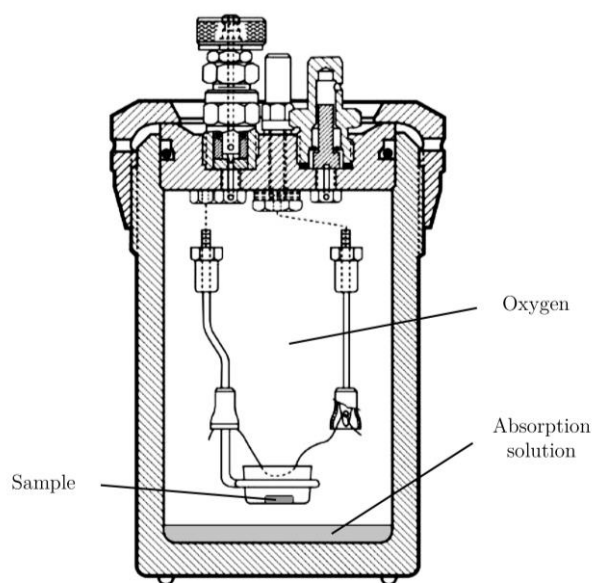


Figure 1.10: Oxygen combustion bomb [114].

1.4.1.3 Pyrohydrolysis

Pyrohydrolysis was first proposed by Warf et al. in 1954 [115]. A platinum boat with a non-combustible sample mixed with an accelerator is inserted into a reaction tube. The reaction tube is placed in an electric furnace ($T \approx 1100^\circ\text{C}$) and a superheated water steam is passed over it. Fluorine is released in the form of gaseous HF and forms hydrofluoric acid in the presence of water vapour, which is condensed in a receiver containing alkaline absorption solution (Fig. 1.11) [105, 116, 117]. The general reaction of the pyrohydrolysis of MF_{2n} ($n = 1/2, 1, 3/2$) can be expressed by Eq. 1.5 [115].



The function of the accelerator (tungsten trioxide (WO_3), triuranium octaoxide (U_3O_8), aluminium oxide (Al_2O_3), silicon dioxide (SiO_2), vanadium(V) oxide (V_2O_5), chromium(III) oxide (Cr_2O_3) or cerium(IV) oxide (CeO_2)) is to speed up the decomposition of particularly resistant materials [105]. WO_3 is most commonly used, and the reaction with MF_{2n} ($n = 1/2, 1, 3/2$) proceeds by Eq. 1.6 [118].

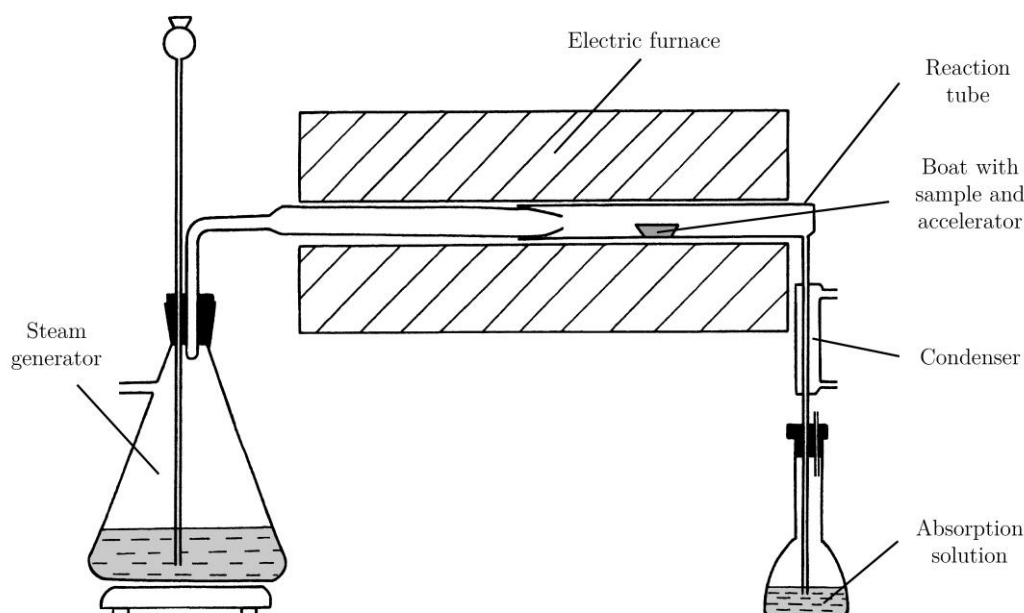
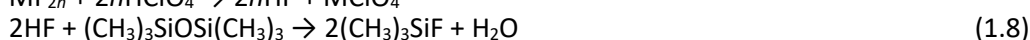


Figure 1.11: Pyrohydrolysis apparatus.

1.4.1.4 Diffusion as Trimethylfluorosilane

A method for the separation of fluorine based on the diffusion of gaseous trimethylfluorosilane was first introduced by Taves in 1968 [119]. The diffusion can be performed in a Conway diffusion cell with two concentric compartments, which is covered by a polypropylene lid. The sample is placed in the inner compartment of the diffusion cell, to which a mixture of perchloric acid (HClO_4) and hexamethyldisiloxane (HMDS; $(\text{CH}_3)_3\text{SiOSi}(\text{CH}_3)_3$) is added. The formed trimethylfluorosilane (TMFS; $(\text{CH}_3)_3\text{SiF}$), which is highly volatile and readily escapes the acid solution, is absorbed in the outer compartment of the diffusion cell containing alkaline solution [120, 121] (Fig. 1.12). The general reaction for the decomposition of MF_{2n} ($n = 1/2, 1, 3/2$) can be expressed by Eqs. 1.7 and 1.8 [121].



In the absorbing solution, TMFS reacts with NaOH according to Eq. 1.9, absorbing fluoride and releasing trimethylsilanol ($(\text{CH}_3)_3\text{SiOH}$) [121].

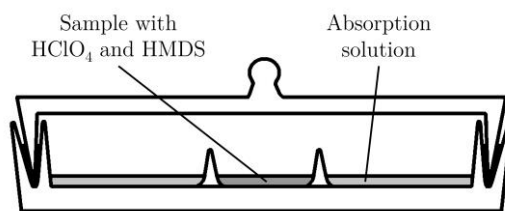


Figure 1.12: Conway diffusion cell.

1.4.2 Methods for the Determination of Fluoride

1.4.2.1 Potentiometry

The introduction of the fluoride ion selective electrode (F-ISE) by Frant and Ross in 1966 [122] constitutes the most significant advance in the determination of F^- ions. The F-ISE has become the most frequently used method for fluoride determination because it is easy to use, cost-effective, highly selective and covers a wide range of concentrations (10^{-5} – 1 mol/l).

The F-ISE is a membrane indicator electrode that consists of a crystal of lanthanum fluoride (LaF_3) doped with europium(II) fluoride (EuF_2) to improve conductivity. Electrode potential depends on the activity of the F^- ions in the sample solution. In ionic solutions of electrolytes, the F^- activity differs from the F^- concentration by a factor called the activity coefficient of F^- ions. The activity coefficient is a function of the total ionic strength of the solution and is therefore usually unknown. By adding sufficiently concentrated electrolyte to the sample solution, the total ionic strength of the solution becomes high and constant. In this case, the activity coefficient is constant, and the electrode potential (E) can be expressed in terms of the F^- concentration in the sample solution (c_F) according to Eq. 1.10 [106, 123].

$$E = I - S \log c_F \quad (1.10)$$

The F^- concentration is usually determined from a calibration curve by plotting the electrode potential against the logarithm of concentrations of standard F^- solutions. The constant S is the slope of the calibration curve and is 58–60 mV per decade when the solution temperature is $(20 \pm 5)^\circ\text{C}$. The constant I is the intercept of the calibration curve and is the sum of several constants, i.e. the F^- activity of internal solution, asymmetry potential, junction potential, reference electrode potential and activity coefficient of F^- [106].

There are some circumstances when the measured concentration may be lower or higher. (1) The measured concentration is lower in acid solutions ($\text{pH} < 5$) because F^- is protonated, forming HF and HF_2^- , which cannot be detected by F-ISE. (2) The measured concentration is higher in alkaline solutions ($\text{pH} > 8$) because the electrode also responds to OH^- ions due to their similar charge and ionic radii [122]. (3) The measured concentration is lower if the sample solution contains elements that can form stable complexes with F^- (Al^{3+} , Fe^{3+}). To overcome these interferences, total ionic strength adjustment buffer (TISAB) is added to calibration standards and sample solutions before the determination of F^- by F-ISE [124]. TISAB contains electrolytes in high concentrations, a pH buffer to adjust pH between 5–8 and masking agents to complex interfering ions (DCTA (diaminocyclohexanetetraacetic acid) [100, 125], citrate [126, 127], tris (tris(hydroxymethyl)aminomethane) [103]).

1.4.2.2 Spectrophotometry

Spectrophotometry is used for the quantitative determination of a number of inorganic and organic compounds that absorb ultraviolet or visible radiation. A beam of monochromatic light with radiation power P_0 passes through the sample solution in a cuvette. Due to the absorption of the light by the solution, the radiation power of the beam decreases from P_0 to P . According to Beer's law, absorbance (A) is proportional to the molar absorptivity (ϵ), path length of the absorbing medium (b) and concentration of the absorbing species in the sample solution (c) (Eq. 1.11) [106].

$$A = \log \frac{P_0}{P} = \epsilon bc \quad (1.11)$$

Spectrophotometry is widely used for the determination of F^- ions, and two methods have been employed most frequently. In the first method, F^- ions replace organic ligands in coloured metal-ligand complexes to give a colourless metal-fluoride complex and a free ligand with a different colour. The most frequently used metal-ligand complex is Zr-SPADNS [128], which was first reported by Bellack and Schouboe in 1958 [129], and the reaction with F^- ions can be expressed by Eq. 1.12.



Other metal-ligand complexes include Al-chrome azurol B [130], Al-malachite green [130], Zr-xyleneol orange [131], Al-xyleneol orange [132] and La-chloranilate [133].

The second method is based on the reaction of F^- ions with a coloured metal-ligand complex, producing a ternary complex of a different colour. The most commonly used metal-ligand complex is La-alizarin complexone [134].

1.4.2.3 Atomic Absorption Spectroscopy

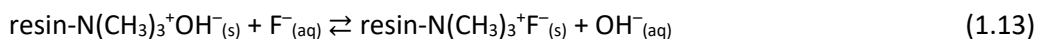
Atomic absorption spectroscopy is used for the determination of trace metals using the absorption of light by free atoms in the gaseous state. In classical line source atomic absorption spectroscopy (LS AAS), the element of interest in the sample solution is atomized in a flame or graphite furnace. The atoms in the gaseous phase are irradiated by a line radiation source (hollow cathode lamp). The transmitted light then passes through a monochromator and is finally measured by a detector. High resolution is provided by the narrow emission line of the line radiation source; therefore, only a medium-resolution monochromator is necessary [106]. LS AAS cannot be used for direct determination of fluorine [135] because its absorption lines are located in the vacuum ultraviolet region of the electromagnetic spectrum, which is not accessible by LS AAS. Fluorine can be determined by LS AAS indirectly by generating diatomic metal monofluoride molecules with absorption lines in the ultraviolet or visible range of the electromagnetic spectrum. However, an appropriate hollow cathode lamp may not be available, as the selected emission line may not precisely overlap with the absorption line of the diatomic molecule, resulting in low resolution.

In high resolution continuum source atomic absorption spectrometry (HR-CS AAS), the atoms are irradiated by a continuum radiation source (xenon short-arc lamp), emitting a spectrum over the entire spectral range. It is therefore crucial to work with a high-resolution double monochromator with a prism for pre-dispersion and an echelle grating. With the introduction of HR-CS AAS, fluorine can be determined indirectly by generating diatomic molecules [135]. The gaseous metal monofluoride molecules (gallium monofluoride (GaF) [136, 137], calcium monofluoride (CaF) [138], strontium monofluoride (SrF) [139] or aluminium monofluoride (AlF) [140]) are generated in a flame or graphite furnace in the presence of an excess of metal, and the molecular absorption is measured.

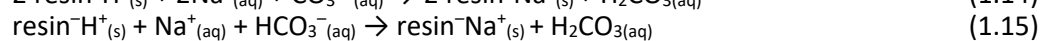
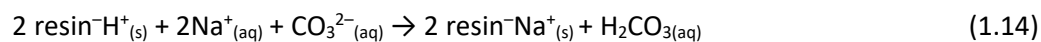
1.4.2.4 Ion Chromatography

Ion chromatography is used for the separation and quantitative analysis of anions and cations. The ions in the sample solution are carried through a column packed with ion-exchange resin by the flow of an eluent. The ions attach and detach from the resin according to their affinities to the active sites. Conductivity, which is proportional to the concentration of eluting ions, is measured by a detector at the end of the column [106].

Ion chromatography for F^- determination presents some problems [141]. Because F^- is very weakly retained by anion-exchange resins, its peak tends to overlap with the water dip negative peak. Another problem is interference from monocarboxylic acids that are co-eluted with or only partly resolved from F^- . A combination of strong base anion-exchange resin (with quaternary ammonium groups ($-\text{N}(\text{CH}_3)_3^+\text{OH}^-$)) and weak eluent (mixture of Na_2CO_3 and NaHCO_3) has shown increased F^- retention and therefore effective separation of the fluoride peak [111, 112]. The reaction on the anion-exchange resin can be illustrated by Eq. 1.13.



The conductivity of the mobile phase is due to high electrolyte concentrations so high that it swamps the conductivity of the analyte ions, greatly reducing detector sensitivity. Therefore, a suppressor column (acid form of cation-exchange resin) has to be introduced after the ion-exchange column, so that the ions of eluent are converted to molecular species and the conductivity is reduced. For $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ solution as the eluent, the reaction on the suppressor column can be illustrated by Eqs. 1.14 and 1.15 [106].



Chapter 2

Aims

Fluoride is ubiquitous in the environment, it is present in the soil, air, water and subsequently in plants, animals and humans. It is toxic to plants causing necrosis and chlorosis and it is also toxic to animals and humans as it can cause fluorosis and damage to various body tissues and organs. Therefore, accurate methods for fluorine determination are of crucial importance. A typical fluorine analysis consists of a total sample decomposition and determination of F^- ions in aqueous solution. The aim of the first part of this thesis (Chapters 4.1, 4.2 and 4.3) was to gain a broader understanding of some aspects of fluorine analysis, i.e. the choice of a total sample decomposition method and evaluation of measurement uncertainty. The aim of the second part (Chapters 4.4 and 4.5) was to determine the F content in some samples with plant matrix, i.e. plants in the vicinity of pollution source and plant-based superfoods.

The most frequently used method for fluorine determination nowadays is F-ISE. This method relies on the determination of fluorine in the form of the free F^- ion in aqueous solution, therefore total sample decomposition is necessary prior to F^- measurement. Several methods for sample decomposition have been proposed in the literature; however, the majority of papers have not incorporated the analysis of the certified reference material (CRM), resulting in a reasonable doubt in the reported results. The aim of our study was therefore to check the applicability and accuracy of three methods (alkaline carbonate fusion, oxygen bomb combustion and pyrohydrolysis) for total decomposition of soil and plant samples by the analysis of appropriate CRMs (Chapters 4.1 and 4.2). In addition, we were interested in the reactions proceeding during these decomposition methods, since there is only limited information available in the literature. Thus, another aim was to suggest possible reactions of metal fluorides proceeding during these three decomposition methods and to estimate the Gibbs energies in order to establish whether these reactions are thermodynamically favourable or not (Chapter 4.1).

Obligatory part of every analysis is the evaluation of measurement uncertainty (MU). MU has generally been expressed as one standard deviation, thus including only random effects. We wanted to estimate also systematic effects by the analysis of appropriate CRM. An approach for MU evaluation where in addition to random effects, systematic effects are also considered, and all uncertainty components of each step of the analytical procedure are identified and quantified, is described in the "Guide to the Expression of Uncertainty in Measurement" (GUM). The aim was therefore to develop a procedure for MU evaluation according to the GUM for fluorine determination in plant samples by F-ISE after prior total sample decomposition (Chapter 4.3).

The newly acquired knowledge regarding total sample decomposition methods and MU evaluation was applied to some samples with plant matrix. The F content in plants is generally low; however, in fluoride polluted areas, plants can uptake considerable amounts of fluoride. In the vicinity of the Rogaška glassworks (Rogaška Slatina, Slovenia), where hydrofluoric acid is used for polishing and etching of the glass, necrotic and chlorotic lesions were observed on some plants. The initial sampling and F determination was conducted by another laboratory and showed low F content, thus the reason for the lesions remained unknown. On the request of the inter-municipal inspector of the area, our laboratory repeated the sampling of different plants species in the vicinity of the glassworks and determined the F content. The aim of the study was to confirm or disprove the uncontrolled release of gaseous fluorides from the glassworks and to

evaluate if the consumption of forage and crops in the influenced area can pose a health risk to animals or humans (Chapter 4.4).

After this study, we searched for other samples with plant matrix that could contain elevated F content. We focused on food samples as diet is considered as an important source of fluoride for adults. While examining different teas and herbs that are sold in the supermarket, we noticed a wide selection of so-called superfoods. This caught our attention, because superfoods have become increasingly popular in recent years due to their supposed extraordinary health benefits. However, their packaging labels do not include any quality certificates and there is practically no information on their F content in databases or scientific papers. The aim was therefore to determine the F content in several plant-based superfoods available on the Slovenian market and to establish if F intake through the consumption of superfoods can significantly contribute to the intake of fluorine (Chapter 4.5).

Chapter 3

Methodology

Fluorine analysis involves five steps: (1) sampling, (2) physical sample preparation, (3) total sample decomposition, (4) measurement of the F^- concentration and (5) interpretation of the results. In physical sample preparation, samples are dried on polyethylene trays in a Heraeus T6 laboratory oven at 60 °C, ground in an agate ball mill and finally passed through a 0.25 mm sieve. Three methods for total sample decomposition are used in our laboratory: alkaline carbonate fusion, oxygen bomb combustion and pyrohydrolysis. Each sample is decomposed at least three times. The F^- concentration in the aqueous solution after sample decomposition is measured by F-ISE at least twice.

3.1 Alkaline Carbonate Fusion

The sample is powdered and weighed into a platinum crucible. It is mixed with 1 ml of 50 % NaOH solution, and the mixture is evaporated to dryness on a sand bath. The alkaline sample is then decomposed by fusion with approximately 6 g of Na_2CO_3 (Fig. 3.1 and Fig. 1.9). The fusion is heated with a modified Bunsen burner slowly, carefully and evenly to avoid losses due to small particles being blown away and to prevent ignition of the sample. The fusion is completed when a transparent melt without any solid remains is obtained. The crucible is then swirled gently so that, as the melt is cooling, a thin layer of solid melt is formed on the inner walls. The solid melt is cracked into small pieces and quantitatively transferred to a 100 ml volumetric flask with distilled water. Before the determination of F^- by F-ISE, the melt is dissolved and the solution acidified to pH 2–3 by the addition of concentrated H_2SO_4 .



Figure 3.1: Evaporation on a sand bath (left) and fusion with Na_2CO_3 (right).

3.2 Oxygen Bomb Combustion

The decomposition is performed in an 1108 Oxygen Combustion Bomb from Parr Instrument Company. A combustible sample is powdered, pressed into a pellet (8 tons), weighed and then placed into a combustion capsule. Compression into a pellet is necessary because small particles can be easily blown out of the combustion capsule during violent combustion, causing incomplete combustion. A fuse wire is fastened between the two electrodes. The combustion capsule is placed in the electrode loop and the wire is bent downwards toward the surface of the pellet so that the loop of wire holds the pellet in place. Absorption solution (20 ml of 0.5 M NaOH) is placed in the bottom of the bomb. The bomb is assembled and closed (Fig. 3.2 and Fig. 1.10). The oxygen tank and ignition unit are connected to the bomb, and the gas outlet valve is closed. The bomb is pressurized with 30 atm oxygen, immersed in a cold water bath and checked for gas leaks before firing. If no bubbles are released from any part of the bomb, the bomb is fired by pressing the firing button on the ignition unit. The bomb is left in the water bath for 30 minutes to cool and then at room temperature for 90 minutes. It is shaken intensively every 30 minutes. Then pressure is released slowly and the bomb is opened. All interior surfaces of the bomb and combustion capsule are washed with distilled water. All washings are collected and quantitatively transferred to a 100 ml volumetric flask. Before the determination of F^- by F-ISE, the solution is acidified to pH 2–3 by the addition of concentrated H_2SO_4 and filtered to remove particles of ash.



Figure 3.2: Oxygen combustion bomb (left) and combustion capsule with sample pellet and a wire between two electrodes (right).

3.3 Pyrohydrolysis

A non-combustible powdered sample is weighed into a platinum boat and mixed with 0.5–1 g of WO_3 accelerator. The boat is inserted into a reaction tube, which is placed in an electric furnace. The temperature of the furnace is controlled with a variable transformer and is gradually increased to approximately 1100 °C. The entrance of the reaction tube is connected to a steam generator. The steam generator is an Erlenmeyer flask containing water that is heated by an electric hot plate. The outlet of the reaction tube is connected to a water-cooled condenser with a brass jacket. The tip of the condenser is immersed into an absorption solution (20 ml of 0.1 M NaOH) placed in a 100 ml volumetric flask (Fig 3.3 and 1.11). The sample is pyrohydrolysed for approximately 30 minutes.



Figure 3.3: Pyrohydrolysis apparatus.

3.4 Fluoride Ion Selective Electrode

An Orion 960 Autochemistry System with an Orion 96-09 Combination F-ISE is used for potentiometric determination of F^- by a calibration curve method (Fig. 3.4). Calibration standards are prepared in 50 ml polyethylene volumetric flasks from an appropriate amount of certified 100 $\mu g/ml$ F^- solution and 25 ml of TISAB buffer. Generally, two calibration standards are used, 0.2 and 1.0 μg F^-/ml or 3.0 and 4.0 μg F^-/ml , depending on the predicted F^- concentration in the sample solution. The accuracy of the calibration curve is checked after its construction and then every 2 hours using 100 $\mu g/ml$ certified F^- solution from another bottle. The sample solutions for the measurement of the F^- concentration are prepared in 50 ml polyethylene volumetric flasks from appropriate sample aliquots obtained after total decomposition and 25 ml of TISAB buffer.

The TISAB buffer is prepared in a 2 l volumetric flask according to Peters et al. [142]. First, 35.3 g of DCTA is dissolved in 1 l of distilled water and 50 % NaOH is added dropwise until it is dissolved. Then, 600 g of trisodium citrate dihydrate and 120 g of sodium chloride (NaCl) are added. The solution is diluted to 1.9 l, the pH is adjusted with concentrated hydrochloric acid (HCl) to 6.0 and water is added to the mark.



Figure 3.4: Orion Autochemistry System (left) and Orion combination F-ISE (right).

Chapter 4

Results and Discussion

4.1 Thermochemistry of Reactions Proceeding During Total Sample Decomposition

In this chapter the article entitled "Paradoxes and paradigms: observations on pyrohydrolysis, oxygen bomb combustion, and alkaline carbonate fusion, most frequently used decomposition methods for subsequent determination of fluorine and accompanying thermochemistry" by D. Štepec, M. Ponikvar-Svet and J. F. Liebman, published in Structural Chemistry in 2018, is presented [113]. D. Štepec, the author of this thesis, performed the experiments, analysed the data and participated in the interpretation of the results and writing of the manuscript.

Alkaline carbonate fusion, pyrohydrolysis and oxygen bomb combustion are widely used methods for total sample decomposition. Pyrohydrolysis is suitable for the decomposition of non-combustible samples only, oxygen bomb combustion is suitable for the decomposition of combustible samples only and alkaline carbonate fusion is suitable for the decomposition of both non-combustible and combustible samples. To check the applicability and accuracy of these three methods, the certified reference material (CRM) "Fluorine in clay" (BCR-461 from the Institute of Reference Materials and Measurements – IRMM) was decomposed by alkaline carbonate fusion and pyrohydrolysis, and the CRM "Fluoride in vegetation" (SRM-2695 from the National Institute of Standards and Measurements – NIST) was decomposed by alkaline carbonate fusion and oxygen bomb combustion. The F^- concentration was then measured by F-ISE. Good agreement was found between the obtained and certified values for both CRMs, indicating that alkaline carbonate fusion and pyrohydrolysis are applicable for the determination of fluorine in soil samples, and alkaline carbonate fusion and oxygen bomb combustion are applicable for the determination of fluorine in plant samples.

Limited information is available in the literature about the reactions of MF_{2n} proceeding during total decomposition by alkaline carbonate fusion, pyrohydrolysis and oxygen bomb combustion. Therefore, possible reactions of selected MF_{2n} (AgF , BaF_2 , CaF_2 , CdF_2 , CoF_2 , CrF_3 , CuF_2 , FeF_2 , FeF_3 , MgF_2 , MnF_2 , NiF_2 , PbF_2 , ZnF_2) were proposed and their Gibbs energies were calculated to establish if these reactions are thermodynamically favourable [143]. The Gibbs energies for the reactions of MF_{2n} proceeding during alkaline carbonate fusion and pyrohydrolysis were negative, indicating that these reactions are thermodynamically favourable. However, Gibbs energies for the reactions of MF_{2n} proceeding during oxygen bomb combustion were positive, suggesting that these reactions are not thermodynamically favourable and that fluorine remains bound.



Paradoxes and paradigms: observations on pyrohydrolysis, oxygen bomb combustion, and alkaline carbonate fusion, most frequently used decomposition methods for subsequent determination of fluorine and accompanying thermochemistry

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Abstract

Pyrohydrolysis, oxygen bomb combustion, and alkaline carbonate fusion are the most frequently used methods for decomposition of fluorine containing materials. The efficiency of these methods was proven by the determination of fluorine content in certified reference materials of clay and vegetation. Possible reactions proceeding during decomposition were suggested and accompanying thermochemistry discussed. The Gibbs energies were estimated to establish if suggested reactions are thermodynamically favorable or not. In addition, linear relationships between the enthalpies of formation of metal fluorides and the balanced values of the enthalpies of formation of the plausible reaction products (metal tungstates, metal oxides, or metal carbonates), electronegativity of metals, and number of fluorine atoms in metal fluorides were established. These equations were suggested for the estimation of the enthalpies of formation of metal tungstates, metal oxides, or metal carbonates, for which experimental data are not available.

Keywords Fluorine · Decomposition · Thermochemistry · Regression analysis

Introduction

Fluorine is one of several trace elements receiving much attention because small amounts of fluoride have proven benefits for dental health, while continuous exposure to high intakes can result in severe adverse effects in humans (and animals), which include development of dental fluorosis in children or skeletal fluorosis in both, children and adults [1–3]. Fluoride emissions represent a threat to vegetation and subsequently entire food chain in many parts of the world.

Monitoring of human and environmental load with fluorine is therefore prerequisite to avoid its negative impacts. A brief note on terminology is in order. In this article, the term “fluorine” (F) is used to denote the element in any of its forms and “fluoride” (F[−]) to denote predominant chemical form inorganic fluoride in which the element is found in the nature.

At most used methods for determination of F[−] nowadays are fluoride ion selective electrode (ISE) and ion chromatography (IC) [4]. The ISE remains a method of choice for determination of F[−] because it is cost-effective, highly selective, and covers a wide range of concentrations (1–10^{−5} mol l^{−1}). Because ISE responds to free F[−] ions only, F-containing samples must be decomposed first in order to assure release of F from inorganic and organic compounds and enable formation of F[−]. Different acids and mixtures of acids dissolve the F in samples only to varying degrees. On the other hand, total decomposition methods of the sample assure decomposition of entire F and enable a comparison of the results from different studies. Among the various decomposition methods, the most important nowadays are pyrohydrolysis [5, 6], oxygen bomb combustion [7, 8], and fusion with alkali metal hydroxides or carbonates [9, 10].

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While pyrohydrolysis is appropriate for the decomposition of noncombustible samples only, like raw materials or soil, oxygen bomb combustion is applicable to the decomposition of combustible samples only, like vegetation. Fusion with alkaline carbonates is applicable to (non)combustible samples and can be used as a reference method to pyrohydrolysis or oxygen bomb combustion method.

There is a lack of literature related to reactions proceeding during decomposition of fluorine-containing compounds and related thermochemistry. In this paper, we report on determination of the contents of fluorine in soil and vegetation samples with ISE after preceding total decomposition with three different methods (pyrohydrolysis, oxygen bomb combustion, alkaline carbonate fusion). The results were verified by the analyses of certified reference materials (CRMs). Chemistry of plausible decomposition reactions and associated thermochemistry are discussed.

Experimental

All reagents were of analytical grade and all solutions were prepared using double-distilled water. Approximately 1 g of soil, spruce needles, or CRM was weighed prior to the decomposition. Pyrohydrolysis [11] and alkaline carbonate fusion decompositions [12, 13] were conducted as reported previously. In oxygen bomb combustion method, the sample was ignited at 30 atm oxygen (Messer, 99.99%) in a Model 1108 Oxygen Combustion Vessel (Parr Instrument Company) containing 20 ml of 0.5 M NaOH absorption solution (prepared by dissolving reagent pellets (Merck) in water). After firing, the bomb was left standing for 2 h. So obtained sample was quantitatively transferred into a 100-ml volumetric flask and before analysis acidified (H_2SO_4 , Merck) and filled with water to the mark. The contents of fluoride in decomposed samples were determined in corresponding aliquots by direct potentiometry using fluoride ISE (Orion model 96-09), calibrated at 0.2 and $1.0 \mu\text{g ml}^{-1}$, on Orion 960 autochemistry system with a temperature sensor following the previously reported procedure [14].

The accuracy and precision of the methods employed were checked by the analysis of a certified reference material, BCR-461, Fluorine in clay (Institute for Reference Materials and Measurements) and SRM-2695, Fluoride in Vegetation (National Institute of Standards and Technology), consisting of a standard with a high and a low level of fluoride.

The statistical analysis of the data was made by regression analysis using OriginPro 7.5 program from OriginLab Corporation. The “adjusted R square” (R^2), which in comparison to “ R square” (R^2) increases only if a new term improves the model more than would be expected by chance, was used to estimate the goodness-of-fit [15].

Results and discussion

The total F contents were determined in samples of soil and vegetation by the ISE after preceding total decomposition. Incombustible soil sample was decomposed by pyrohydrolysis and combustible spruce needles by oxygen bomb combustion. As a reference decomposition method, alkaline carbonate fusion with NaKCO_3 was employed for both soil and spruce needles. The accuracy of the methods was verified by the analyses of CRMs with matrix and fluorine level comparable to the samples. For soil sample, BCR-461 and for vegetation samples, SRM-2695 was used. The results of the analyses are presented in Table 1.

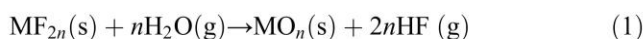
The results from the analyses of the CRMs were compared to the certified values using statistical test recommended by the European Reference Materials [16]. For both CRMs, the results of determinations agreed well with the certified values. This is a proof of high accuracy and precision of the F determinations in soil and vegetation material with no interferences caused by the sample matrix.

There is a lack of literature related to the reactions proceeding during decomposition of fluorine-containing compounds and their thermochemistry. Thus, thermochemistry of plausible reactions proceeding during each type of decomposition was studied and discussed.

Pyrohydrolysis

High-temperature hydrolysis (pyrohydrolysis) was introduced by Warf et al. in 1954 [17]. It is a fast, reliable, and therefore very convenient method for decomposition of incombustible solid samples. The decomposition proceeds in the reaction tube by passing over a boat containing a sample a superheated steam at around 700–800 °C or even higher (≈ 1100 °C). The distillate is cooled and collected in the receiver containing absorbing solution. The susceptibility to pyrohydrolysis for different fluorides was found to decrease in the following order: CuF_2 , FeF_3 , AgF , FeF_2 , CrF_3 , ZnF_2 , NiF_2 , CoF_2 , CdF_2 , PbF_2 , MnF_2 , MgF_2 , CaF_2 , BaF_2 [17].

The general reaction of pyrohydrolysis of metal fluorides (MF_{2n} , $n = 1/2, 1, 3/2$) can be expressed as follows [17]:



The resulting oxides in addition react with accelerator. The WO_3 , U_3O_8 , Al_2O_3 , Cr_2O_3 , CeO_2 , SiO_2 , V_2O_5 , and the salt $\text{Na}_2\text{W}_2\text{O}_7$ have all been recommended as accelerators to speed up the reactions of decomposition but the first two are most commonly used [10]. The reactions with WO_3 proceed as follows [18]:

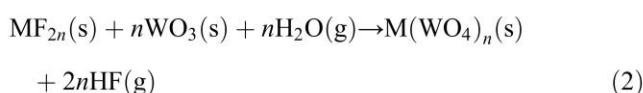
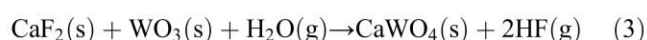


Table 1 The contents of fluorine determined in CRMs and samples

Sample	$w(\text{F})$ (mg kg ⁻¹)			
	Certified value ^a	Pyrohydrolysis ^b	Oxygen bomb combustion ^b	Alkaline carbonate fusion ^b
BCR-461	568 ± 60	569 ± 4	/	579 ± 4
Soil	/	423 ± 38	/	465 ± 27
SRM-2695, high level	277 ± 27	/	267 ± 7	295 ± 6
SRM-2695, low level	64.0 ± 8.4	/	62.3 ± 2.6	68.0 ± 1.7
Spruce needles, high level	/	/	195 ± 4	219 ± 5
Spruce needles, low level	/	/	59.2 ± 1.1	67.8 ± 1.2

^a The uncertainties of certified values are given at 95% level of confidence^b Mean of three and five parallel determinations for the samples and CRMs, respectively, ± standard deviation of measurements

The enthalpy (ΔH), entropy (ΔS), and Gibbs energy (ΔG) have been estimated for the reactions of MF_{2n} as studied by Warf et al. [17]. All available data were taken from the Wagman compendium of measured quantities [19]. The ΔG for the reactions of all MF_{2n} are negative indicating that pyrohydrolysis is a thermodynamically favored reaction. Estimation of ΔG is now briefly discussed on an example of CaF_2 :



Under standard temperature and pressure (STP), the ΔH of this reaction is 117 kJ mol⁻¹. According to Campbell's rule [20–22], the entropy of the reaction can be estimated as 140 J/(K mol rxn) for each net mole of gas produced in the balanced chemical reaction. In our reaction, 1 mol of gas is produced and by multiplying the ΔS by the temperature of interest ($T_{\text{decomposition}} = 1373$ K) and subtracting it from the ΔH , the negative ΔG is estimated (−75 kJ mol⁻¹).

Some other aspects of thermochemistry of exemplary reactions proceeding during pyrohydrolysis in the presence of other accelerators were discussed by two authors of the current paper [11] (JFL and MPS) and will be therefore not discussed in detail.

We recall now the brief assertion [23] that for sulfur-fluorine species that “the replacement of OH by F results in an increase of ΔH_f° of about 7 kcal” (30 kJ) and the subsequent discussions of this suggested near constant change for numerous hydroxides (oxides) and fluorides [24–27]. It was also extended to the understanding gas phase organofluorine species [28, 29] and gas phase binary fluorides [30]. But what about the solid phase metal fluorides and tungstates that are of interest in the current study? Is it possible to assume that there is a relationship between them?

An attempt to establish a relationship between the enthalpy of formation (ΔH_f°) of metal fluorides and of metal tungstates ($\text{M}(\text{WO}_4)_n$) was made using regression analysis.

In addition to $\Delta H_f^\circ(\text{M}(\text{WO}_4)_n)$ as a parameter, also the enthalpies of formation of other reactants and products, which were all balanced according to the stoichiometry of the reactions (ΔH_f°), electronegativity of the metals (χ) as defined by Allen [31], and a number of fluorine atoms in metal fluorides $n(\text{F})$ were considered. Thus, the equations for the estimation of the enthalpy of formation of metal fluorides ($\Delta H_f^\circ(\text{MF}_{2n}(\text{s}))_{\text{calc.}}$) were suggested. The MF_{2n} considered in this study and the studied parameters are listed in Table 2; the arrangement of species following that is given by Warf et al. [17].

By simple linear regression analysis using the data from Table 2, Eq. 4 was suggested for the ΔH_f° s of metal fluorides depending on the ΔH_f° s of metal tungstates:

$$\begin{aligned} \Delta H_f^\circ(\text{MF}_{2n}(\text{s}))_{\text{calc.}} &= (294.8 \pm 92.3) \\ &+ (0.8820 \pm 0.0710) \cdot \Delta H_f^\circ(\text{M}(\text{WO}_4)_n(\text{s})) \\ R^2 &= 0.9504 \end{aligned} \quad (4)$$

Further consideration of the $\Delta H_f^\circ(\text{HF}(\text{g}))$, $\Delta H_f^\circ(\text{H}_2\text{O}(\text{g}))$, and $\Delta H_f^\circ(\text{WO}_3(\text{s}))$ with ΔH_f° of −271, −242, and −843 kJ mol⁻¹, respectively showed that these parameters are correlated because of the reaction stoichiometry; it is adequate to use only one of them to establish the next equation. Thus, Eq. 5 was calculated choosing $\Delta H_f^\circ(\text{HF}(\text{g}))$ as an additional parameter:

$$\begin{aligned} \Delta H_f^\circ(\text{MF}_{2n}(\text{s}))_{\text{calc.}} &= (16 \pm 102) + (1.0809 \pm 0.0745) \cdot \Delta H_f^\circ(\text{M}(\text{WO}_4)_n(\text{s})) \\ &- (1.030 \pm 0.307) \cdot \Delta H_f^\circ(\text{HF}(\text{g})) \\ R^2 &= 0.9799 \end{aligned} \quad (5)$$

In addition, the electronegativity was considered as a parameter:

$$\begin{aligned} \Delta H_f^\circ(\text{MF}_{2n}(\text{s}))_{\text{calc.}} &= (366 \pm 323) + (1.435 \pm 0.319) \cdot \Delta H_f^\circ(\text{M}(\text{WO}_4)_n(\text{s})) \\ &- (1.891 \pm 0.814) \cdot \Delta H_f^\circ(\text{HF}(\text{g})) - (224 \pm 197) \cdot \chi \\ R^2 &= 0.9809 \end{aligned} \quad (6)$$

Table 2 Standard enthalpies of formation of some MF_{2n} , and parameters associated to the reactions proceeding during pyrohydrolysis, oxygen bomb combustion, or alkaline carbonate fusion (balanced values for the enthalpies of formation of $\text{MF}_{2n}(\text{s})$, $\text{M}(\text{WO}_4)_n(\text{s})$, $\text{MO}_n(\text{s})$, $\text{M}(\text{CO}_3)_n(\text{s})$, $n(\text{F})$, and χ)

Cation (M)	ΔH_f° (kJ mol ⁻¹)	$\Delta H_f^{\circ\circ}$ (kJ mol ⁻¹)			$n(\text{F})$	χ^a [32, 33]
	$\text{MF}_{2n}(\text{s})$	$\text{M}(\text{WO}_4)_n(\text{s})$	$\text{MO}_n(\text{s})$	$\text{M}(\text{CO}_3)_n(\text{s})$		
Cu ^{II}	-543	-1105	-157		2	1.85
Fe ^{III}	-990 [34]		-412		3	1.80
Ag ^I	-205	-463	-16	-253	1	1.87
Fe ^{II}	-711	-1155	-272	-741	2	1.80
Cr ^{III}	-1159		-570		3	1.65
Zn ^{II}	-764	-1226	-348	-813	2	1.59
Ni ^{II}	-651	-1133	-240		2	1.88
Co ^{II}	-692		-238	-713	2	1.84
Cd ^{II}	-700		-258	-751	2	1.52
Pb ^{II}	-664		-217	-699	2	1.854
Mn ^{II}	-860 [35]	-1305	-385	-894	2	1.75
Mg ^{II}	-1123	-1533	-602	-1096	2	1.293
Ca ^{II}	-1220	-1645	-635	-1207	2	1.034
Ba ^{II}	-1207	-1703	-554	-1216	2	0.881

^a Electronegativity is given in Pauling units

Finally, this equation was simplified by replacing $\Delta H_f^{\circ\circ}(\text{HF}(\text{g}))$ by a number of fluorine atoms in metal fluorides ($n(\text{F})$):

$$\Delta H_f^\circ(\text{MF}_{2n}(\text{s}))_{\text{calc.}} = (366 \pm 323) + (1.435 \pm 0.319) \cdot \Delta H_f^{\circ\circ}(\text{M}(\text{WO}_4)_n(\text{s})) + (513 \pm 221) \cdot n(\text{F}) - (224 \pm 197) \cdot \chi \quad (7)$$

$R^2 = 0.9880$

It is worth mentioning that the ratio between the coefficients of $n(\text{F})$ parameter (see Eq. 7) and $\Delta H_f^{\circ\circ}(\text{HF}(\text{g}))$ parameter (see Eq. 6) equals to $\Delta H_f^\circ(\text{HF}(\text{g}))$, i.e., -271 kJ mol⁻¹.

Already by considering only $\Delta H_f^{\circ\circ}(\text{M}(\text{WO}_4)_n(\text{s}))$ as a parameter, a surprisingly high value of $R^2 = 0.9504$ was calculated (Eq. 4). By adding additional parameters, the R^2 was gradually increasing and finally a value of 0.9880 was estimated (Eq. 7). The calculated values of $\Delta H_f^\circ(\text{MF}_{2n}(\text{s}))$ were compared to the available experimental values reported in Wagman [19] by constructing residuals ($\Delta H_f^\circ(\text{MF}_{2n}(\text{s}))_{\text{calc.}} - \Delta H_f^\circ(\text{MF}_{2n}(\text{s}))_{\text{exp.}}$) vs. order fit for Eqs. 4 and 7, see Fig. 1.

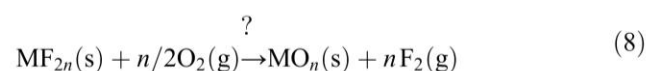
The largest difference in estimating $\Delta H_f^\circ(\text{MF}_{2n}(\text{s}))$ by using Eq. 4 is for AgF (91 kJ mol⁻¹) and by using Eq. 7 for CuF_2 (-65 kJ mol⁻¹). These differences represent about 45 and 12% of the experimental values, respectively. The differences in calculated values of ΔH_f° as opposed to the experimental values range for other $\text{MF}_{2n}(\text{s})$ between 0 and 25% (Eq. 4) and 0–6% (Eq. 7). Given our interest primarily in the current paper is the analytical chemistry (as opposed to thermochemistry) of inorganic fluorides, the aforementioned deviations are comfortably small. Equation 7 can be therefore suggested for the estimation of the enthalpies of formation of $\text{M}(\text{WO}_4)_n$ for which experimental data are not available.

Oxygen bomb combustion

Combustion in a closed vessel at high pressure was first suggested by Berthold in 1892 [10]. In this method, a powdered

sample is placed in an ignition capsule supported from the lid of the bomb. The bomb is filled with oxygen to the pressure of about 25–40 atm. The sample is ignited and the gaseous products are absorbed in a suitable solution in the bomb itself. This is a fast and simple method applicable to the decomposition of combustible samples.

In vegetation, fluorine is mainly present in the form of metal fluorides [36]. A plausible reaction of combustion of MF_{2n} can be written as follows:



The ΔH , ΔS , and ΔG have been then estimated for the reactions. The ΔG s are positive for reactions of all MF_{2n} studied. As such, reaction 8 is not expected to proceed as written and fluorine remains bound in the form of MF_{2n} .

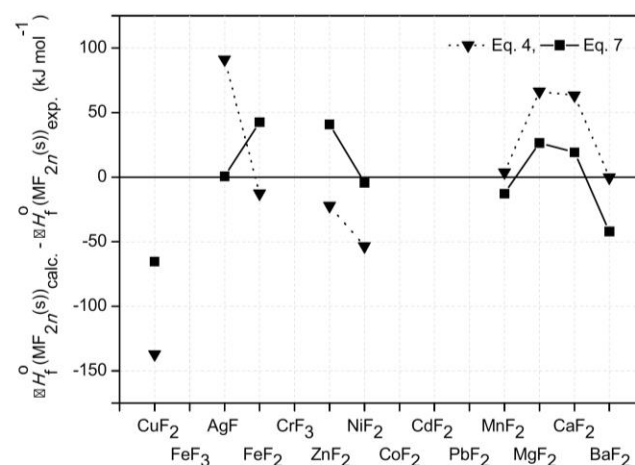
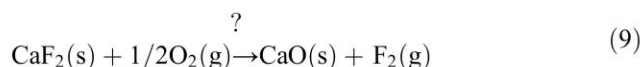


Fig. 1 Plot of differences between calculated and experimental values of $\Delta H_f^\circ(\text{MF}_{2n}(\text{s}))$ considering $\Delta H_f^{\circ\circ}(\text{M}(\text{WO}_4)_n(\text{s}))$ as a parameter (Eq. 4) and $\Delta H_f^{\circ\circ}(\text{M}(\text{WO}_4)_n(\text{s}))$, $n(\text{F})$ and χ as parameters (Eq. 7)

Estimation of ΔG is now briefly discussed on an example of combustion of CaF_2 :



Under STP, the reaction is endothermic by 585 kJ mol^{-1} and is favored entropically because there is 0.5 mol of gas more products than reactants. Let the temperature of the reaction be chosen as the “hotter than experimental practice and reality” 1500 K. By considering Campbell’s rule [20–22], the entropy increase over standard temperature is ca. $140 \cdot 0.5 \cdot 1500/1000 \approx 105 \text{ kJ mol}^{-1}$. This entropy change is not insignificant, but it is not large enough to favor the reaction thermodynamically ($\Delta G \approx 480 \text{ kJ mol}^{-1}$).

A possible relationship between the enthalpy of formation of MF_{2n} and of metal oxides (MO_n) was then investigated (the parameters listed in Table 2). In addition to $\Delta H_f^\circ(\text{MO}_n(\text{s}))$ as a parameter, also earlier suggested parameters $n(\text{F})$ and χ were considered. The following equations (Eqs. 10–12) were suggested:

$$\begin{aligned} \Delta H_f^\circ(\text{MF}_{2n}(\text{s})) &= (-279.5 \pm 33.1) + (1.5450 \pm 0.0843) \cdot \Delta H_f^\circ(\text{MO}_n(\text{s})) \\ R^2 &= 0.9626 \end{aligned} \quad (10)$$

$$\begin{aligned} \Delta H_f^\circ(\text{MF}_{2n}(\text{s})) &= (-148.3 \pm 58.1) + (1.4305 \pm 0.0827) \cdot \Delta H_f^\circ(\text{MO}_n(\text{s})) \\ &\quad - (82.7 \pm 32.3) \cdot n(\text{F}) \\ R^2 &= 0.9745 \end{aligned} \quad (11)$$

and

$$\begin{aligned} \Delta H_f^\circ(\text{MF}_{2n}(\text{s})) &= (-421 \pm 115) + (1.089 \pm 0.148) \cdot \Delta H_f^\circ(\text{MO}_n(\text{s})) \\ &\quad - (152.7 \pm 37.5) \cdot n(\text{F}) + (183.9 \pm 70.8) \cdot \chi \\ R^2 &= 0.9832 \end{aligned} \quad (12)$$

The calculated values of $\Delta H_f^\circ(\text{MF}_{2n}(\text{s}))$ were compared to the experimental values reported in Wagman [19] by constructing residuals vs. order fit for Eqs. 10 and 12, see Fig. 2.

The largest difference between calculated and experimental ΔH_f° values, when comparing Eqs. 10 and 12, can be observed for AgF . The differences are -99 and -42 kJ mol^{-1} , which represent 48 and 32% of the experimental value, respectively. The differences in calculated values of ΔH_f° as opposed to the experimental values range for other studied MF_{2n} between 0–7% (Eq. 10) and 1–6% (Eq. 12). Again, these deviations can be considered as comfortably small. Thus, both equations can be suggested for the estimation of the enthalpies of formation of MO_n for which experimental data are not available.

Alkaline carbonate fusion

This is a method of choice for decomposition of a variety of combustible or incombustible sample matrixes. An

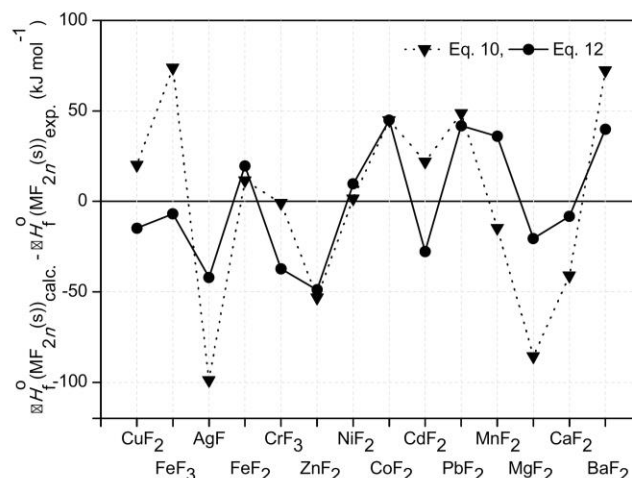
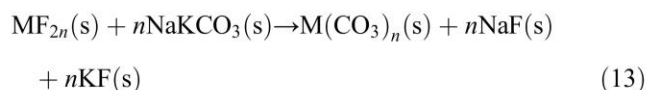


Fig. 2 Plot of differences between calculated and experimental values of $\Delta H_f^\circ(\text{MF}_{2n}(\text{s}))$ considering $\Delta H_f^\circ(\text{MO}_n(\text{s}))$ as a parameter (Eq. 10) and $\Delta H_f^\circ(\text{MO}_n(\text{s}))$, $n(\text{F})$, and χ as parameters (Eq. 12)

opening out procedure involving fusion with potassium carbonate was first described in 1767 by Marggraf [10]. An excess of alkali carbonate, usually sodium carbonate ($T_{m.p.} = 853^\circ \text{C}$), or the equimolar mixture of sodium and potassium carbonates ($T_{m.p.} = 712^\circ \text{C}$), the major advantage of which is lower melting point than either of the single compounds, is used for the fusion. Temperature and duration of the fusion depend on the nature of the sample and particle size. Use of carbonate fluxes is widespread in investigation and important method for dealing with insoluble or complex fluorides such as metal fluorides. During fusion, the cationic constituents of the sample are generally converted to acid-soluble carbonates or oxides and the anionic constituents are converted to soluble alkali metal salts.

The general reaction of decomposition of metal fluorides using NaKCO_3 can be expressed as follows:



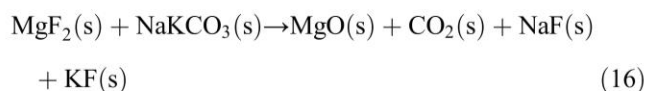
The ΔH , ΔS , and ΔG have been estimated for all metal fluorides studied. Since ΔH_f° and S° of NaKCO_3 were not found in Wagman [19] it was assumed that they can be calculated as mean values of Na- and K-salts. Under STP, the ΔG s are negative for the reactions of all MF_{2n} s. The exception to this is MgF_2 , CaF_2 , and BaF_2 .

Estimation of ΔG is now briefly discussed on two examples: thermodynamically unfavorable reaction of CaF_2 and thermodynamically favorable reaction of CdF_2 :



Under STP, reaction 14 is endothermic by 13 kJ mol^{-1} and reaction 15 is exothermic by -50 kJ mol^{-1} . Both reactions are not entropically favored since ΔS is $-3 \text{ J mol}^{-1} \text{ K}^{-1}$ for reaction 14 and $-12 \text{ J mol}^{-1} \text{ K}^{-1}$ for reaction 15. By assuming that the temperature at which the decomposition proceeds is 985 K (*m.p.* of NaKCO_3) then the calculated ΔG is 16 kJ mol^{-1} for the reaction of CaF_2 and -38 kJ mol^{-1} for the reaction of CdF_2 .

It is well known that chemical reactions usually proceed more readily at elevated temperatures. Therefore, the ΔG for MgF_2 , which has the most positive ΔG among MgF_2 , CaF_2 , and BaF_2 and for which data is available in NIST Chemistry WebBook [37] was calculated at $T = 1000 \text{ K}$. The calculated ΔG is still positive indicating that the reactions of MgF_2 , CaF_2 , and BaF_2 are thermodynamically unfavorable also at the actual temperature of decomposition. However, the result of F determination in MgF_2 after alkaline carbonate fusion conducted in our laboratory was however in good agreement with theoretical content, i.e., $(60.7 \pm 0.2) \%$ as opposed to 61.0% . This can be considered as an evidence of a high decomposition efficacy of alkaline carbonate fusion for metal fluorides despite negative ΔG s, which however, as must be admitted, are rather low. As reasonably explained earlier [38], the reactions in the molten salt do not proceed to completion but only to equilibrium. However, at high temperatures, the MgCO_3 is decomposed to MgO and CO_2 , which is evolved, thereby permitting more of the reaction to continue until the conversion of MgF_2 to MgO is complete. The process proceeds according to the reaction



The same type of the reaction is expected to proceed also for CaF_2 and BaF_2 . The ease of decomposition of MF_{2n} can be correlated by their ionic-covalent nature. In the compounds with a more ionic character, the decomposition seems to proceed more easily than in compounds with a more covalent character. This is in accordance with the calculated values of ΔG for MgF_2 , CaF_2 , and BaF_2 at STP which are 46 , 16 , and 2 kJ mol^{-1} , respectively.

As next, an attempt to establish a relationship between the enthalpy of formation of MF_{2n} and of metal carbonates ($\text{M}(\text{CO}_3)_n(\text{s})$) was made. The studied parameters are listed in Table 2. By considering only $\Delta H_f^\circ(\text{M}(\text{CO}_3)_n(\text{s}))$ as a parameter, Eq. 17 was suggested:

$$\Delta H_f^\circ(\text{MF}_{2n}(\text{s})) = (80.6 \pm 19.5) + (1.0681 \pm 0.0221) \cdot \Delta H_f^\circ(\text{M}(\text{CO}_3)_n(\text{s})) \quad (17)$$

$$R^2 = 0.9962$$

In addition, parameter $1/2[\Delta H_f^\circ(\text{NaF}(\text{s})) + \Delta H_f^\circ(\text{KF}(\text{s}))]$ was considered, which represents a mean value of the ΔH_f° of $\text{NaF}(\text{s})$ and $\text{KF}(\text{s})$, i.e., -570 kJ mol^{-1} . The suggested equation is

$$\Delta H_f^\circ(\text{MF}_{2n}(\text{s})) = (38.0 \pm 39.4) + (1.0951 \pm 0.0306) \cdot \Delta H_f^\circ(\text{M}(\text{CO}_3)_n(\text{s})) - (0.0602 \pm 0.0488) \cdot 1/2[\Delta H_f^\circ(\text{NaF}(\text{s})) + \Delta H_f^\circ(\text{KF}(\text{s}))] \quad (18)$$

$$R^2 = 0.9972$$

The $\Delta H_f^\circ(\text{NaKCO}_3(\text{s}))$ is correlated variable and so redundant. By replacing $1/2[\Delta H_f^\circ(\text{NaF}(\text{s})) + \Delta H_f^\circ(\text{KF}(\text{s}))]$ with $n(\text{F})$, the following equation was obtained:

$$\Delta H_f^\circ(\text{MF}_{2n}(\text{s})) = (38.0 \pm 39.4) + (1.0951 \pm 0.0306) \cdot \Delta H_f^\circ(\text{M}(\text{CO}_3)_n(\text{s})) + (34.4 \pm 27.9) \cdot n(\text{F}) \quad (19)$$

$$R^2 = 0.9972$$

The ratio between the coefficients of $n(\text{F})$ parameter and $1/2[\Delta H_f^\circ(\text{NaF}(\text{s})) + \Delta H_f^\circ(\text{KF}(\text{s}))]$ parameter equals to mean value of the ΔH_f° of $\text{NaF}(\text{s})$ and $\text{KF}(\text{s})$, i.e., -570 kJ mol^{-1} . Finally, χ was added as parameter:

$$\Delta H_f^\circ(\text{MF}_{2n}(\text{s})) = (129.6 \pm 87.1) + (1.1855 \pm 0.0827) \cdot \Delta H_f^\circ(\text{M}(\text{CO}_3)_n(\text{s})) + (72.4 \pm 42.3) \cdot n(\text{F}) - (57.1 \pm 48.8) \cdot \chi \quad (20)$$

$$R^2 = 0.9977$$

High linearity was observed already by using simple linear regression only ($R^2 = 0.9962$, Eq. 17). By adding additional parameters, the R^2 increased to 0.9977 (Eq. 20). The calculated values of $\Delta H_f^\circ(\text{MF}_{2n}(\text{s}))$ were compared to the experimental values reported in Wagman [19] by constructing residuals vs. order fit for Eqs. 17 and 20, see Fig. 3.

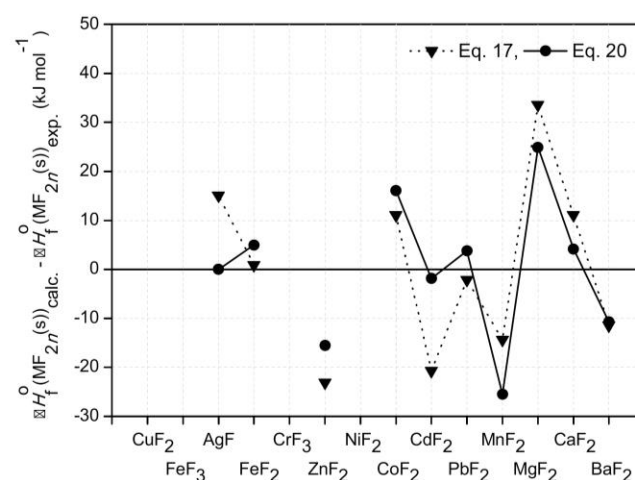


Fig. 3 Plot of differences between calculated and experimentally determined values of $\Delta H_f^\circ(\text{MF}_{2n})$ considering $\Delta H_f^\circ(\text{M}(\text{CO}_3)_n(\text{s}))$ as a parameter (Eq. 17) and $\Delta H_f^\circ(\text{M}(\text{CO}_3)_n(\text{s}))$, χ , and $n(\text{F})$ as parameters (Eq. 20)

The largest difference in estimating $\Delta H_f^\circ(\text{MF}_{2n})$ by using Eq. 17 is for AgF (15 kJ mol^{-1}) and using Eq. 20 for MnF_2 (-25 kJ mol^{-1}). The differences for all MF_{2n} s range between 0–7% (Eq. 17) and 0–3% (Eq. 20) of experimental values, which suggests excellent prediction power of Eq. 20 for estimation of the ΔH_f° s of $\text{M}(\text{CO}_3)_n$ for which experimental data are not available.

Conclusions

Plausible reactions proceeding during pyrohydrolysis, oxygen bomb combustion, or alkaline carbonate fusion with NaKCO_3 of fluorine-containing samples were suggested and discussed from the perspective of analytical chemistry and thermochemistry. Good efficiency of these decomposition methods was proven by the analyses of CRMs of clay and vegetation. By calculating ΔG s, pyrohydrolysis and alkaline carbonate fusion of MF_{2n} were proven as thermodynamically favorable reactions and oxygen combustion as thermodynamically unfavorable process. In addition, linear relationships between the enthalpies of formation of metal fluorides and the balanced values of the enthalpies of formation of the plausible products (metal tungstates, metal oxides, or metal carbonates), electro-negativity of the metals, and number of fluorine atoms in metal fluorides were established.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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4.2 Applicability of Oxygen Bomb Combustion for the Decomposition of Plant Samples

This chapter describes the evaluation of the applicability and accuracy of oxygen bomb combustion for the decomposition of plant samples for the subsequent determination of fluorine. This work has not yet been published; however, the results are important and therefore briefly outlined herein.

Introduction

Alkaline fusion is the most widely used method for total sample decomposition for the determination of fluorine. It has been proven to be effective for a variety of samples, i.e. plants [121], silicate samples [103], food [100], tea and soil [144]. However, it has two main disadvantages: it is time-consuming and needs constant operator supervision. Therefore, faster and easier methods are preferred. The oxygen bomb combustion method fulfils these two requirements. A few papers have reported its use for the determination of fluorine in different samples (coal [110], fuel and organic wastes [109], petroleum products [145], blood serum [146]), but no papers have reported its use for the determination of fluorine in plant samples.

A simple, fast and efficient way to evaluate the applicability and accuracy of the method employed is the incorporation of an appropriate CRM into the analytical procedure. The CRM selected must resemble as closely as possible the actual sample being analysed, since the method performance can vary dramatically with different types of matrixes and different concentration levels of the analyte.

In our laboratory alkaline carbonate fusion has been successfully used for the decomposition of plant samples for the determination of total fluorine. However, due to its tedious procedure, oxygen bomb combustion was recently introduced. The CRM and several plant samples (Norway spruce, black tea, matcha, green tea, gotu kola, ginkgo biloba, tulsi, neem and brahmi) were decomposed by both decomposition methods in order to (1) check the applicability and accuracy of oxygen bomb combustion for decomposition of plant samples and (2) compare the results obtained by the recently-introduced oxygen bomb combustion with the results obtained by the well-established alkaline carbonate fusion.

Methods

Samples of Norway spruce contaminated with fluoride were collected in the vicinity of the Rogaška glassworks (Rogaška Slatina, Slovenia). Black tea (Twinings Original English Breakfast) and matcha (dmBio Matcha Tee) were bought in the Mercator and dm-drogerie markt retail stores, respectively. Green tea (Sencha) was bought in Chai teahouse. Neem was bought from supplement provider Herbana, and gotu kola, ginkgo biloba, tulsi and brahmi were bought from supplement provider Nutrislim. The CRM "Fluoride in vegetation" (SRM-2695), consisting of a standard with high ($277 \pm 27 \mu\text{g/g}$) and low ($64.0 \pm 8.4 \mu\text{g/g}$) level of fluorine, was purchased from NIST. Samples were dried on polyethylene trays in a Heraeus T6 (Thermo Fisher Scientific) laboratory oven at 60°C , then ground in an agate ball mill and finally passed through a 0.25 mm sieve (Retsch).

Samples and CRMs were decomposed by alkaline carbonate fusion as reported previously [102] and by oxygen bomb combustion. Oxygen bomb combustion was performed in an 1108 Oxygen Combustion Bomb (Parr Instrument Company). The powdered sample was pressed into a pellet (8 tons). Absorption solution (20 ml of 0.5 M NaOH ; prepared by dissolving reagent pellets (Merck Millipore) in water) was placed in the bottom of the bomb. The bomb was filled with 30 atm oxygen (99.99 %, Messer) and ignited. The bomb was left standing for 2 hours and shaken intensively every 30 min.

Sample solutions obtained after decompositions were quantitatively transferred to 100 ml volumetric flasks. Prior to measurement, they were acidified with concentrated H_2SO_4 (Merck Millipore) to pH 2–3 and filtered through 589/1 black ribbon paper (Whatman). The F^- concentration was measured potentiometrically using an Orion 960 Autochemistry System with Orion 96-09 combination F-ISE (Thermo Fisher Scientific). The F-ISE was calibrated at $0.2 \mu\text{g F}^-/\text{ml}$ and $1.0 \mu\text{g F}^-/\text{ml}$, and the F^- concentration was measured in a corresponding sample aliquot following the previously reported procedure [147]. Each CRM was analysed in quintuplicate and each sample in triplicate.

Results and Discussion

The CRM "Fluoride in vegetation" (SRM-2695) and several plant samples (Norway spruce, black tea, matcha, green tea, gotu kola, ginkgo biloba, tulsi, neem and brahmi) were decomposed by alkaline carbonate fusion and oxygen bomb combustion, and the F^- concentration was determined by F-ISE. The results of fluorine determination show high precision and are presented in Table 4.1.

Table 4.1: The F content (w_F) with one standard deviation (s) determined in CRMs and plant samples after prior total decomposition with alkaline carbonate fusion and oxygen bomb combustion.

	$w_F \pm s$ [$\mu\text{g/g}$]	
	Alkaline carbonate fusion	Oxygen bomb combustion
SRM-2695 - high level ^a	295 $\pm$ 6	267 $\pm$ 7
SRM-2695 - low level ^b	68.0 $\pm$ 1.7	62.3 $\pm$ 2.6
Norway spruce - high level	219 $\pm$ 4	195 $\pm$ 4
Norway spruce - low level	67.8 $\pm$ 1.2	59.2 $\pm$ 1.1
Black tea	413 $\pm$ 4	363 $\pm$ 4
Matcha	373 $\pm$ 3	324 $\pm$ 3
Green tea	192 $\pm$ 4	156 $\pm$ 3
Gotu kola	111 $\pm$ 2	76.2 $\pm$ 1.0
Ginkgo biloba	91.0 $\pm$ 1.1	78.5 $\pm$ 0.8
Tulsi	68.8 $\pm$ 1.5	53.4 $\pm$ 1.4
Neem	67.0 $\pm$ 2.1	53.9 $\pm$ 1.3
Brahmi	35.3 $\pm$ 1.1	29.2 $\pm$ 1.1

^a Certified value for F: 277 $\pm$ 27 $\mu\text{g/g}$

^b Certified value for F: 64.0 $\pm$ 8.4 $\mu\text{g/g}$

Evaluation of the accuracy of oxygen bomb combustion

The results of the analyses of the CRMs were compared to certified values using a statistical test recommended by the European Reference Materials (ERM) [148]. No statistically significant difference was observed between the determined and certified values for both decomposition methods at both concentration levels, suggesting that both decomposition methods are accurate and appropriate for the decomposition of plant samples.

However, it is evident that the results obtained by oxygen bomb combustion are lower than those obtained by alkaline carbonate fusion for both CRMs and all samples. These differences have been proved to be statistically significant by a *t*-test for comparison of two experimental means at a 0.05 level of confidence. The results of the analyses of CRMs obtained by both decomposition methods are nevertheless within the certified interval because it is very wide, representing 10 % and 13 % of the certified value for the high and low level CRM, respectively.

At this point, some remarks regarding CRMs should be made. In the search for an appropriate CRM, we noticed a serious lack of CRMs for fluorine. At the time of the study, only one CRM with a certified value for fluorine in plant matrix was commercially available, i.e. SRM-2695 from NIST. This CRM has, in addition to a wide certified interval, two major drawbacks. First, the method for the evaluation of measurement uncertainty is not stated and is certainly not based on the GUM, since the SRM-2695 was produced four years before the GUM was published. Furthermore, it is not fully in accordance with the "ISO Guide 31" [149], meaning that instructions for its correct storage and use (temperature of storage, possible storage in a desiccator, mixing before use, minimum sample intake) are not stated, and a stability study was not performed.

Comparison of oxygen bomb combustion and alkaline carbonate fusion

The ratio between the results obtained by oxygen bomb combustion and alkaline carbonate fusion was 91–92 % and 69–89 % for the CRMs and samples, respectively. The results are influenced either by a negative bias in the case of oxygen bomb combustion or by a positive bias in the case of alkaline carbonate fusion.

The possibility that the results obtained by oxygen bomb combustion are influenced by a negative bias was examined. Firstly, 40 µg and 200 µg of F⁻ (prepared from 100 µg/ml certified F⁻ standard solution) modelling low and high level standard, respectively, were placed in the combustion capsule, ignition was performed and the F⁻ concentration was determined by F-ISE. The recovery was 95 % and 97 % for the low level and high level standard, respectively, indicating that soluble fluorides with free F⁻ ions are efficiently determined. Secondly, the conditions of oxygen bomb combustion were varied for green tea analysis to establish if it is possible to achieve a higher recovery of combustion. Therefore, (1) the pellets were pressed to 5 and 10 tons, (2) the bomb was filled to 25 and 35 atm pressure of oxygen, (3) a higher concentration of absorption solution was used (1.0 M NaOH), (4) the time of absorption was longer (7 h), (5) the interior surfaces of the bomb and combustion capsule were washed by a 3-times larger volume of water and (6) the pellet was soaked with methanol. The determined F content was higher only in the case of the prolonged time of absorption but was still significantly lower than the result obtained by alkaline carbonate fusion.

The possibility that the results obtained by alkaline carbonate fusion are influenced by a positive bias was also examined. During the infusion of tea leaves (*Camellia sinensis* L.), up to 100 % of fluorine is released into the water in the form of F⁻ [89]. Therefore, green tea was infused in boiling water, which was made alkaline by the addition of NaOH to avoid a possible loss of fluorine in the form of HF. The determined F content was statistically not significantly different from that obtained by alkaline carbonate fusion. These results indicate that the oxygen bomb combustion method is influenced by a negative bias.

Conclusions

Oxygen bomb combustion, as well as alkaline carbonate fusion, assured accurate results within the certified intervals of CRM. However, lower results were observed in the case of oxygen bomb combustion in comparison to alkaline carbonate fusion (69–89 %). In our paper [113] it was demonstrated that the reactions of metal fluorides proceeding during alkaline carbonate fusion are thermodynamically favourable, while reactions proceeding during oxygen bomb combustion are not and fluoride remains bound. Therefore, the results in the case of oxygen bomb combustion are probably lower because only the content of soluble fluorides is determined by F-ISE. Insoluble fluorides present in the leaves (CaF₂, MgF₂) or on the leaves as a result of deposition of particulate fluorides from industrial emissions (AlF₃, cryolite, fluorapatite) are not decomposed during the combustion and their content cannot be determined. Another possible reason is the loss of unabsorbed HF when the bomb is opened after combustion, since it has been demonstrated that the determined F content is higher in the case of a prolonged time of absorption.

Future Aspects

Higher recovery in oxygen bomb combustion could possibly be obtained by the use of a proper catalyst system. Geng et al. [107] reported that the use of a catalyst (tungsten trioxide (WO₃), molybdenum trioxide (MoO₃) or vanadium(V) oxide (V₂O₅)) and co-catalyst tin (Sn) resulted in an improvement in recovery in the determination of fluorine in coal (> 95 % recovery).

To give an illustration, CaF₂ reacts with WO₃ and forms WF₆ according to Eq. 4.1. Complete fluoride exchange does not occur, resulting in the formation of very volatile oxyfluorides (WOF₄, WO₂F₂).



Tin is supposed to improve the recovery due to Eqs. 4.2 and 4.3.



Considering the results gathered up to this point, it is possible to suggest that oxygen bomb combustion does not give satisfactory results. However, before making a final conclusion that this method is not applicable for the decomposition of plant samples, further experiments with different catalyst systems should be carried out.

4.3 Measurement Uncertainty Evaluation for Total Fluorine Determination in Vegetation

In this chapter the article entitled "Measurement uncertainty evaluation and traceability assurance for total fluorine determination in vegetation by fluoride ion selective electrode" by D. Štepec, G. Tavčar and M. Ponikvar-Svet, published in the Journal of Fluorine Chemistry in 2019, is presented [147]. D. Štepec, the author of this thesis, performed the experiments, analysed the data, participated in the interpretation of results and wrote the first draft of the manuscript.

A measurement result has to be reported with measurement uncertainty (MU). In analytical chemistry, MU has generally been expressed as one standard deviation, including only random effects. A preferable approach is described in the "Guide to the Expression of Uncertainty in Measurement" (GUM) [150], where in addition to random effects, systematic effects are also considered, and all uncertainty components of each step of the analytical procedure are identified and quantified. All these components are then combined, and the result is reported with a MU that is called expanded uncertainty.

In this paper a procedure for MU evaluation according to the GUM for the determination of fluorine in tea by F-ISE after prior total decomposition by alkaline carbonate fusion was developed. The most obvious uncertainty components are those correlated with the fluoride measurement step and were identified as the uncertainty of weighing, uncertainty of volumetric glassware and uncertainty of the cell potential of F-ISE. In addition, all uncertainties associated with the preparation of the calibration curve were considered, i.e. the uncertainty of the concentration and density of standard F^- solution and uncertainty of volumetric glassware. The uncertainty of precision (random effects) was estimated by replicate analysis of the sample. The uncertainty of bias (systematic effects) was estimated by replicate analysis of the CRM "Fluoride in vegetation" (SRM-2695 from NIST), which consists of the uncertainty of the obtained value and uncertainty of the certified value. All uncertainty components were then quantified, and the expanded uncertainty was calculated.

The expanded uncertainties were estimated to represent 7–15 % of the result for different types of tea (black tea, matcha, green, oolong, Pu-erh). The uncertainty of bias had the largest contribution (76–98 %), the contribution of the uncertainty of precision was 2–23 % and the other uncertainties were negligible. The contribution of the uncertainty of bias was so large because of the wide certified interval. A CRM with a narrower certified interval would be a better choice, but unfortunately SRM-2695 was the only CRM with a certified value of fluorine in plant matrixes available at the time of the study.

This procedure can be used for all samples with a plant matrix containing comparable F content to those in SRM-2695 and can be easily modified to samples with other matrixes by employing an appropriate CRM.



Measurement uncertainty evaluation and traceability assurance for total fluorine determination in vegetation by fluoride ion selective electrode

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ABSTRACT

The evaluation of measurement uncertainty (MU) according to the *Guide to the Expression of Measurement Uncertainty* (GUM) is of great importance, because in addition to random effects, which are estimated by one standard deviation, also systematic effects are included. Furthermore, all uncertainty components are quantified and traceability to SI units is assured. In research laboratories, there is a lack of knowledge on how to introduce the GUM principles into analytical measurements, which is also illustrated by the scarcity of papers reporting results together with the MU. In order to clarify some of the issues related to this topic, a procedure for the MU evaluation based on the GUM for determination of fluorine in solid samples was suggested. The procedure was applied to determination of fluorine in tea as a model sample, because it is known to accumulate large amounts of fluorine in the leaves. Fluoride was determined with fluoride ion selective electrode after preceding total decomposition by alkaline carbonate fusion. The uncertainties according to the GUM represent 7–15% of the reported result. They are larger than standard deviations (1–5%) but only such results are trustworthy and have metrological value.

1. Introduction

The result of every measurement has to be accurate and reported together with a measurement uncertainty (MU) [1]. Traditionally in analytical chemistry, the MU has been expressed as one standard deviation (s), thus including only random effects. A superior, more comprehensive approach is outlined in a definite document in the field of metrology a *Guide to the expression of uncertainty in measurement* (GUM) [2]. In this approach, in addition to random also systematic effects are taken into account and all uncertainty components of each step of analytical procedure are quantified. All these contributions are then combined and the result is reported together with an expanded uncertainty. Such uncertainty is larger than s , but it certainly includes all possible uncertainty sources and the result is considered traceable [1] to SI units.

The GUM is accepted by many international organizations and techniques used to calculate the MU according to the GUM are well known, however the number of papers reporting the results together with expanded uncertainty are scarce. Chemists from different fields apparently encounter problems when trying to apply the GUM principles into their measurements, mainly because: (1) the GUM guidelines are very general and supported by only a limited number

of practical examples; (2) it is difficult to incorporate the principles, which were prepared from the perspective of physics, to actual chemical measurements; and (3) evaluation of the MU is a challenging mathematic task.

In this work an attempt was made to solve some of the outlined issues. In order to show how the GUM guidelines can be applied to actual chemical measurements, the procedure for the MU evaluation was suggested on the case of determination of fluorine (F). This element is receiving much attention owing to its beneficial and possible harmful effects on humans and environment. Small daily intakes have proven benefits for dental health, while excessive intakes can cause development of dental fluorosis in children or skeletal fluorosis in both children and adults [3]. Predominating methods nowadays for determination of fluoride are fluoride ion selective electrode (F-ISE) and ion chromatography (IC). The F-ISE introduced by Frant and Ross in 1966 [4] is easy to use and cost effective, whereas IC is more expensive and requires better trained personnel [5].

The only paper reporting procedure for evaluation of the MU according to the GUM for F is on determination of fluoride (F^-) in water [6]. In this case, the MU is composed of an uncertainty of a measurement step only, because the entire F is present in the ionic form as F^- and can be directly determined by F-ISE. In our work, F was determined

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in solid samples, where F is present in both bound and ionic forms. The samples must undergo a total decomposition before measurement to assure conversion of the entire F to F^- and bring the solid sample into a water-soluble form. This is a crucial step of entire analytical procedure and makes evaluation of the MU much more complex, because: (1) in addition to the uncertainty of the measurement step also uncertainty of the decomposition step must be included; and (2) transformation of the sample from one chemical form to another cannot be traceable to any reference and therefore the highest form of traceability can be achieved by the use of matrix matched certified reference material (CRM) [7]. Tea was chosen as a model solid vegetation sample, because many papers [8–12] are reporting that tea plant (*Camellia sinensis* L.) uptakes large amounts of F from the soil and accumulates it in the leaves. Since long-term consumption of high fluoride tea can result in chronic fluoride intoxication [13] accurate measurements of F should be mandatory.

The objective of this work was therefore to develop a procedure for the MU evaluation based on the GUM for the determination of F in solid vegetation samples by F-ISE after preceding total decomposition with alkaline carbonate fusion. The MU was evaluated for five types of tea selected based on earlier reported paper [8]. The main features of suggested procedure are: (1) assurance of traceability to the SI units at all steps of the analytical procedure; (2) use of the CRM to evaluate the uncertainty of a bias; (3) suggestion of an approach to a precision study applicable to research chemical laboratories analyzing a variety of samples with different matrixes; and (4) construction of an alternative to a Fishbone diagram, where in addition to uncertainty components also equations for their calculations and main steps of the analytical procedure are illustrated. The procedure can be applied without modifications to all vegetation samples containing comparable amounts of F to those in CRM. It can be easily adapted to the samples with other matrixes by choosing CRM with matrix and analyte concentration similar to the analyzed samples.

2. Results

The approach for the MU evaluation that covers all types of measurements is presented in the definite document in the field of metrology GUM. A guide *Quantifying uncertainty in analytical measurement* (QUAM) [14] was prepared in the light of MU evaluation in quantitative chemical analysis.

The GUM recommends to estimate the MU in four steps: (1) specification of the measurand and writing down the expression used to calculate the value of the measurand; (2) identifying all uncertainty components - all parameters in the expression used to calculate the value of the measurand and other uncertainty components affecting the value of the measurand, e.g. calibration curve, precision, bias; (3) quantifying the uncertainty components; and (4) calculating the combined and expanded uncertainty. The uncertainties have to be propagated by applying the rules for the evaluation of standard uncertainty through functional relationships [15].

Listing all uncertainty components is often based on an elaboration of a "Cause and Effect" (Fishbone) Diagram. This diagram is illustrative in terms of visualizing the uncertainty components but it is "dry" in terms of mathematical expressions and analytical procedure steps. In this work, a flowchart diagram (Scheme 1) for the MU evaluation for determination of F by F-ISE after preceding total decomposition was constructed.

The flowchart illustrates: (1) the key steps of the analytical procedure; (2) all uncertainty components contributing to the MU; (3) the mathematical equations used to calculate these uncertainties; and (4) how the uncertainty components are combined. The diagram is easily extendable/adaptable to any other measurands or analytical methods.

The evaluation of the MU according to the GUM involves

mathematical equations with a great number of nonconventional abbreviations. To assist the reader, all are clearly listed¹.

2.1. Specification of measurand and expression for calculation of its value

It is crucial to clearly define the measurand, i.e. is the objective to determine the analyte concentration and evaluate the associated MU in the sampling target or in the sample being delivered to the laboratory. In each case different uncertainties are taken into account [16] (S1 in Supporting information). In this paper, the focus was on the determination of F content and evaluation of associated MU in the laboratory sample. After physical preparation and total sample decomposition, the content of F^- was determined by direct potentiometry using F-ISE. A cell potential (E_{cell}) is linearly related to a logarithm of a mass concentration of F^- in a sample solution ($\gamma_{F(\text{sam})}$) according to Eq. 1, where S_{sam} and I are a slope and an intercept of a calibration curve, respectively.

$$\gamma_{F(\text{sam})} = 10^{\frac{-E_{\text{cell}} + I}{S_{\text{sam}}}} \quad (1)$$

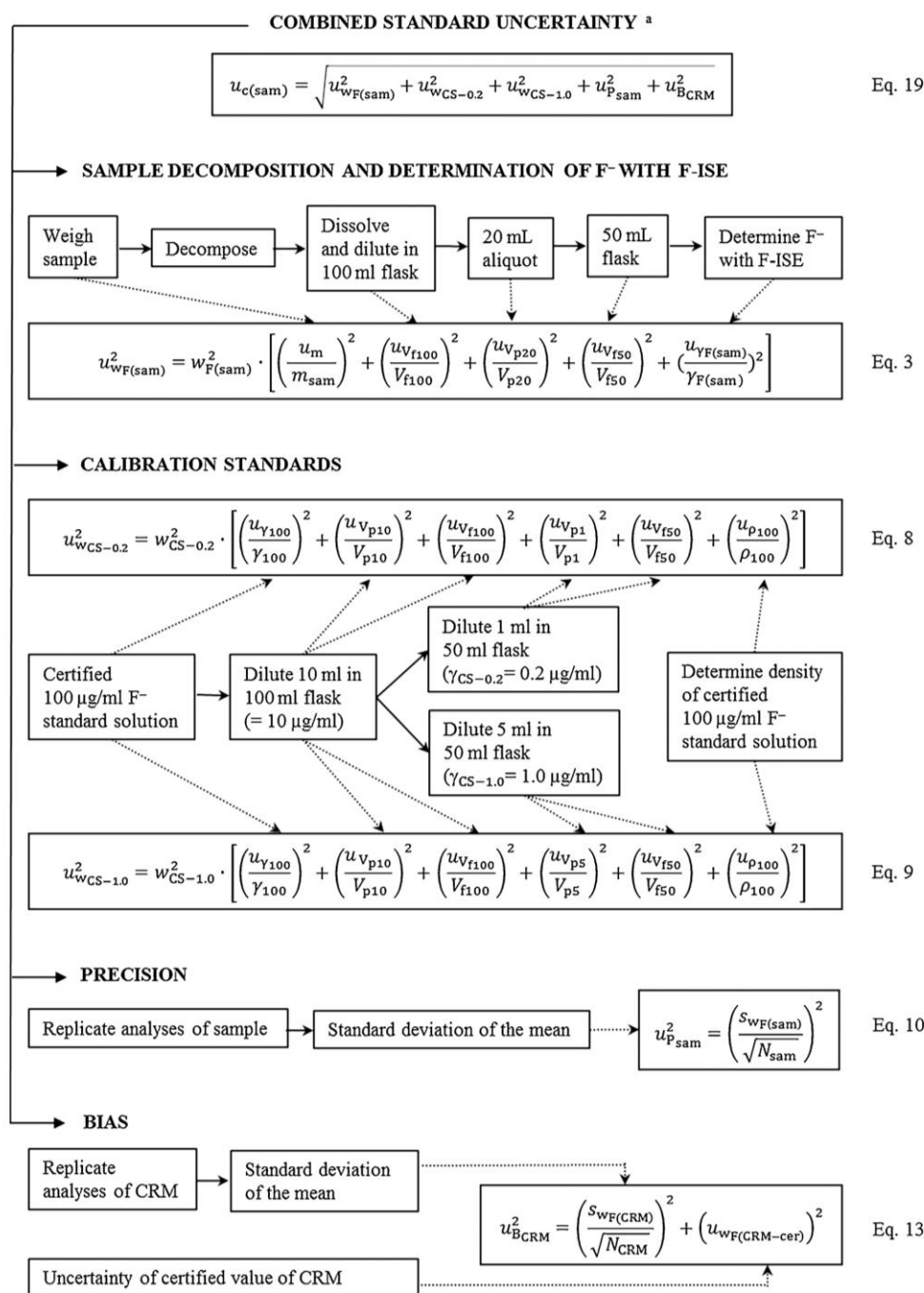
The decomposed sample was transferred to a 100 ml volumetric flask (V_{f100}) and $\gamma_{F(\text{sam})}$ was measured in a 50 ml volumetric flask (V_{f50}) containing a 20 ml sample aliquot (V_{p20}) and CBS (see 4.4 and 4.5). A mass fraction of F in the sample ($w_{F(\text{sam})}$) is given by Eq. 2.

$$w_{F(\text{sam})} = \frac{\gamma_{F(\text{sam})} \cdot V_{f50} \cdot V_{f100}}{V_{p20} \cdot m_{\text{sam}}} \quad (2)$$

2.2. Identification of uncertainty components

In addition to the parameters in the equation used to calculate the value of the measurand (Eq. 2), also the parameters, which do not appear directly in this equation but do affect the measurement result

¹ Abbreviations: B_{CRM}^a , bias determined by CRM analysis ($\mu\text{g/g}$); CRM, certified reference material; CRM(H), CRM with high F level; CRM(L), CRM with low F level; E_{cell} , cell potential (mV); F, fluorine; F^- , fluoride; F-ISE, fluoride ion selective electrode; GUM, guide *Guide to the expression of uncertainty in measurement*; I , intercept of calibration curve (mV); k , coverage factor; m_{empt} , empty vial weight (g); m_{fil} , filled vial weight (g); m_{sam}^b , sample weight (g); MU, measurement uncertainty; N_{CRM}^a , number of replicate analyses of CRM; N_{sam}^b , number of replicate analyses of sample; P_{sam}^b , precision of sample analysis ($\mu\text{g/g}$); QUAM, guide *Quantifying uncertainty in analytical measurement*; s , standard deviation; sam, sample; S_{sam}^b , slope of calibration curve (mV); $s_{wF(\text{CRM})}^a$, standard deviation of replicate analyses of CRM ($\mu\text{g/g}$); $s_{wF(\text{sam})}^b$, standard deviation of replicate analyses of sample ($\mu\text{g/g}$); u^c , standard uncertainty; $u_{c(\text{sam})}^b$, combined standard uncertainty ($\mu\text{g/g}$); u_m , standard uncertainty of weighing operations (g); u_{mcal} , balance calibration uncertainty (g); u_{mdd} , balance daily drift (g); $u_{\text{mrep(empt)}}$, repeatability of weighing of empty vials (g); $u_{\text{mrep(fill)}}$, repeatability of weighing of filled vials (g); u_{msys} , balance systematic error (g); U_{sam}^b , expanded uncertainty ($\mu\text{g/g}$); u_v , standard uncertainty of volumetric operations (ml); u_{vcal} , calibration uncertainty of volumetric equipment (ml); u_{vrep} , repeatability of volumetric operations (ml); $u_{\text{V(empt)}}$, uncertainty of volume because of temperature differences (ml); V , volume of laboratory equipment (ml); V_{disp} , volume of dispensed certified 100 $\mu\text{g/ml}$ F^- standard solution (ml); V_{f50} , volume of 50 ml volumetric flask (ml); V_{f100} , volume of 100 ml volumetric flask (ml); V_{p1} , volume of 1 ml pipette (ml); V_{p5} , volume of 5 ml pipette (ml); V_{p10} , volume of 10 ml pipette (ml); V_{p20} , volume of 20 ml pipette (ml); $w_{\text{CS-0.2}}$, mass fraction of 0.2 $\mu\text{g/ml}$ calibration standard ($\mu\text{g/g}$); $w_{\text{CS-1.0}}$, mass fraction of 1.0 $\mu\text{g/ml}$ calibration standard ($\mu\text{g/g}$); $w_{F(\text{CRM})}^a$, obtained mass fraction of F in CRM ($\mu\text{g/g}$); $w_{F(\text{CRM-cer})}^d$, certified mass fraction of F in CRM ($\mu\text{g/g}$); $w_{F(\text{sam})}^b$, mass fraction of F in sample ($\mu\text{g/g}$); α , water volume expansion coefficient ($^{\circ}\text{C}^{-1}$); γ_{100} , mass concentration of certified 100 $\mu\text{g/ml}$ F^- standard solution ($\mu\text{g/ml}$); $\gamma_{F(\text{sam})}$, mass concentration of F^- in sample solution ($\mu\text{g/ml}$); ΔT , temperature difference ($^{\circ}\text{C}$); ρ_{100} , density of certified 100 $\mu\text{g/ml}$ F^- standard solution (g/ml); ^a subscript CRM refers to CRM(H) or CRM(L); ^b subscript sam refers to black, matcha, green, oolong or Pu-erh; ^c standard uncertainties of each uncertainty component were not listed separately; ^d subscript CRM-cer refers to CRM(H)-cer or CRM(L)-cer.



Scheme 1. Flowchart diagram for the evaluation of MU.

were considered, i.e. calibration curve, precision, bias. Uncertainties arising from sampling and physical sample preparation (milling, sieving, homogenization and drying) were not included in the study, because the objective was to evaluate the MU of chemical analysis (S1 in Supporting information).

2.2.1. Mass fraction of F in sample

The standard uncertainty of $w_{F(sam)}$ depends on the standard uncertainty of $\gamma_{F(sam)}$ and the standard uncertainties of volumetric ($u_{V_{f100}}$, $u_{V_{f50}}$, $u_{V_{p20}}$) and weighing (u_m) operations (Eq. 3, Scheme 1).

The standard uncertainty of $\gamma_{F(sam)}$ depends on the standard uncertainties of E_{cell} , S_{sam} and I and is calculated by Eq. 4. The S2 in Supporting information provides information about its derivation.

$$\left(\frac{u_{\gamma_{F(sam)}}}{\gamma_{F(sam)}} \right)^2 = \left(\frac{2.303}{S_{sam}} \right)^2 \cdot u_{E_{cell}}^2 + \left(\frac{2.303}{S_{sam}} \right)^2 \cdot u_I^2 + \left(\frac{2.303 \cdot (-E_{cell} + I)}{S_{sam}^2} \right)^2 \cdot u_{S_{sam}}^2 \quad (4)$$

Since standard uncertainties of S_{sam} and I are zero (see 2.3.4), the Eq. 4 can be simplified to Eq. 5.

$$\left(\frac{u_{\gamma_{F(sam)}}}{\gamma_{F(sam)}} \right)^2 = \left(\frac{2.303}{S_{sam}} \right)^2 \cdot u_{E_{cell}}^2 \quad (5)$$

2.2.2. Calibration standards

The calibration standards (0.2 µg/ml and 1.0 µg/ml of F⁻) were prepared by successive dilution of a certified 100 µg/ml F⁻ standard

solution (γ_{100}) (see 4.5). The concentration of the calibrations standards is expressed in $\mu\text{g}/\text{ml}$ unit, but the final result and expanded uncertainty in $\mu\text{g}/\text{g}$ unit. According to the rules for propagation of uncertainties all groups of contributors must have the same unit. Thus, the mass concentrations of the calibration standards were converted to the mass fractions ($w_{\text{CS-0.2}}$ and $w_{\text{CS-1.0}}$) by considering the density of certified 100 $\mu\text{g}/\text{ml}$ F^- standard solution (ρ_{100}) (Eqs. 6 and 7, respectively).

$$w_{\text{CS-0.2}} = \frac{V_{\text{p1}} \cdot V_{\text{p10}} \cdot \gamma_{100}}{V_{\text{f50}} \cdot V_{\text{f100}} \cdot \rho_{100}} \quad (6)$$

$$w_{\text{CS-1.0}} = \frac{V_{\text{p5}} \cdot V_{\text{p10}} \cdot \gamma_{100}}{V_{\text{f50}} \cdot V_{\text{f100}} \cdot \rho_{100}} \quad (7)$$

Standard uncertainties of $w_{\text{CS-0.2}}$ and $w_{\text{CS-1.0}}$ were calculated by Eqs. 8 and 9, respectively (Scheme 1).

2.2.3. Precision

When the method is used for the analyses of a single matrix type with the analyte at a single concentration, the precision can be determined as a standard deviation (s) of replicate analyses of a typical sample over an extended period of time, with several different batches, and by varying method parameters, e.g. different calibration standards, more than one analyst, different F-ISEs [17]. The time and cost restrains restrict such approach, if certain sample matrix is analyzed occasionally only. For this reason, an alternative approach to the precision study was suggested. The precision (P_{sam}) was determined for each tea sample separately by 3 replicate analyses of the same batch. The measurements were conducted on the same day under the same method parameters. Confidence intervals for the variance at a 0.05 level of confidence were calculated to identify eventual outliers and run, if necessary, additional analyses [18]. Standard uncertainty of P_{sam} was then calculated as a standard deviation of a mean according to Eq. 10 (Scheme 1).

2.2.4. Bias

In addition to random effects evaluated by precision study, also systematic effects have to be taken into account. In solid sample analysis, sample is transformed from one chemical form to another during decomposition step, which always results in a doubt, if all the analyte is present in the measuring system. Bias, which includes also the bias of the decomposition, can be determined by the analysis of a CRM, which resembles as closely as possible the actual samples being analyzed with respect to a matrix and analyte concentration. The CRM analyses should be spread over a short period of time and carried out with one batch and under the same method parameters [17]. Bias (B_{CRM}) is defined by Eq. 11 as a difference between the obtained and the certified value. In our study it was determined as a run bias by a small number of replicate analyses of CRM [19].

$$B_{\text{CRM}} = w_{\text{F(CRM)}} - w_{\text{F(CRM-cer)}} \quad (11)$$

The significance of the bias was tested by Eq. 12, by taking coverage factor (k) as 2 [19].

$$B_{\text{CRM}} > k \cdot u_{B_{\text{CRM}}} \quad (12)$$

The bias was determined as insignificant, so no corrections of the results needed to be made. As the measurement of the bias and concluding that it is zero adds uncertainty, the MU should include the uncertainty of the bias. The standard uncertainty of B_{CRM} was calculated according to the Eq. 13 (Scheme 1) [19]. It consists of the uncertainty of the obtained value calculated as the standard deviation of the mean and the uncertainty of the certified value.

2.3. Quantifying uncertainty components

A size of each uncertainty component was estimated. All the components of uncertainty were expressed as standard uncertainties before they were combined. The standard uncertainties were estimated by: (1)

Type A evaluation from series of measurements as s ; or (2) Type B evaluation from probability density functions based on experience or other information [1].

2.3.1. Volumetric operations

A standard uncertainty of volumetric operations (u_v) is given by Eq. 14 and is associated with: (1) a calibration uncertainty of volumetric equipment (u_{Vcal}) stated in the manufacturer calibration certificate; (2) an uncertainty due to a use of volumetric equipment at a temperature different from that at which it was calibrated (u_{Vtemp}); and (3) a repeatability (u_{Vrep}) determined experimentally as s of 10 fill-and-weigh operations. A rectangular distribution was assumed for u_{Vcal} and u_{Vtemp} .

$$u_v = \sqrt{u_{\text{Vcal}}^2 + u_{\text{Vtemp}}^2 + u_{\text{Vrep}}^2} \quad (14)$$

According to the manufacturer, the volumetric equipment is calibrated at 20 °C but the temperature in the laboratory varies ($\Delta T = \pm 5$ °C). The u_{Vtemp} was therefore calculated by considering a water volume expansion coefficient ($\alpha = 2.1 \times 10^{-4} \text{ } ^\circ\text{C}^{-1}$ at 20 °C) by Eq. 15.

$$u_{\text{Vtemp}} = \Delta T \cdot \alpha \cdot V \quad (15)$$

2.3.2. Weighing operations

A standard uncertainty of weighing operations (u_m) is given by Eq. 16 and is associated with: (1) a balance systematic error (u_{msys}) stated in a calibration certificate together with a k ; (2) a balance calibration uncertainty (u_{mcal}) stated in a calibration certificate together with k ; and (3) a balance daily drift (u_{mdd}) determined from a control chart obtained using a certified one-gram weight (211 measurements in a period of 1 year).

$$u_m = \sqrt{u_{\text{msys}}^2 + u_{\text{mcal}}^2 + u_{\text{mdd}}^2} \quad (16)$$

2.3.3. Cell potential

A standard uncertainty of E_{cell} is associated with a resolution of the digital potentiometer. A rectangular distribution was assumed.

2.3.4. Calibration curve

The F-ISE has a Nernstian response for F^- concentration in a range from 10^{-6} M to 1 M [4]. The usual procedure for calibration of F-ISE is by using two calibration standards only, one below and one above the expected F^- concentrations in the samples. Since the line goes straight through these two points, there is no deviation of these points from the line. Therefore, the standard uncertainties of the S_{sam} and I are zero. Uncertainty of preparation of calibration standards is discussed in Section 2.2.2.

2.3.5. Certified 100 $\mu\text{g}/\text{ml}$ F^- standard solution

A standard uncertainty of γ_{100} is associated with the uncertainty stated in a manufacturer's certificate. A rectangular distribution was assumed.

2.3.6. Density of certified 100 $\mu\text{g}/\text{ml}$ F^- standard solution

A ρ_{100} was calculated by Eq. 17 and determined experimentally by dispensing 5 aliquots (V_{disp}) of the certified 100 $\mu\text{g}/\text{ml}$ F^- standard solution into the vials.

$$\rho_{100} = \frac{m_{\text{fill}} - m_{\text{empt}}}{V_{\text{disp}}} \quad (17)$$

A standard uncertainty of ρ_{100} is associated with an uncertainty of V_{disp} and an uncertainty of weighing operations. The uncertainty of weighing operations is associated in addition to all the parameters given in Eq. 16 also with a repeatability of weighing of filled ($u_{\text{mrep(fill)}}$) and empty ($u_{\text{mrep(empt)}}$) vials. The standard uncertainty of ρ_{100} is given by Eq. 18, S3 in Supporting information provides information about its derivation. A rectangular distribution was assumed for the V_{disp} .

Table 1

Values of uncertainty components and their standard uncertainties, combined and expanded uncertainties.

value (standard uncertainty)		black tea	matcha	green tea	oolong tea	Pu-erh tea
sample decomposition and determination of F⁻ by F-ISE						
$w_{F(sam)}$ ($u_{wF(sam)}$)	($\mu\text{g/g}$)	413 (1.17)	373 (1.07)	192 (0.548)	125 (0.357)	80.9 (0.230)
m_{sam} (u_m)	(g)	0.491 ($9.11 \cdot 10^{-6}$)	0.514 ($9.11 \cdot 10^{-6}$)	0.511 ($9.11 \cdot 10^{-6}$)	1.02 ($9.11 \cdot 10^{-6}$)	1.02 ($9.11 \cdot 10^{-6}$)
V_{f100} (u_{Vf100})	(ml)			100 (0.0839)		
V_{p20} (u_{Vp20})	(ml)			20.0 (0.0212)		
V_{f50} (u_{Vf50})	(ml)			50.0 (0.0470)		
$\gamma_{F(sam)}$ ($u_{\gamma F(sam)}$)	($\mu\text{g/ml}$)	0.812 (0.00187)	0.768 (0.00179)	0.392 (0.000916)	0.510 (0.00118)	0.329 (0.000764)
calibration standards						
$w_{CS-0.2}$ ($u_{wCS-0.2}$)	($\mu\text{g/g}$)			0.201 (0.00210)		
$w_{CS-1.0}$ ($u_{wCS-1.0}$)	($\mu\text{g/g}$)			1.00 (0.00890)		
γ_{100} ($u_{\gamma 100}$)	($\mu\text{g/ml}$)			100 (0.289)		
V_{p10} (u_{Vp10})	(ml)			10.0 (0.0131)		
V_{f100} (u_{Vf100})	(ml)			100 (0.0839)		
V_{f50} (u_{Vf50})	(ml)			50.0 (0.0470)		
V_{p1} (u_{Vp1})	(ml)			1.00 (0.00818)		
V_{p5} (u_{Vp5})	(ml)			5.00 (0.0300)		
ρ_{100} ($u_{\rho 100}$)	(g/ml)			0.996 (0.00553)		
precision ($N_{sam} = 3$)						
u_{psam}	($\mu\text{g/g}$)	2.09	1.87	2.44	1.78	2.41
$s_{wF(sam)}$	($\mu\text{g/g}$)	3.61	3.23	4.23	3.08	4.17
bias ($N_{CRM} = 5$)						
u_{bCRM}	($\mu\text{g/g}$)	14.0	14.0	14.0	4.35	4.35
$s_{wF(CRM)}$	($\mu\text{g/g}$)	5.72	5.72	5.72	1.65	1.65
$u_{wF(CRM-cer)}$	($\mu\text{g/g}$)	13.8	13.8	13.8	4.29	4.29
combined standard uncertainty						
$u_{c(sam)}$	($\mu\text{g/g}$)	14.2	14.2	14.2	4.71	4.98
expanded uncertainty ($k = 1.96$)						
U_{sam}	($\mu\text{g/g}$)	27.9	27.8	27.9	9.24	9.75

$$\left(\frac{u_{\rho 100}}{\rho_{100}}\right)^2 = \left(\frac{u_{Vdisp}}{V_{disp}}\right)^2 + \frac{u_{mrep(fill)}^2 + u_{mrep(empty)}^2 + 2u_{msys}^2 + 2u_{mcal}^2 + 2u_{mdd}^2}{(m_{fill} - m_{empty})^2} \quad (18)$$

2.3.7. Certified value of CRM

A standard uncertainty of $w_{F(CRM-cer)}$ is associated with the stated uncertainty in the CRM certificate together with k .

2.3.8. Quantification

The values of the uncertainty components and their standard uncertainties for tea samples analyzed were quantified and listed (Table 1). A detailed procedure for quantification of all uncertainty components is presented in S4 in Supporting information.

2.4. Calculation of combined and expanded uncertainty

The next step is to combine the standard uncertainties to obtain a combined standard uncertainty ($u_{c(sam)}$) given at a 68.3% level of confidence. How the individual uncertainty components are combined depends on whether or not they are proportional to the analyte concentration [17]. Assumed was, that all sources of uncertainty are independent of the analyte concentration, thus $u_{c(sam)}$ is given by Eq. 19 (Scheme 1). The $u_{c(sam)}$ was finally multiplied by $k = 1.96$ to obtain an expanded uncertainty (U_{sam}) at a 95% level of confidence (Eq. 20).

$$U_{sam} = k \cdot u_{c(sam)} \quad (20)$$

Table 1 lists the values of the $u_{c(sam)}$ and U_{sam} for all tea samples analyzed.

2.5. Reporting the result

The GUM recommends reporting the result together with the expanded uncertainty calculated using an appropriate k . The result must include a statement of k and the level of confidence. In accordance to this, the results are reported as:

Mass fractions of F in tea samples:

Black tea: $(413 \pm 28) \mu\text{g/g}^*$
 Matcha: $(373 \pm 28) \mu\text{g/g}^*$
 Green tea: $(192 \pm 28) \mu\text{g/g}^*$
 Oolong tea: $(125 \pm 9) \mu\text{g/g}^*$
 Pu-erh tea: $(81 \pm 10) \mu\text{g/g}^*$

* The reported uncertainties are expanded uncertainties calculated using $k = 1.96$, which corresponds to the 95% level of confidence.

3. Discussion

In analytical chemistry, there has been great emphasis on a precision of the results obtained using a specified method. Thus, the MU has been traditionally reported in a form of: $(1) \pm s$, which corresponds to a 68.3% confidence level; or $(2) \pm$ confidence interval for the mean to give a higher confidence level, calculated as s multiplied by the appropriate Student's t value and divided by the square root of a number of measurements (N). Reporting results in such way is incomplete because they: (1) do not include systematic biases; and (2) are not linked to any firm references. More comprehensive approach which overcomes these issues is outlined in the GUM where all uncertainty components are combined and MU is then expressed as expanded uncertainty.

In this work, MU evaluation according to GUM was illustrated on the case of determination of F with F-ISE in different types of tea (black, matcha, green, oolong, Pu-erh) after preceding total sample decomposition with alkaline carbonate fusion. The main steps of the analytical procedure, uncertainty components and equations used for their calculations are outlined in Scheme 1. The values of uncertainty components, their standard uncertainties, combined standard uncertainties and the expanded uncertainties are presented in Table 1.

To see the differences between three different types of MU evaluation, in addition to expanded uncertainties according to GUM, also standard deviations and confidence intervals for the mean were calculated. The results are presented in Table 2.

Results listed in Table 2 demonstrate considerable differences between the expanded uncertainties, s and confidence intervals for the mean. The expanded uncertainties according to the GUM are considerably higher than the values calculated by traditional approaches

Table 2

Contents of F determined in teas together with calculated expanded uncertainties, standard deviations and confidence intervals for the mean.

sample	$w_{\text{F(sam)}}$	$U_{\text{sam}}^{\text{a}}$ ($\mu\text{g/g}$)	s^{b}	$ts/\sqrt{N_{\text{sam}}}^{\text{c}}$
black tea	413	28	4	9
matcha	373	28	3	8
green tea	192	28	4	11
oolong tea	125	9	3	8
Pu-erh tea	81	10	4	10

^a $k = 1.96$ to give expanded uncertainty at 95% confidence level.

^b 68.3% confidence level.

^c $t = 4.3$ to give 95% confidence level ($N = 3$).

and represent 7–15% of the result in comparison to s and confidence interval for the mean representing 1–5% and 2–12% of the result, respectively. Nevertheless, expanded uncertainties, in contrast to the traditional approaches, which are based on the repeatability measurements only, include systematic biases and the results can be considered traceable to the SI units. In our approach, the traceability to the SI units was assured in every single step of analytical procedure, i.e. the calibration of F-ISE, weighing and use of volumetric equipment. The weakest step of the entire procedure was the sample decomposition, because the transformation of the sample from one chemical form to another cannot be traceable to any reference. The matrix matched CRM was used to support the traceability of this step.

The contribution of each uncertainty component (uncertainty of the mass fraction of F in the sample ($u_{w_{\text{F(sam)}}}$), uncertainty of the calibration standards ($u_{w_{\text{CS-0.2}}}$ and $u_{w_{\text{CS-1.0}}}$), uncertainty of the precision ($u_{p_{\text{sam}}}$) and uncertainty of the bias ($u_{b_{\text{CRM}}}$) to the combined standard uncertainty ($u_{c(\text{sam})}$) for each tea sample was examined. In our case, the u_c^2 is calculated as a sum of the squares of all uncertainty components (Eq. 19 in Scheme 1) assuming that all sources of uncertainty are independent of the analyte concentration. The impact of each contributor to combined standard uncertainty was visualized in a form of a bar chart (Chart 1).

The largest contribution to the $u_{c(\text{sam})}$ for all tea samples is $u_{b_{\text{CRM}}}$ representing between 76–98%. The contribution of $u_{p_{\text{sam}}}$ is 2–23% and of $u_{w_{\text{F(sam)}}}$ less than 1%. The contributions of $u_{w_{\text{CS-0.2}}}$ and $u_{w_{\text{CS-1.0}}}$ are negligible. The contribution $u_{b_{\text{CRM}}}$ is that large due to large uncertainty of the certified value of CRM ($u_{w_{\text{F(CRM-cer)}}$). Since the CRM should resemble as closely as possible the analyte concentration of the samples being analyzed, the CRM with high level of F was employed for the calculation of $u_{b_{\text{CRM}}}$ for tea samples with high content of F (black, matcha and green tea), and CRM with low level of F was used for the

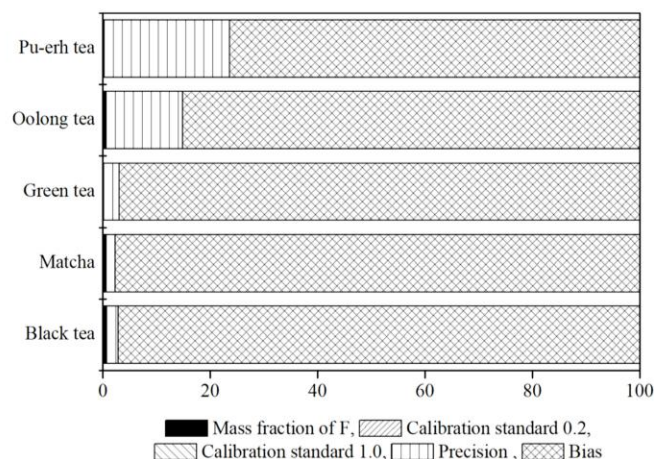


Chart 1. Percent contributions of uncertainty components to combined standard uncertainty.

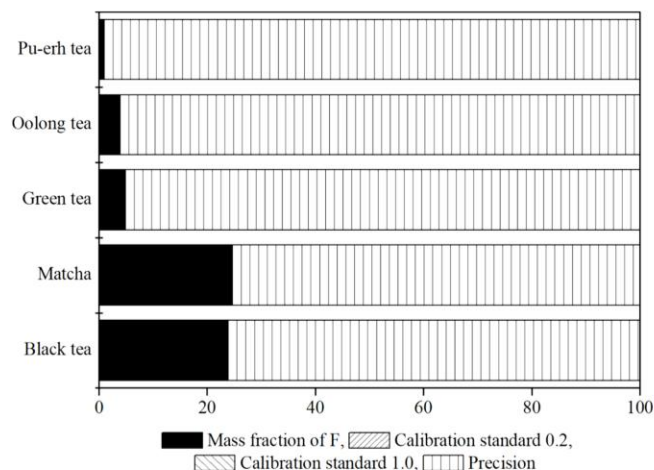


Chart 2. Percent contributions of uncertainty components to combined standard uncertainty (bias excluded).

calculation of $u_{b_{\text{CRM}}}$ for tea samples with low content of F (oolong and Pu-erh tea). The value of $u_{w_{\text{F(CRM-cer)}}}^2$ is much lower in the case of low F level CRM than in the case of high F level CRM. Therefore, also the contribution of $u_{b_{\text{CRM}}}$ is lower in the case of tea samples with low level F (76–85%) than in the case of tea samples with high level of F (97–98%).

The only way to lower the contribution of $u_{b_{\text{CRM}}}$ to the $u_{c(\text{sam})}$ and consequently expanded uncertainty would be to choose a vegetation based CRM with narrower certified intervals. Unfortunately, at the time of the study, SRM 2695 was the only available CRM on the market with certified values of F in vegetation.

Since the contribution of $u_{b_{\text{CRM}}}$ is that large, the impact of uncertainty components other than the bias cannot be seen from Chart 1. The impacts of only $u_{w_{\text{F(sam)}}}$, $u_{w_{\text{CS-0.2}}}$, $u_{w_{\text{CS-1.0}}}$ and $u_{p_{\text{sam}}}$ to $u_{c(\text{sam})}$ were therefore visualized in a form of a bar chart (Chart 2).

The largest contribution to the $u_{c(\text{sam})}$ for all tea samples is $u_{p_{\text{sam}}}$ (75–99%). The contribution of $u_{w_{\text{F(sam)}}}$ is 1–25% and the contributions of $u_{w_{\text{CS-0.2}}}$ and $u_{w_{\text{CS-1.0}}}$ are negligible. The ratio between contributions of $u_{p_{\text{sam}}}$ and $u_{w_{\text{F(sam)}}}$ varies depending on the value of $w_{\text{F(sam)}}$ and s in sample analysis. Taking green tea as an illustrative example, the contribution of $u_{p_{\text{sam}}}$ is higher than 50%, if relative s is higher than approximately 0.2% and the contribution of $u_{p_{\text{sam}}}$ is higher than 90% if relative s is higher than approximately 2%. Accordingly, for a quick and rough estimation of the MU, the $u_{w_{\text{F(sam)}}}$, $u_{w_{\text{CS-0.2}}}$ and $u_{w_{\text{CS-1.0}}}$ can be neglected, and reporting the MU based on $u_{p_{\text{sam}}}$ and $u_{b_{\text{CRM}}}$ only, is justified.

In conclusion, the MU evaluation in accordance with the GUM is a challenging and demanding task, but it is important to identify all possible uncertainty components, to include random and systematic effects and to assure traceability to SI units. Sadly, the GUM laid the foundations for the MU evaluation already in 1995, but papers reporting the results together with expanded uncertainty are still scarce. Our contribution is aimed to increase the awareness of the importance of MU evaluation, to help and encourage chemists from different analytical fields to evaluate the MU for their measurements and to add a small pebble to a mosaic of the MU evaluation in chemistry.

4. Material and methods

4.1. Reagents and standards

All reagents were of EMSURE® grade and all solutions were prepared using double-distilled water. A 50% (w/w) solution of NaOH (Merck) was prepared by dissolving the reagent pellets in water. For alkaline carbonate fusion, solid NaKCO₃ (Merck) was used. For the acidification

of the samples, conc. H_2SO_4 (Merck) was used. Calibration standards for calibration of F-ISE were prepared by successive dilution of a certified $100\text{ }\mu\text{g/ml}$ F^- standard solution (Orion). The accuracy of calibration curve was tested by certified $100\text{ }\mu\text{g/ml}$ F^- standard solution from a different bottle (Orion). A citrate buffer solution (CBS buffer) was prepared as reported previously [20]. A CRM Fluoride in Vegetation (SRM 2695) from National Institute of Standards and Technology (NIST), consisting of a standard with a high ($277\text{ }\mu\text{g/g}$) and low ($64.0\text{ }\mu\text{g/g}$) level of F was used for the bias study.

4.2. Instrumentation

An analytical balance (Mettler Toledo model AT201) was used for weighing. The samples were decomposed by alkaline carbonate fusion in platinum crucibles using a Bunsen burner. An Orion 960 Autochemistry System with a temperature sensor and a combination F-ISE (Orion 96-09) was used for a potentiometric determination of F^- and density of the certified $100\text{ }\mu\text{g/ml}$ F^- standard solution.

4.3. Sample collection and preparation

The proposed procedure was applied to the determination of F in different types of tea. Black tea (Twinings Original English Breakfast) and matcha (dmBio Matcha Tee) were bought in retail stores Mercator and dm-drogerie markt, respectively, while green tea (Sencha), oolong tea (Milky Oolong) and Pu-erh (Pu-erh Superior) were bought in tea-house Chai. Samples were dried on Teflon plates in a laboratory oven (Thermo, T 6) at $60\text{ }^\circ\text{C}$ then ground, homogenized in an agate ball mill and finally passed through 0.25 mm sieve (Retsch).

4.4. Sample decomposition

Approximately 0.5 g of the CRM (high level), black tea, matcha and green tea and approximately 1.0 g of the CRM (low level), oolong tea and Pu-erh tea was weighed on a balance with readability of 0.00001 g prior to the decomposition by alkaline carbonate fusion [21]. The samples were then cooled to a room temperature and quantitatively transferred to 100 ml volumetric flasks. Before determining the F^- content, the samples were acidified with the addition of conc. H_2SO_4 to pH 2–3 and diluted with water to the mark. Samples were filtered through a 589/1 black ribbon paper (Whatman) prior to the analysis.

4.5. Determination of fluoride

The F^- was determined by direct potentiometry using the F-ISE at room temperature ($T = (20 \pm 5)\text{ }^\circ\text{C}$) calibrated using two calibration standards: one below and one above the expected concentration of F^- in the sample, i.e. at $0.2\text{ }\mu\text{g/ml}$ and $1.0\text{ }\mu\text{g/ml}$ of F^- . These standards were prepared by adding 1.0 ml and 5.0 ml of $10\text{ }\mu\text{g/ml}$ standard F^- solution into two 50 ml flasks. The $10\text{ }\mu\text{g/ml}$ standard F^- solution was prepared by dilution of certified $100\text{ }\mu\text{g/ml}$ F^- standard solution (10 ml in 100 ml volumetric flask). A 20 ml of a mixture of all the reagents used during decomposition by alkaline carbonate fusion was added to each flask. Finally, to assure a constant and high ionic strength of the solution, the appropriate pH and complexation of interfering ions, a 20 ml of CBS buffer was added to each flask before diluting with water to the mark. Polyethylene volumetric flasks and beakers were used throughout.

The F^- content in the decomposed samples and CRMs was determined in a 20 ml aliquot. Each sample was analyzed according to the suggested procedure for 3 times and CRMs for 5 times. The content of F^- in each portion was measured at least twice. The repetitive analyses of the same sample were carried out on the same day using one calibration curve. The accuracy of the calibration curve was checked after its

construction and then every two hours.

4.6. Determination of density of certified F^- standard solution

The density of the certified $100\text{ }\mu\text{g/ml}$ F^- standard solution was determined by dispensing five 5.214 ml aliquots of solution, whose weights were averaged and used to calculate the density.

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Declarations of interest

None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jfluchem.2018.08.010>.

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Supplementary data

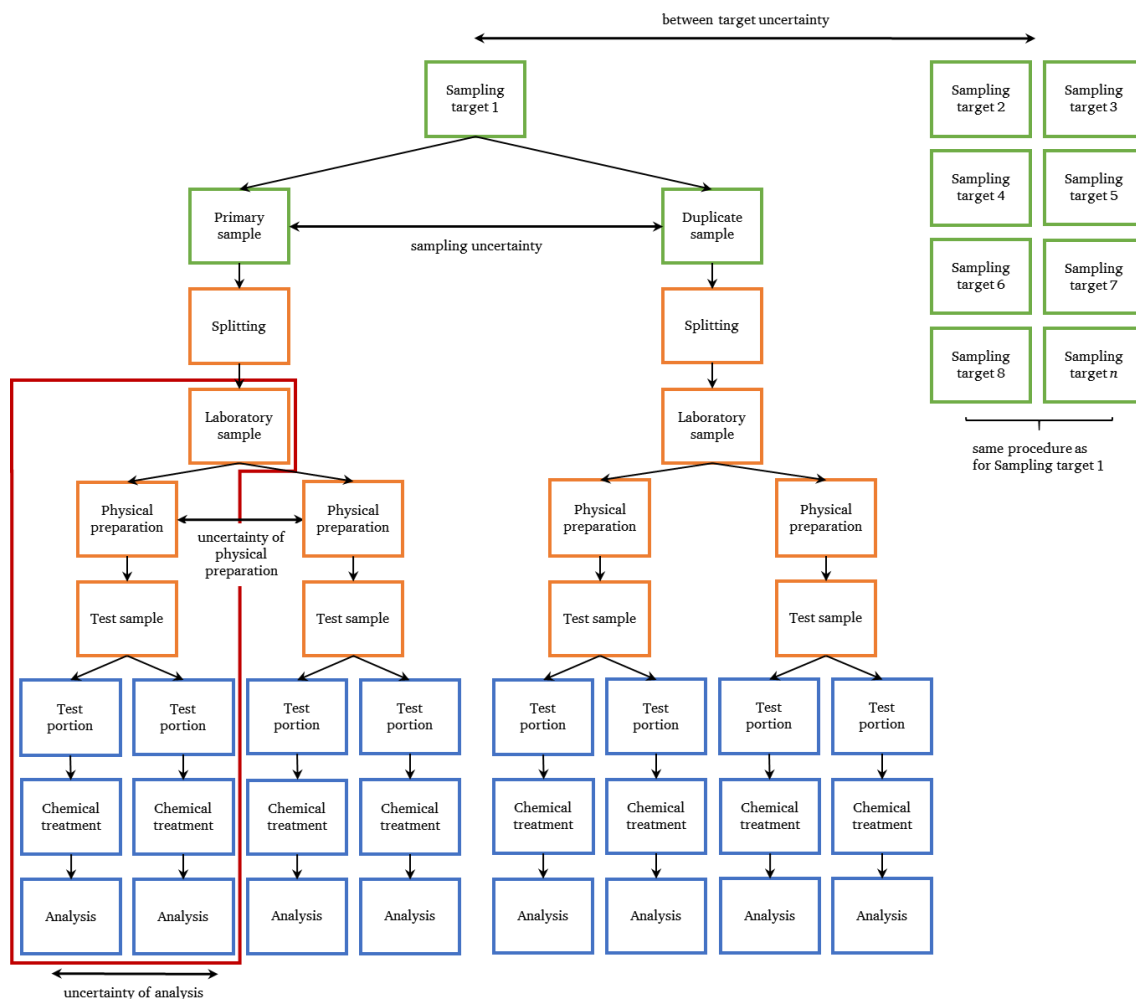
S1. Measurement uncertainty arising from sampling and physical sample preparation

The whole measurement process is presented in Scheme S1 and is divided into three steps: (1) sampling (depicted in green), (2) physical sample preparation (depicted in orange) and (3) chemical analysis (depicted in blue). The measurement uncertainty (MU) evaluation arising from sampling and physical sample preparation is described in the guide "Measurement uncertainty arising from sampling" [1] and from the chemical analysis in the guide "Quantifying uncertainty in analytical measurement" [2].

First a sampling target is chosen and from it a primary sample is taken as a single sample or by applying a particular sampling protocol (e.g. W shape) a number of increments, which are then combined. Minimum eight sampling targets are selected and duplicate samples are taken (e.g. different orientation of the W shape). The primary sample is split to form a laboratory sample, which is then divided in two portions that go separately through several steps of physical sample preparation (drying, milling and sieving) to obtain two test samples. At least two test portions from each test sample are taken, chemically treated and analysed. Duplicate samples go through the exact same steps as primary samples.

In such methodology of duplicated sampling, duplicated physical sample preparation and duplicated analysis, four different uncertainties are obtained with analysis of variance (ANOVA): (1) between target uncertainty due to heterogeneity between sampling targets, (2) sampling uncertainty due to heterogeneity within sampling target and uncertainty of a particular sampling protocol, (3) uncertainty of physical sample preparation and (4) uncertainty of analysis.

The objective of this paper was to develop a procedure for the determination of the analyte content and evaluation of associated measurement uncertainty in laboratory samples (depicted in red frame on Scheme S1). Evaluation of uncertainties arising from sampling and physical sample preparation was therefore out of the scope of the paper.



Scheme S1. Measurement process and associated uncertainties.

References

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 [2] Eurachem/CITAC, Quantifying uncertainty in analytical measurement, 2012.

S2. Derivation of Eq. 4

$$\begin{aligned}
 \gamma_{F(\text{sam})} &= 10 \frac{-E_{\text{cell}} + I}{S_{\text{sam}}} \\
 \frac{u_{\gamma_{F(\text{sam})}}}{\gamma_{F(\text{sam})}} &= 2.303 u \left(\frac{-E_{\text{cell}} + I}{S_{\text{sam}}} \right) \\
 \frac{u_{\gamma_{F(\text{sam})}}}{\gamma_{F(\text{sam})}} &= 2.303 \sqrt{\frac{u_{E_{\text{cell}}}^2}{S_{\text{sam}}^2} + \frac{u_I^2}{S_{\text{sam}}^2} + \frac{(-E_{\text{cell}} + I)^2 u_{S_{\text{sam}}}^2}{S_{\text{sam}}^4}} \\
 \left(\frac{u_{\gamma_{F(\text{sam})}}}{\gamma_{F(\text{sam})}} \right)^2 &= \left(\frac{2.303}{S_{\text{sam}}} \right)^2 u_{E_{\text{cell}}}^2 + \left(\frac{2.303}{S_{\text{sam}}} \right)^2 u_I^2 + \left(\frac{2.303 (-E_{\text{cell}} + I)}{S_{\text{sam}}^2} \right)^2 u_{S_{\text{sam}}}^2
 \end{aligned}$$

$$\begin{aligned}
u\left(\frac{-E_{\text{cell}} + I}{S_{\text{sam}}}\right) &=? \\
\left(\frac{u\left(\frac{-E_{\text{cell}} + I}{S_{\text{sam}}}\right)}{\frac{-E_{\text{cell}} + I}{S_{\text{sam}}}}\right)^2 &= \left(\frac{u(-E_{\text{cell}} + I)}{-E_{\text{cell}} + I}\right)^2 + \left(\frac{u(S_{\text{sam}})}{S_{\text{sam}}}\right)^2 \\
\left(\frac{u\left(\frac{-E_{\text{cell}} + I}{S_{\text{sam}}}\right)}{\frac{-E_{\text{cell}} + I}{S_{\text{sam}}}}\right)^2 &= \frac{u_{E_{\text{cell}}}^2 + u_I^2}{(-E_{\text{cell}} + I)^2} + \frac{u_{S_{\text{sam}}}^2}{S_{\text{sam}}^2} \\
\left(u\left(\frac{-E_{\text{cell}} + I}{S_{\text{sam}}}\right)\right)^2 &= \frac{u_{E_{\text{cell}}}^2}{S_{\text{sam}}^2} + \frac{u_I^2}{S_{\text{sam}}^2} + \frac{(-E_{\text{cell}} + I)^2 u_{S_{\text{sam}}}^2}{S_{\text{sam}}^4}
\end{aligned}$$

S3. Derivation of Eq. 18

$$\begin{aligned}
\left(\frac{u_{\rho_{100}}}{\rho_{100}}\right)^2 &= \left(\frac{u_{V_{\text{disp}}}}{V_{\text{disp}}}\right)^2 + \left(\frac{u(m_{\text{fill}} - m_{\text{empt}})}{m_{\text{fill}} - m_{\text{empt}}}\right)^2 \\
\left(\frac{u_{\rho_{100}}}{\rho_{100}}\right)^2 &= \left(\frac{u_{V_{\text{disp}}}}{V_{\text{disp}}}\right)^2 + \frac{u_{m_{\text{fill}}}^2 + u_{m_{\text{empt}}}^2}{(m_{\text{fill}} - m_{\text{empt}})^2}
\end{aligned}$$

Standard uncertainty of weighing operations is associated with: (1) balance systematic error ($u_{m_{\text{sys}}}$), (2) balance calibration uncertainty ($u_{m_{\text{cal}}}$), (3) balance daily drift ($u_{m_{\text{dd}}}$) and (4) repeatability of weighing of filled ($u_{m_{\text{rep}}(\text{fill})}$) and empty vials ($u_{m_{\text{rep}}(\text{empt})}$).

$$u_m^2 = u_{m_{\text{sys}}}^2 + u_{m_{\text{cal}}}^2 + u_{m_{\text{dd}}}^2 + u_{m_{\text{rep}}}^2$$

Therefore:

$$\left(\frac{u_{\rho_{100}}}{\rho_{100}}\right)^2 = \left(\frac{u_{V_{\text{disp}}}}{V_{\text{disp}}}\right)^2 + \frac{u_{m_{\text{rep}}(\text{fill})}^2 + u_{m_{\text{rep}}(\text{empt})}^2 + 2u_{m_{\text{sys}}}^2 + 2u_{m_{\text{cal}}}^2 + 2u_{m_{\text{dd}}}^2}{(m_{\text{fill}} - m_{\text{empt}})^2}$$

S4. Detailed procedure for quantification of all uncertainty components

The uncertainty components and their standard uncertainties were quantified and are presented in Table S4.

Table S4: Detailed procedure for quantification of all uncertainty components.

Contributor	Abbr.	Unit	(Average) value (V)	Uncertainty component	Abbr.	Reference	Uncertainty	Type	Standard uncertainty (u_i)	u_i^2	u_i/V	$(u_i/V)^2$
Sample decomposition and determination of F ⁻ with F-ISE												
Mass fraction of F ⁻												
Black tea	$W_{f(\text{black})}$	µg/g	413.3		$U_{f(\text{black})}$	Eq.3						
	$W_{f(\text{black})}$	µg/g		Calibration	$U_{f(\text{black})}$	Eq.3	1.168	B, $k=1.96$	$5.102 \cdot 10^{-6}$	$2.603 \cdot 10^{-11}$	$1.854 \cdot 10^{-5}$	$3.438 \cdot 10^{-10}$
Matcha	$W_{f(\text{matcha})}$	µg/g	373.4	Daily drift	$U_{f(\text{matcha})}$	Certificate	1.510 ⁵	B, $k=2$	$7.500 \cdot 10^{-6}$	$5.625 \cdot 10^{-11}$	$1.772 \cdot 10^{-5}$	$3.140 \cdot 10^{-10}$
	$W_{f(\text{matcha})}$	µg/g			U_{dri}	Control chart	$1.190 \cdot 10^{-5}$	A, $\sqrt{2}11$	$8.194 \cdot 10^{-7}$	$6.713 \cdot 10^{-13}$	$1.783 \cdot 10^{-5}$	$3.180 \cdot 10^{-10}$
Green tea	$W_{f(\text{green})}$	µg/g	192.0		$U_{f(\text{green})}$	Eq.3						
	$W_{f(\text{green})}$	µg/g		Calibration	$U_{f(\text{green})}$	Eq.3	0.5484	B, $\sqrt{3}$	0.005376	$2.890 \cdot 10^{-5}$	$8.389 \cdot 10^{-4}$	$7.037 \cdot 10^{-7}$
Oolong tea	$W_{f(\text{oolong})}$	µg/g	125.2	Temperature	$U_{f(\text{oolong})}$	Eq.15	0.1050	B, $\sqrt{3}$	0.06062	0.003675	$8.948 \cdot 10^{-6}$	$8.006 \cdot 10^{-11}$
Pu-erh tea	$W_{f(\text{puerh})}$	µg/g	80.89	Repeatability	$U_{f(\text{puerh})}$	Fill-and-weigh	0.017	A, $\sqrt{10}$	0.005376	$2.890 \cdot 10^{-5}$	$8.968 \cdot 10^{-6}$	$8.043 \cdot 10^{-11}$
	$W_{f(\text{puerh})}$	µg/g			U_{rep}	Eq.14						
Sample weight												
Black tea	m_{black}	g	0.49123	Systematic error	U_m	Eq.16			$9.108 \cdot 10^{-6}$			
Matcha	m_{matcha}	g	0.51395	Calibration	$U_{m(\text{black})}$	Eq.3						
	m_{matcha}	g			$U_{m(\text{black})}$	Eq.3	1.066	B, $\sqrt{3}$	0.001866	0.001200	$9.399 \cdot 10^{-4}$	$8.835 \cdot 10^{-7}$
Green tea	m_{green}	g	0.51075	Daily drift	$U_{m(\text{matcha})}$	Certificate	1.510 ⁵	B, $k=2$	$7.500 \cdot 10^{-6}$	$5.625 \cdot 10^{-11}$	$1.772 \cdot 10^{-5}$	$3.140 \cdot 10^{-10}$
Oolong tea	m_{oolong}	g	1.01789		U_{dri}	Control chart	$1.190 \cdot 10^{-5}$	A, $\sqrt{2}11$	$8.194 \cdot 10^{-7}$	$6.713 \cdot 10^{-13}$	$1.783 \cdot 10^{-5}$	$3.180 \cdot 10^{-10}$
Pu-erh tea	m_{puerh}	g	1.01557	Calibration	$U_{m(\text{oolong})}$	Eq.3						
	m_{puerh}	g			$U_{m(\text{oolong})}$	Eq.3	0.3565	B, $\sqrt{3}$	0.0011848	0.0011848	$5.397 \cdot 10^{-6}$	$5.410 \cdot 10^{-6}$
100 ml volumetric flask												
	V_{100}	ml	100.0	Calibration	$U_{f(\text{puerh})}$	Eq.3	0.2305	B, $\sqrt{3}$	0.0011848	0.0011848	$5.397 \cdot 10^{-6}$	$5.410 \cdot 10^{-6}$
20 ml pipette												
	V_{20}	ml	20.00	Calibration	$U_{f(\text{puerh})}$	Eq.3	0.2305	B, $\sqrt{3}$	0.0011848	0.0011848	$5.397 \cdot 10^{-6}$	$5.410 \cdot 10^{-6}$
50 ml volumetric flask												
	V_{50}	ml	50.00	Calibration	$U_{f(\text{puerh})}$	Eq.3	0.2305	B, $\sqrt{3}$	0.0011848	0.0011848	$5.397 \cdot 10^{-6}$	$5.410 \cdot 10^{-6}$
Mass concentration of F ⁻												
Black tea	$Y_{f(\text{black})}$	µg/ml	0.8121	Calibration	$U_{f(\text{black})}$	Eq.3						
	$Y_{f(\text{black})}$	µg/ml		Temperature	$U_{f(\text{black})}$	Eq.3	1.066	B, $\sqrt{3}$	0.001866	0.001200	$9.399 \cdot 10^{-4}$	$8.835 \cdot 10^{-7}$
Matcha	$Y_{f(\text{matcha})}$	µg/ml	0.7676	Repeatability	$U_{f(\text{matcha})}$	Fill-and-weigh	0.0029	A, $\sqrt{10}$	0.04700	$8.410 \cdot 10^{-7}$	$9.399 \cdot 10^{-4}$	$8.835 \cdot 10^{-7}$
	$Y_{f(\text{matcha})}$	µg/ml			U_{rep}	Eq.14						
Green tea	$Y_{f(\text{green})}$	µg/ml	0.3921		$U_{f(\text{green})}$	Certificate	0.06	B, $\sqrt{3}$	0.03464	0.001200	$9.399 \cdot 10^{-4}$	$8.835 \cdot 10^{-7}$
Oolong tea	$Y_{f(\text{oolong})}$	µg/ml	0.5100		$U_{f(\text{green})}$	Eq.15	0.05250	B, $\sqrt{3}$	0.03031	$9.188 \cdot 10^{-4}$	$9.399 \cdot 10^{-4}$	$8.835 \cdot 10^{-7}$
Pu-erh tea	$Y_{f(\text{oolong})}$	µg/ml	0.3286		$U_{f(\text{oolong})}$	Fill-and-weigh	0.030	A, $\sqrt{10}$	0.009487	$9.000 \cdot 10^{-5}$	$9.399 \cdot 10^{-4}$	$8.835 \cdot 10^{-7}$
	$Y_{f(\text{oolong})}$	µg/ml			U_{rep}	Eq.14						
	$Y_{f(\text{puerh})}$	µg/ml			$U_{f(\text{puerh})}$	Eq.3						

Precision									
Black tea	P_{black}	$\mu\text{g/g}$	Eq.10	U_{Pblack}	$S_{wPblack}$	3.615	$A, \sqrt{N}_{black}$	2.087	4.355
Matcha	P_{matcha}	$\mu\text{g/g}$	Eq.10	$U_{Pmatcha}$	$S_{wPmatcha}$	3.231	$A, \sqrt{N}_{matcha}$	1.865	3.479
Green tea	P_{green}	$\mu\text{g/g}$	Eq.10	U_{Pgreen}	$S_{wPgreen}$	4.231	$A, \sqrt{N}_{green}$	2.443	5.967
Oolong tea	P_{oolong}	$\mu\text{g/g}$	Eq.10	$U_{Poolong}$	$S_{wPoolong}$	3.084	$A, \sqrt{N}_{oolong}$	1.781	3.170
Pu-erh tea	P_{puerh}	$\mu\text{g/g}$	Eq.10	U_{Ppuerh}	$S_{wPpuerh}$	4.172	$A, \sqrt{N}_{puerh}$	2.409	5.803
Bias									
CRM-H	$B_{CRM(H)}$	$\mu\text{g/g}$	Observed value	$U_{BCRM(H)}$	Eq. 13	5.720	$A, \sqrt{N}_{CRM-H}$	14.01	196.3
			Certified value	$S_{wB(CRM(H))}$	s	27	$B, k = 1.96$	2.558	6.544
CRM-L	$B_{CRM(L)}$	$\mu\text{g/g}$	Observed value	$U_{BCRM(L)}$	Eq. 13	1.653	$A, \sqrt{N}_{CRM-L}$	4.349	18.91
			Certified value	$S_{wB(CRM(L))}$	s	8.4	$B, k = 1.96$	0.7393	0.5465
Combined standard uncertainty									
Black tea	$U_{C(black)}$	$\mu\text{g/g}$	14.21	$U_{BwP(black)}$					
Matcha	$U_{C(matcha)}$	$\mu\text{g/g}$	14.17	$U_{BwP(matcha)}$					
Green tea	$U_{C(green)}$	$\mu\text{g/g}$	14.23	$U_{BwP(green)}$					
Oolong tea	$U_{C(oolong)}$	$\mu\text{g/g}$	4.713	$U_{BwP(oolong)}$					
Pu-erh tea	$U_{C(puerh)}$	$\mu\text{g/g}$	4.977	$U_{BwP(puerh)}$					
Expanded uncertainty ($k = 1.96$)									
Black tea	U_{black}	$\mu\text{g/g}$	27.86						
Matcha	U_{matcha}	$\mu\text{g/g}$	27.78						
Green tea	U_{green}	$\mu\text{g/g}$	27.90						
Oolong tea	U_{oolong}	$\mu\text{g/g}$	9.237						
Pu-erh tea	U_{puerh}	$\mu\text{g/g}$	9.755						

4.4 Fluorine in Vegetation due to an Uncontrolled Release of Fluorides from a Glassworks

In this chapter the article entitled "Fluorine in vegetation due to an uncontrolled release of gaseous fluorides from a glassworks: a case study of measurement uncertainty, dispersion pattern and compliance with regulation" by D. Štepec, G. Tavčar and M. Ponikvar-Svet, published in *Environmental Pollution* in 2019, is presented [151]. D. Štepec, the author of this thesis, participated in performing the experiments, analysing the data, interpreting the results and writing the manuscript.

In the glass industry, hydrofluoric acid is used for polishing and etching of the glass. Gaseous HF emissions are controlled using scrubbers and filters; however, accidental releases may occur due to routine maintenance or failure of emissions control devices. Such releases can have serious consequences, as exposure to high fluoride concentrations can cause necrosis and chlorosis in plants and fluorosis in animals and humans.

This study was initiated because local inhabitants in the vicinity of the Rogaška glassworks (Rogaška Slatina, Slovenia) observed the appearance of necrotic and chlorotic lesions on different plants. The first sampling and determination of F was conducted by another laboratory and the results revealed low F content, thus the cause of damage has not been identified. On the request of the inter-municipal inspector of the area, our laboratory repeated the sampling and determination of F content. Four plant species, i.e. peach, common hornbeam, common bean and common grape vine were collected at a single distance from the emitter. In addition, Norway spruce was collected at various distances from the emitter with the purpose of plotting the F content against radial distance from the emitter.

The F content in these plant species grown at comparable distances from the emitter was 87–676 $\mu\text{g/g}$ and highly exceeded the F content in area unpolluted by fluoride (10 $\mu\text{g/g}$), thus confirming the uncontrolled release of fluorides from the glassworks. The initial F content determined by another laboratory was probably low, because total sample decomposition was not performed. Samples were only extracted with water and therefore only water-soluble fluoride was determined.²

The maximum permissible F content in animal feedstuffs is 50 $\mu\text{g/g}$ [152]. The determined F content was above this limit for peach, common hornbeam, common bean and common grape vine. By using the suggested inverse-power function equation, it was estimated that the F content in the Norway spruce was above this limit at distances up to approximately 380 m from the emitter. The maximum permissible F content in food for humans was estimated at 21 $\mu\text{g/g}$ for an adult of 70 kg body weight. The determined F content was above this limit for peach, common hornbeam, common bean and common grape vine. By using the suggested equation, it was estimated that the F content in the Norway spruce was above this limit at distances up to approximately 570 m.

² The results of our analyses were delivered to inter-municipal inspector of the area, who requested the analyses. The Direction of the glassworks was unofficially informed by us about the results.



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Fluorine in vegetation due to an uncontrolled release of gaseous fluorides from a glassworks: A case study of measurement uncertainty, dispersion pattern and compliance with regulation[☆]

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ABSTRACT

This study was initiated after the appearance of chlorotic and necrotic lesions on vegetation in the vicinity of a glassworks. The aim was to establish whether the cause was an uncontrolled release of gaseous fluorides. Five different plant species (Norway spruce, peach, common hornbeam, common bean, common grape vine) were collected in the influenced area, and the fluorine (F) content was determined by a fluoride ion selective electrode after prior total sample decomposition by alkaline carbonate fusion. The measurement results were reported together with their measurement uncertainties (MUs), which were evaluated according to the *Guide to the Expression of Uncertainty in Measurement*. The F contents at comparable distances from the emitter and in a clean area, free from natural or anthropogenic fluoride emissions, were 87–676 and 10 $\mu\text{g g}^{-1}$, respectively, thereby confirming the release of gaseous fluorides from the glassworks. The F contents in samples of Norway spruce taken at various radial distances from the emitter suggest that the emitted gaseous fluorides were spread about evenly in all directions from the source following an inverse-power function. Estimated distances at which the F content would decrease to 50 $\mu\text{g g}^{-1}$ (allowed maximum content of F in feeding stuffs) and 21 $\mu\text{g g}^{-1}$ (maximum fluoride content in vegetables and fruits in relation to the upper limit of fluoride intake for humans) were 378 m and 571 m, respectively, from the emitter. Evaluation of our results for compliance with specification revealed a lack of regulation on fluoride content in the diet of humans and animals as well as a lack of guidelines on how to take into account MU.

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1. Introduction

Fluorine (F) is ubiquitous in our environment. In nature it occurs in the anionic form as fluoride (F^-) in rocks or soils, air and water. The F is released into the air in the form of gaseous (e.g., HF, SiF_4) or particulate fluorides (e.g., CaF_2 , NaF, Na_3AlF_6) from natural sources, such as volcanoes, marine aerosols and forest fires, and from various industrial activities, such as phosphate fertilizer and elemental phosphorus production, aluminium smelting, petroleum refining, and glass and brick manufacture. Gaseous HF and SiF_4 are between 1 and 3 orders of magnitude more toxic than other

common pollutants (O_3 , SO_2 , peroxyacyl nitrates, Cl_2 , Cl^-) (Weinstein and Davison, 2003). The Industrial Emissions Directive (EU, 2010) is the main European Union (EU) instrument regulating pollutant emissions, including fluorine, from industrial installations. Fluoride emission measurements are periodic to assure continued compliance with emission standards. Periods of higher than normal emissions can, however, occur in most industries due to routine maintenance or failure of scrubbing equipment.

An accidental release of F^- can have severe consequences because of its toxicity to vegetation, animals and humans (Ponikvar, 2008; Barbier et al., 2010; Agalakova and Gusev, 2012). The background F content in plants is low (often as low as 1 and less than 10 $\mu\text{g g}^{-1}$ in most species) (Weinstein and Davison, 2003) and very unlikely to exceed 30 $\mu\text{g g}^{-1}$ (Kabata-Pendias and Pendias, 2001). Exposure to higher amounts can cause F^- intoxication resulting in the appearance of chlorotic and necrotic lesions (McNulty and Newman, 1961). As F^- compounds easily penetrate tissue and

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accumulate in biomass almost immediately after exposure, the determination of total F in vegetation is of help in estimating a measure of an exposure (Zimny, 1984; Koblar et al., 2011).

Animals normally ingest small amounts of F^- in their diet without experiencing any adverse effects, but increased consumption of high-fluoride forage can cause fluorosis. In the European Union, the maximum content of F in feeding stuffs is regulated by EC and EU directives (EC, 2002; EC, 2005; EU, 2015) concerning undesirable substances in animal feed. The prescribed limit for complete feed for cattle, sheep and goats is 50 mg kg^{-1} of F. However, suggested threshold values of F in forage are $30\text{--}40 \text{ mg kg}^{-1}$ (Kabata-Pendias and Pendias, 2001).

F^- intake by humans has both beneficial and negative effects, and the ranges of intakes producing these opposing effects do not differ greatly from each other. Because of the beneficial effect of F^- on the prevention of caries, the adequate intake of F^- from all sources for both children and adults is set at $0.05 \text{ mg kg body weight}^{-1}$ per day (EFSA, 2017) (3.5 mg for a 70 kg person). Excessive intake during a longer period can cause the development of dental fluorosis in children and skeletal fluorosis in both children and adults (Ponikvar, 2008). The upper limit of F^- intake from all sources is set at $0.12 \text{ mg kg body weight}^{-1}$ per day (EFSA, 2005) (8.4 mg for a 70 kg person).

Thus, accurate and precise analytical methods for determination of F^- are of crucial importance. The most commonly used current methods for the determination of F^- are a fluoride ion selective electrode (ISE) (Frant and Ross, 1966) and ion chromatography (Gleisner et al., 2010). ISE remains the method of choice because it is cost-effective, highly selective and covers a wide range of concentrations ($1\text{--}10^{-5} \text{ mol l}^{-1}$). Because ISE responds to free F^- ions only, F-containing samples must be decomposed first. Different acids and mixtures of acids can be used to extract and dissolve the F present in the samples but only to varying degrees; therefore, total decomposition of the samples by employing pyrohydrolysis, oxygen bomb combustion or fusion with alkali metal hydroxides or carbonates (Pavlović et al., 2018) is a prerequisite prior to F^- measurements.

A truly informed decision based on a measurement result can only be made with the consideration of measurement uncertainty (MU) (JCGM, 2012). Classically, in analytical chemistry, MU has been expressed as one standard deviation (s), thus including only random effects. A superior, more comprehensive approach is outlined in the *Guide to the Expression of Uncertainty in Measurement* (GUM), the definitive document in the field of metrology (JCGM, 2008). In this approach, in addition to random effects, systematic effects are also taken into account, and all uncertainty components of each step of an analytical procedure are identified, quantified and traceable (JCGM, 2012) to the SI units. All of these contributors are then combined, and MU is reported as an expanded uncertainty corresponding to the 95% level of confidence. Recently Štepec et al. (2019) addressed the importance of evaluating MU according to the GUM in research laboratories and suggested a procedure for MU evaluation for determination of F in vegetation.

The evaluation of MU according to the GUM is also important for stating compliance with a specification of quantitative measurements, as the statement of compliance is generally based on a 95% coverage probability for the expanded uncertainty. To satisfy the requirements of the International Organization for Standardization/International Electrotechnical Commission Standard 17025 (ISO and IEC, 2017), laboratories should provide customers with the statements of measurement results, their uncertainties and the assessment of compliance/noncompliance with specification, when requested to do so, in accordance with the guidelines prepared by International Laboratory Accreditation Cooperation (ILAC, 2009).

The present study was initiated after the appearance of chlorotic

and necrotic lesions on vegetation in the vicinity of the Rogaška Slatina glassworks (Slovenia). There are many climatic, edaphic and biological factors that could cause such symptoms. The focus of this study was to establish whether the cause was an uncontrolled release of gaseous fluorides from the glassworks via the determination of F content in five different plant species (Norway spruce, peach, common hornbeam, common bean and common grape vine) from the influenced area. The main objectives of this paper were to present the results from the perspective of (1) MU evaluation according to the GUM, (2) the pattern of gaseous fluoride dispersion at ground level and (3) compliance with regulations on the allowed maximum content of F in feeding stuffs for cattle, sheep and goats (EC, 2002; EC, 2005; EU, 2015) and upper limit of F^- intake for humans (EFSA, 2005). By fulfilling the objectives, it should be possible to identify (1) issues and concerns related to natural or anthropogenic fluoride emissions and (2) needs for future actions regarding fluoride guidelines and regulation.

2. Materials and methods

2.1. Reagents and standards

All reagents were of EMSURE® grade, and all solutions were prepared using double-distilled water.

A certified reference material (CRM), Fluoride in Vegetation (SRM 2695) from the National Institute of Standards and Technology (NIST), consisting of a standard with a high ($(277 \pm 27) \mu\text{g g}^{-1}$) and low ($(64.0 \pm 8.4) \mu\text{g g}^{-1}$) level of F was used (1) to check the accuracy of the method employed and (2) for the bias study in the evaluation of MU according to the GUM (Štepec et al., 2019).

2.2. Instrumentation

An analytical balance (Mettler Toledo model AT201) was used for weighing. An Orion 960 Autochemistry System with a temperature sensor and a combination ISE (Orion 96–09) was used for potentiometric determination of F^- .

2.3. Procedures

2.3.1. Sampling area and weather conditions

Rogaška Slatina is a tourist, medium-sized municipality (Slovenia, EU). The most important economic activities are tourism, glass industry, construction and manufacture of cosmetics, which does not include manufacture of dental care products. The first signs of F^- injuries to the vegetation in the surroundings of the Rogaška Slatina glassworks were observed on the morning of May 25, 2015. During the previous night, temperatures were $11\text{--}16^\circ\text{C}$, relative humidity was 66–95%, wind speeds were $0.1\text{--}1.3 \text{ m s}^{-1}$ and there was no rainfall (Table S1). During the next two weeks, temperatures were $6\text{--}30^\circ\text{C}$, relative humidity was 30–98%, wind speeds were $0.0\text{--}4.7 \text{ m s}^{-1}$ and rainfall was $0.0\text{--}1.0 \text{ mm}$ (Table S1) (ARSO, 2015).

2.3.2. Sample collection and preparation

Five different species of vegetation, i.e. Norway spruce needles (*Picea abies*), peach leaves (*Prunus persica*), common hornbeam leaves (*Carpinus betulus*), common bean leaves (*Phaseolus vulgaris*) and common grape vine leaves (*Vitis vinifera*), were sampled on September 11, 2015. Needles of Norway spruce were collected at various radial distances from the emitter ($d = 90\text{--}393 \text{ m}$) from the side of the tree facing the factory. The control sample was collected in a clean area, i.e. free from natural or anthropogenic fluoride emissions (Razori, Slovenia). Criteria for defining and selecting a clean area are described in more detail in our previous study

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(Koblar et al., 2011). The samples were dried on Teflon plates in a laboratory oven (Thermo, T 6) at $T = 60^\circ\text{C}$ and then ground, homogenized in an agate ball mill and finally passed through a 0.25 mm sieve (Retsch).

2.3.3. Sample decomposition

CRMs and samples were decomposed by alkaline carbonate fusion (Ponikvar et al., 2007; Koblar et al., 2011). Each sample was weighed into a 30 ml platinum crucible and soaked with 1 ml of 50% (w/w) sodium hydroxide (NaOH). After evaporation to dryness in a sand bath, each sample was decomposed by fusion with approximately 4 g of potassium sodium carbonate (KNaCO_3) using a modified Bunsen burner and melted until a transparent melt with no solid remains was obtained. Initial weights were approximately 1.0 g. Based on the results of these analyses, the weight of each CRM and sample for subsequent analyses was adjusted so that the potential readout was within the range of a calibration curve.

2.3.4. Determination of fluoride

The contents of F^- in the decomposed CRMs and samples were determined in corresponding aliquots by direct potentiometry using ISE calibrated at $0.2\ \mu\text{g ml}^{-1}$ and $1.0\ \mu\text{g ml}^{-1}$ following the previously reported procedure (Ponikvar et al., 2000; Štepec et al., 2019). Each CRM was analyzed in quintuplicate and each sample in triplicate. The content of F^- in each digestate was measured at least twice.

2.4. Measurement uncertainty evaluation

MUs according to the GUM were calculated based on a flowchart diagram recently suggested by Štepec et al. (2019) for MU evaluation for the determination of F in vegetation by ISE after prior total decomposition by alkaline carbonate fusion (S2).

3. Results and discussion

On the morning of May 25, 2015, local inhabitants in the vicinity of the Rogaška Slatina glassworks observed the appearance of

chlorotic and necrotic lesions on vegetation typical of F^- intoxication. As can be seen in the photos taken by a local resident on that day (Fig. 1. A and B), the lesions appeared on the side of the Norway spruce facing the glassworks. The camera with which the photos were taken recorded the date and time. The first sampling of damaged vegetation and F determinations were carried out about one week after the damage was observed. The results revealed low F contents (this work was not conducted by our laboratory) and thus did not support the suspicion that the injuries were caused by an uncontrolled release of gaseous fluorides. The cause of the damage was not identified in the subsequent months, prompting the inspector for this area to request the present study, and our laboratory repeated sampling for subsequent F determinations on September 11, 2015.

3.1. Determination of fluorine content and evaluation of measurement uncertainty

Samples of Norway spruce, which is a well-distributed species in the area around the emitter, were collected at various radial distances from the emitter. In addition, representative samples of four other species were collected (peach, common hornbeam, common bean and common grape vine) at a single distance from the emitter. Other plant species were not collected either because their growth was suppressed or their growth started later than the release occurred. The sampling sites and main wind directions in the area are outlined in Fig. 2.

The characteristic signs of F^- phytotoxicity, i.e. chlorosis and necrosis, were still visible in all species, but to varying degrees (Fig. 3).

The F contents were determined by ISE after prior total decomposition by alkaline carbonate fusion. The MUs of the measurement results were evaluated by two classical approaches and the GUM approach: (1) one standard deviation (s), which corresponds to a 68.3% confidence level; (2) a confidence interval for the mean to give a higher confidence level, calculated as s multiplied by the appropriate Student's t value and divided by the square root of a



Fig. 1. Photos of damaged Norway spruce at a distance of 214 m from the emitter: distant photo (A) and close photo (B) (taken on May 25, 2015, photo: local resident Sajko, F.).



Fig. 2. Map of the Rogaška Slatina glassworks area showing the location of the emitter, sampling locations and the main wind directions (Kartendaten © 2018 Google Bilder © 2018, CNES/Airbus, DigitalGlobe). (Legend: ■ emitter, → main wind directions, ▲ Norway spruce at various distances from the emitter (d) ($d_1 = 90$ m, $d_2 = 214$ m, $d_3 = 258$ m, $d_4 = 295$ m, $d_5 = 393$ m), □ peach ($d = 242$ m), ★ common hornbeam ($d = 256$ m), ● common grape vine ($d = 238$ m), ◆ common bean ($d = 241$ m)).

number of measurements (N_{sam}); and (3) expanded uncertainties according to the GUM (U_{sam}). The last approach is briefly presented in S2, and all the details are discussed in the original paper (Štepec et al., 2019). The determined F contents in the samples ($w_{\text{F(sam)}}$) at different distances from the emitter (d) together with their MUS evaluated by three different approaches are listed in Table 1.

First, the results of the CRM analyses were compared with the certified values using the statistical test recommended by the European Reference Materials (ERM, 2010). For both CRMs, the results of determinations agreed well with the certified values, demonstrating that the method employed is accurate.

The F contents determined in the samples taken in the influenced area of the glassworks were 3–125 times higher than those measured in the clean area, thereby confirming the sudden release of gaseous fluorides. Since no additional damage to vegetation was observed after May 25, 2015, it was assumed that the high F contents found in vegetation after sampling on September 11, 2015 were due to the May 25th accident.

The reason for the low F contents initially found in the damaged vegetation (not conducted in our laboratory) and subsequent exclusion of the release of gaseous fluoride as a possible cause can be ascribed to the employed analytical method. The F content in the samples was determined after prior extraction with water, which assures only the release of water-soluble fluoride, representing only a fraction of total F. On the other hand, in our laboratory, total decomposition of the sample by alkaline carbonate fusion, which assures the release of the entire F present in the sample, was carried out. In addition, it is important to note that the efficacy of the

extraction procedure with water for subsequent F^- determination cannot be supported by CRM analysis because a suitable CRM is not available.

Considerable differences in the MUS (Table 1) with respect to the approach used for their evaluation were observed. The s and $t \cdot s / \sqrt{N_{\text{sam}}}$, which are classically used in the scientific literature to indicate measurement precision, were notably lower in comparison to the expanded uncertainties (U_{sam}) because the value of precision is limited by random effects only. The only way of reporting a valid result is together with the U_{sam} estimated according to the GUM (JCGM, 2008) because (1) in addition to the random effects, systematic effects are also included; (2) traceability to SI units is assured; and (3) all uncertainty components of each step of the analytical procedure are considered. As stated by the GUM (JCGM, 2008), “Without such an indication, measurement results cannot be compared, either among themselves or with reference values given in a specification or standard.”

3.2. Pattern of gaseous fluoride dispersion

Studies reporting a pattern of gaseous fluoride dispersion based on the determination of F content in different vegetation species (or air) in relation to the distance from the pollution source are relatively sparse (Sidhu, 1979; Perkins et al., 1980; Haidouti et al., 1993; Vike, 1999; Mezghani et al., 2005; Franzaring et al., 2006; Elloumi et al., 2016). In general, the pattern of gaseous fluoride dispersion and the concentration gradients at ground level depend on the height of the emission, the temperature of the plume, wind

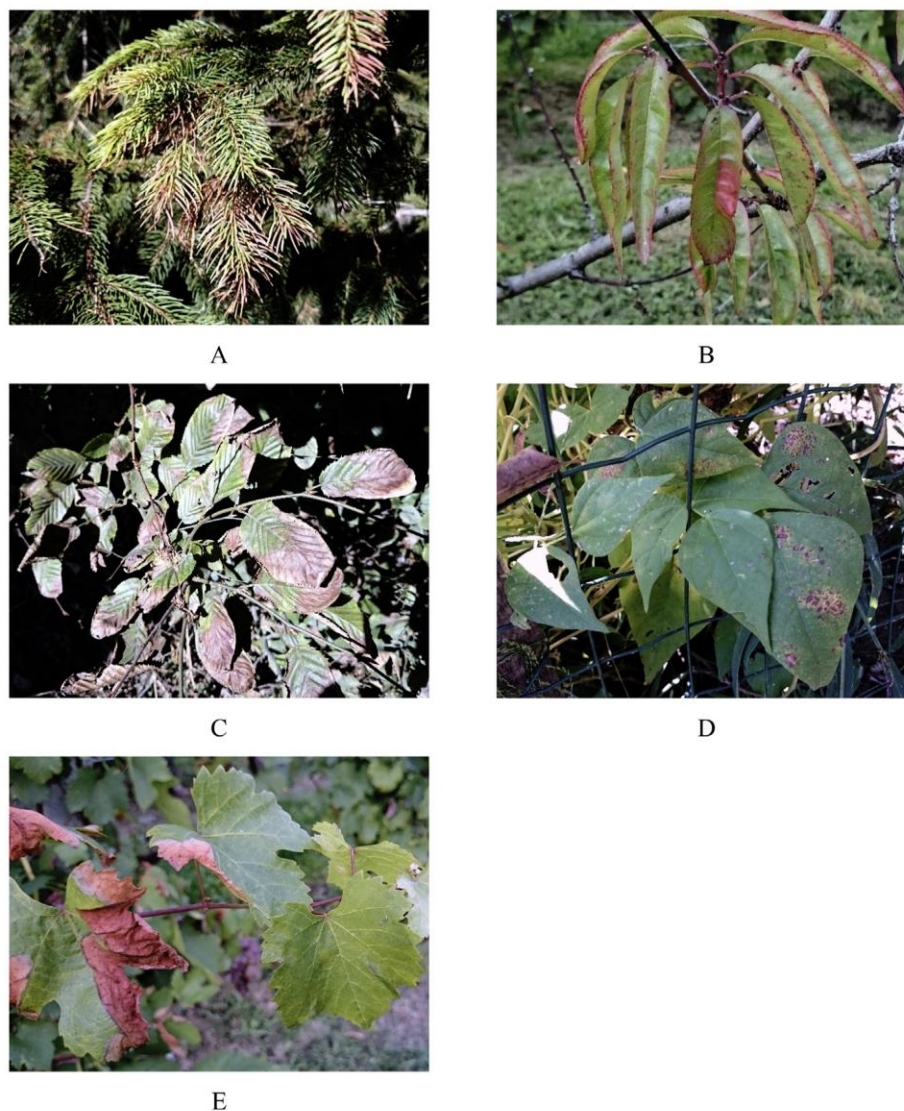


Fig. 3. Photos of damaged vegetation at different distances from the emitter (d): Norway spruce needles at $d = 214$ m (A), peach leaves at $d = 242$ m (B), common hornbeam leaves at $d = 256$ m (C), common bean leaves at $d = 241$ m (D) and common grape vine leaves at $d = 238$ m (E) (taken on September 11, 2015, photo: Ponikvar-Svet, M.).

Table 1
The F contents determined in CRMs, Norway spruce, peach, common hornbeam, common bean and common grape vine together with their associated expanded uncertainties, standard deviations and confidence intervals for the mean.

Sample	d [m]	$W_{F(sam)}$ [$\mu\text{g g}^{-1}$]	U_{sam}^a [$\mu\text{g g}^{-1}$]	s^b [$\mu\text{g g}^{-1}$]	$t \cdot s / \sqrt{N_{sam}}^c$ [$\mu\text{g g}^{-1}$]
Norway spruce	90	1209	69	55	137
Norway spruce	214	219	28	4	11
Norway spruce	258	87.4	9.6	3.9	9.7
Norway spruce	295	67.8	8.6	1.2	2.9
Norway spruce	393	30.5	8.6	0.9	2.3
Norway spruce, control	95032	9.7	8.5	0.3	0.8
Peach	242	132	10	4	9
Common hornbeam	256	676	36	20	51
Common bean	241	539	33	16	40
Common grape vine	238	264	28	5	12
SRM-2695, high level		295		6	11
SRM-2695, low level		68.0		1.7	3.2

^a Coverage factor $k = 1.96$ to give expanded uncertainty at a 95% confidence level.

^b 68.3% confidence level.

^c $t = 4.3$ to give a 95% confidence level ($N = 3$).

direction, wind speed, turbulence, precipitation, topography and characteristics of the vegetation (Weinstein and Davison, 2004).

In our study, the pattern of gaseous fluoride dispersion at ground level was studied via determination of F content in the samples of Norway spruce collected at different radial distances from the emitter (Fig. 4). We expected that, due to the still weather conditions (see 2.3 Procedures – Sampling area and weather conditions and Table S1), gaseous fluorides were spread about evenly in all directions from the source, diffusing both horizontally and vertically. Therefore, the F content in Norway spruce can be fitted with respect to the distances from the emitter with an inverse-power function (Equation (1) and Fig. 4). The use of different forms of inverse power functions for fitting measured pollution levels with respect to the distance from the measuring sites was discussed by Gulliver et al. (2015).

$$w_{F(\text{sam})} = (2.64912 \cdot 10^7 \pm 1.72501 \cdot 10^7) \cdot d^{(-2.22088 \pm 0.14347)}; \\ R^2 = 0.99576 \quad (1)$$

Equation (1) was linearized by a log-log transformation of the variables ($w_{F(\text{sam})}$ and d) into equation (2) because of simpler operation with the logarithmic function than the power function.

$$\log w_{F(\text{sam})} = (7.42310 \pm 0.18631) - (2.22088 \pm 0.14347) \cdot \log d \quad (2)$$

Using equation (2), it was estimated that the F contents in Norway spruce would decrease to 10 and 30 $\mu\text{g g}^{-1}$ (background contents) at a distance of 780 m and 476 m, respectively, from the emitter.

Equations (1) and (2) are applicable only to the estimation of F content in Norway spruce because of different susceptibilities of plants to atmospheric fluorides. The main reasons are differences in (1) accumulation ability for F (Koblar et al., 2011; Koblar et al., 2015) and (2) leaf/needle specific weights (Witkowski and Lamont, 1991). This is also evident from our results, i.e. the F contents in Norway spruce, peach, common hornbeam, common bean and common grape vine sampled at comparable distances from the emitter (238–258 m) were highly variable and ranged from 87 to 676 $\mu\text{g g}^{-1}$ (Table 1).

3.3. Compliance with regulations

Norway spruce, peach, common hornbeam, common bean and common grape vine were selected for determination of F contents

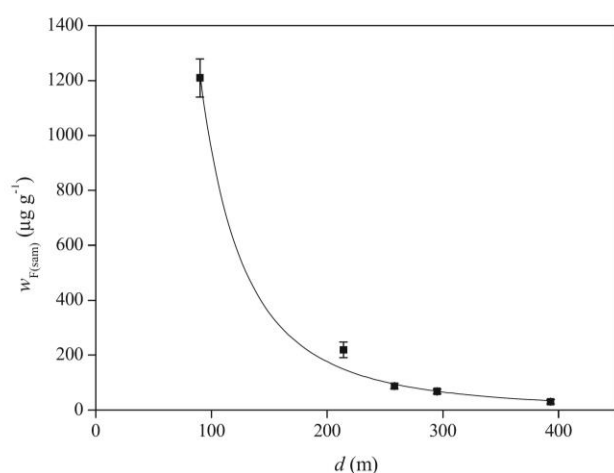


Fig. 4. The F content in Norway spruce ($w_{F(\text{sam})} \pm$ expanded uncertainties) as a function of distance from the emitter (d).

because they still showed symptoms of F^- intoxication three months after the release of gaseous fluorides from the glassworks.

The maximum F content in feeding stuffs set by EU standards (EC, 2002; EC, 2005; EU, 2015) is 50 $\mu\text{g g}^{-1}$ in complete feed for cattle, sheep and goats. Using equation (2), it was estimated that the F content in Norway spruce would decrease to 50 $\mu\text{g g}^{-1}$ at a distance of 378 m from the emitter. The upper limit of F^- intake for humans from all sources is set at 0.12 mg kg body weight $^{-1}$ per day (EFSA, 2005), which is equivalent to 8.4 mg per day for a 70 kg person. Since the recommended consumption of fruit and vegetables is set at more than 400 g day $^{-1}$ (≈ 5 portions) (WHO/FAO, 2003), their F^- content should be 21 $\mu\text{g g}^{-1}$ or less because fruits and vegetables are not the only source of F^- . Using equation (2), it was estimated that the F content in Norway spruce would decrease to 21 $\mu\text{g g}^{-1}$ at a distance of 559 m from the emitter. At a comparable distance, the other analyzed plant species contained a higher F content than that of Norway spruce. Consequently, it can be assumed that forage or crops could contain a similar or even higher F content and that the distance at which the F content would decrease to 50 or 21 $\mu\text{g g}^{-1}$ might be even greater.

The ILAC (2009) and ISO/IEC Standard 17025 (2017) provide guidelines on how to consider MU when reporting compliance with specification. It is straightforward to report compliance/non-compliance when the value of the measurand plus/minus the expanded uncertainty is above or below limit. But in cases when the value is above or below the limit, but the limit is within the expanded uncertainty, it is advised to consult with the client and/or regulator as to the appropriate action to take in such cases (Eurachem and CITAC, 2007).

Our results can be stated as follows: under the measurement and environmental conditions and by taking MU (coverage probability of 95%) into account, the determined F contents are

- (1) above the specification limit of the maximum content of F in feeding stuffs (EC, 2002; EC, 2005; EU, 2015) for peach, common hornbeam, common bean, common grape vine and Norway spruce (at distances up to 295 m) and below the specification limit for Norway spruce at a distance of 393 m, and
- (2) above the specification limit for the upper limit of F^- intake for humans (EFSA, 2005) for peach, common hornbeam, common bean and common grape vine. In the case of Norway spruce, the results are above the specification limit at distances of up to 295 m and at 393 m for a body weight up to 73 kg. Above that weight, it is not possible to state compliance because the result plus/minus expanded uncertainty overlaps the limit.

4. Conclusions

The ISE measurements after prior total decomposition by alkaline carbonate fusion revealed that the F contents in five different plant species (Norway spruce, peach, common hornbeam, common bean and common grape vine) at comparable distances from the Rogaška Slatina glassworks emitter were 87–676 $\mu\text{g g}^{-1}$, which is up to 125 times higher than that in a clean area free from natural or anthropogenic fluoride emissions (10 $\mu\text{g g}^{-1}$). These results confirmed the sudden release of gaseous fluorides from the glassworks. This study highlighted the importance of assuring total sample decomposition prior to F^- analysis by ISE and the importance of MU evaluation according to the GUM. The F contents in samples of Norway spruce taken at various radial distances from the emitter suggested that the release followed an inverse-power function.

It was estimated that the F content in Norway spruce would decrease to 50 $\mu\text{g g}^{-1}$ (allowed maximum content of F in feeding

stuffs) and $21 \mu\text{g g}^{-1}$ (maximum content of F^- in vegetables and fruits in relation to the upper limit of F^- intake for humans) at distances of 378 and 571 m, respectively, from the emitter. Norway spruce contained the lowest F content among the five different species growing at comparable distances from the emitter. Therefore, it can be assumed that forage and crops could contain similar or even higher F content and that the distance at which the F content would decrease to 50 or $21 \mu\text{g g}^{-1}$ could be even greater.

Evaluation of our results for compliance with specifications revealed a lack of regulation regarding F^- content in human and animal diets as well as a lack of guidelines on how to take into account MU.

In conclusion, we should (1) put more emphasis on identifying possible accidental releases of gaseous or particulate fluorides, (2) increase awareness of food as a possible source of F^- for humans and animals, (3) pay more attention to regulation concerning F^- in human or animal food, (4) encourage research laboratories to also report measurement results together with MU estimated in accordance with the GUM and (5) establish firm guidelines on how to take into account MU when reporting compliance with specifications.

Declaration of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2019.02.046>.

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Supplementary data

S1. Weather conditions in Podčetrtek (Atomske toplice) from May 24 to May 25, 2015

Weather conditions in Podčetrtek from May 24 to May 25, 2015 are presented in Table S1. The weather conditions from May 26 to June 7, 2015 can be found in supplementary data at <https://doi.org/10.1016/j.envpol.2019.02.046>.

Table S1. Average temperature, average relative humidity, rainfall and wind speed in Podčetrtek.

Date and time	Average temperature [°C]	Average relative humidity [%]	Rainfall [mm]	Wind speed [m/s]
May 24, 2015, 12:00 AM	11.5	92	0.0	0.5
May 24, 2015, 1:00 AM	11.3	91	0.0	0.5
May 24, 2015, 2:00 AM	11.1	92	0.0	0.5
May 24, 2015, 3:00 AM	10.9	92	0.0	0.3
May 24, 2015, 4:00 AM	10.5	94	0.0	0.3
May 24, 2015, 5:00 AM	10.6	94	0.0	1.0
May 24, 2015, 6:00 AM	11.0	92	0.0	0.3
May 24, 2015, 7:00 AM	11.8	86	0.0	2.1
May 24, 2015, 8:00 AM	12.9	82	0.0	1.1
May 24, 2015, 9:00 AM	14.1	73	0.0	1.5
May 24, 2015, 10:00 AM	15.2	67	0.0	1.2
May 24, 2015, 11:00 AM	16.6	62	0.0	1.8
May 24, 2015, 12:00 PM	17.6	61	0.0	2.6
May 24, 2015, 1:00 PM	17.9	58	0.0	2.7
May 24, 2015, 2:00 PM	18.8	56	0.0	2.8
May 24, 2015, 3:00 PM	18.1	58	0.0	2.2
May 24, 2015, 4:00 PM	18.9	58	0.0	2.6
May 24, 2015, 5:00 PM	18.5	57	0.0	1.3
May 24, 2015, 6:00 PM	17.6	71	0.0	0.3
May 24, 2015, 7:00 PM	16.3	77	0.0	0.3
May 24, 2015, 8:00 PM	15.7	79	0.0	1.5
May 24, 2015, 9:00 PM	14.9	82	0.0	1.0
May 24, 2015, 10:00 PM	14.2	86	0.0	0.5
May 24, 2015, 11:00 PM	13.3	88	0.0	0.1
May 25, 2015, 12:00 AM	12.7	90	0.0	0.4
May 25, 2015, 1:00 AM	12.4	92	0.0	0.1
May 25, 2015, 2:00 AM	12.0	92	0.0	0.2
May 25, 2015, 3:00 AM	11.7	93	0.0	0.3
May 25, 2015, 4:00 AM	11.5	94	0.0	0.5
May 25, 2015, 5:00 AM	11.2	94	0.0	0.4
May 25, 2015, 6:00 AM	11.6	95	0.0	1.1
May 25, 2015, 7:00 AM	12.3	93	0.0	0.9
May 25, 2015, 8:00 AM	13.3	86	0.0	0.3
May 25, 2015, 9:00 AM	15.2	73	0.0	0.6
May 25, 2015, 10:00 AM	16.3	66	0.0	1.1
May 25, 2015, 11:00 AM	17.8	57	0.0	1.3
May 25, 2015, 12:00 PM	18.1	58	0.0	1.0
May 25, 2015, 1:00 PM	17.5	68	0.1	0.5
May 25, 2015, 2:00 PM	17.2	72	0.0	0.9
May 25, 2015, 3:00 PM	17.5	68	0.0	0.6
May 25, 2015, 4:00 PM	17.5	75	0.0	0.5
May 25, 2015, 5:00 PM	16.4	86	0.0	0.5
May 25, 2015, 6:00 PM	15.6	90	0.0	0.7
May 25, 2015, 7:00 PM	14.9	89	0.0	0.2
May 25, 2015, 8:00 PM	14.1	92	0.0	0.5
May 25, 2015, 9:00 PM	13.7	93	0.0	0.0
May 25, 2015, 10:00 PM	13.2	94	0.0	0.2
May 25, 2015, 11:00 PM	13.1	95	0.0	0.4

S2. Measurement uncertainty evaluation

The measurement uncertainty was evaluated according to a "Guide to the expression of uncertainty in measurement" (JCGM, 2008) based on a procedure suggested by Štepec et al. (2018). The values of uncertainty components, their standard uncertainties, combined standard uncertainties and the expanded uncertainties are presented in Table S2.

Table S2. Values of uncertainty components and their standard uncertainties, combined and expanded uncertainties.

Value (standard uncertainty)	Norway spruce, $d = 90$ m	Norway spruce, $d = 214$ m	Norway spruce, $d = 258$ m	Norway spruce, $d = 295$ m	Norway spruce, $d = 393$ m	Norway spruce, control	Peach, $d = 247$ m	Common hornbeam, $d = 256$ m	Common bean, $d = 241$ m	Common grape vine, $d = 238$ m
Sample decomposition and determination of F by F-ISE										
$W_{\text{FSam}}^{\text{CRM}}$ ($U_{W_{\text{FSam}}^{\text{CRM}}}$)	1209 (3.46)	219 (0.621)	87.4 (0.249)	67.8 (0.194)	30.5 (0.0868)	9.65 (0.0275)	132 (0.376)	676 (1.92)	539 (1.54)	264 (0.753)
m_{sam} (U_m)	0.102 (9.11·10 ⁻⁶)	0.504 (9.11·10 ⁻⁶)	2.03 (9.11·10 ⁻⁶)	2.02 (9.11·10 ⁻⁶)	2.90 (9.11·10 ⁻⁶)	5.96 (9.11·10 ⁻⁶)	0.978 (9.11·10 ⁻⁶)	0.196 (9.11·10 ⁻⁶)	0.302 (9.11·10 ⁻⁶)	0.600 (9.11·10 ⁻⁶)
V_{100} (U_{V100})	(ml)				100 (0.0839)					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				20.0 (0.0212)					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				50.0 (0.0470)					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				0.230					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				0.515					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				0.529					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				0.651					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				0.632					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				0.996 (0.00553)					
Calibration standards										
$W_{\text{CS},0.2}$ ($U_{W_{\text{CS},0.2}}$)	($\mu\text{g/g}$)				0.201 (0.00210)					
$W_{\text{CS},1.0}$ ($U_{W_{\text{CS},1.0}}$)	($\mu\text{g/g}$)				1.00 (0.00890)					
V_{100} (U_{V100})	(ml)				100 (0.289)					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				10.0 (0.0131)					
V_{100} (U_{V100})	(ml)				100 (0.0839)					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				50.0 (0.0470)					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				1.00 (0.00818)					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				5.00 (0.0300)					
V_{FSam} ($U_{V_{\text{FSam}}}$)	(ml)				0.996 (0.00553)					
Precision ($N_{\text{sam}} = 3$)										
U_{FSam} ($U_{U_{\text{FSam}}}$)	($\mu\text{g/g}$)	31.9	2.59	2.26	0.685	0.543	0.183	2.14	11.8	9.32
$S_{\text{wF(CRM)}}$ ($U_{S_{\text{wF(CRM)}}}$)	($\mu\text{g/g}$)	55.3	4.49	3.91	1.19	0.940	0.318	3.71	20.5	16.1
Bias ($N_{\text{CRM}} = 5$)										
U_{CRM} ($U_{U_{\text{CRM}}}$)	($\mu\text{g/g}$)	14.0	14.0	4.35	4.35	4.35	4.35	4.35	14.0	14.0
$S_{\text{wF(CRM)}}$ ($U_{S_{\text{wF(CRM)}}}$)	($\mu\text{g/g}$)	5.72	5.72	1.65	1.65	1.65	1.65	1.65	5.72	5.72
U_{CRM} ($U_{U_{\text{CRM}}}$)	($\mu\text{g/g}$)	13.8	13.8	4.29	4.29	4.29	4.29	4.29	13.8	13.8
Combined standard uncertainty										
U_{CRM} ($U_{U_{\text{CRM}}}$)	($\mu\text{g/g}$)	35.1	14.3	4.91	4.41	4.38	4.35	4.86	18.4	16.9
Expanded uncertainty ($k = 1.96$)										
U_{CRM} ($U_{U_{\text{CRM}}}$)	($\mu\text{g/g}$)	68.7	28.0	9.62	8.64	8.59	8.53	9.53	36.1	33.1
										28.0

Abbreviations

B_{CRM}	bias determined by CRM analysis ($\mu\text{g/g}$)
m_{sam}^a	sample weight (g)
N_{CRM}	number of replicate analyses of CRM
N_{sam}^a	number of replicate analyses of sample
P_{sam}^a	precision of sample analysis ($\mu\text{g/g}$)
$S_{\text{wF(CRM)}}$	standard deviation of replicate analyses of CRM ($\mu\text{g/g}$)

$S_{wF(sam)}^a$	standard deviation of replicate analyses of sample ($\mu\text{g/g}$)
u^b	standard uncertainty
$u_{c(sam)}^a$	combined standard uncertainty ($\mu\text{g/g}$)
u_m	standard uncertainty of weighting operations (g)
U_{sam}^a	expanded uncertainty ($\mu\text{g/g}$)
V_{f50}	volume of 50 ml volumetric flask (ml)
V_{f100}	volume of 100 ml volumetric flask (ml)
V_{p1}	volume of 1 ml pipette (ml)
V_{p5}	volume of 5 ml pipette (ml)
V_{p10}	volume of 10 ml pipette (ml)
V_{p20}	volume of 20 ml pipette (ml)
$w_{CS-0.2}$	mass fraction of 0.2 $\mu\text{g/ml}$ calibration standard ($\mu\text{g/g}$)
$w_{CS-1.0}$	mass fraction of 1.0 $\mu\text{g/ml}$ calibration standard ($\mu\text{g/g}$)
$w_{F(CRM-cer)}$	certified mass fraction of F in CRM ($\mu\text{g/g}$)
$w_{F(sam)}^a$	mass fraction of F in sample ($\mu\text{g/g}$)
γ_{100}	mass concentration of certified 100 $\mu\text{g/ml}$ F^- standard solution ($\mu\text{g/ml}$)
$\gamma_{F(sam)}^a$	mass concentration of F^- in sample solution ($\mu\text{g/ml}$)
ρ_{100}	density of certified 100 $\mu\text{g/ml}$ F^- standard solution (g/ml)

^a subscript sam refers to the samples of Norway spruce collected at different distances from the emitter (90 m, 214 m, 258 m, 295 m, 393 m, control), peach, common hornbeam, common bean or common grape vine

^b standard uncertainties of each uncertainty component are not listed separately

References

- Joint Committee on Guides for Metrology (JCGM), 2008. Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement.
- Štepec, D., Tavčar, G., Ponikvar-Svet, M., 2019. Measurement uncertainty evaluation and traceability assurance for total fluorine determination in vegetation by fluoride ion selective electrode. J. Fluorine Chem. 217, 22–28.

4.5 High Fluorine Content in Some Exotic Superfoods

In this chapter the article entitled "Surprisingly high fluorine content in some exotic superfoods" by D. Štepec, G. Tavčar and M. Ponikvar-Svet, published in the Journal of Fluorine Chemistry in 2020, is presented [153]. D. Štepec, the author of this thesis, performed the experiments, analysed the data, participated in the interpretation of the results and wrote the first draft of the manuscript.

One of the biggest food trends in the recent years has been the consumption of so-called superfoods. The term "superfood" is used to describe foods from remote parts of the world that supposedly have extraordinary health benefits (e.g. acai, chia, goji, ginkgo, maca). Such foods have been imported into Europe, labelled as superfoods and have become very popular among consumers due to excellent marketing.

There is practically no information on the F content in superfoods in databases [86, 87], scientific papers or on their packaging labels. The F content was therefore determined in some exotic superfoods and among the many different types available on the market (algae, grasses, mushrooms, roots, seeds, fruits), two types were selected: (1) superfoods made from the leaves of different plants, shrubs and trees (alfalfa, barley grass, brahmi, ginkgo biloba, gotu kola, gynostemma, matcha, moringa, neem, stevia, tulsi, wheatgrass) and (2) algae superfoods (chlorella, Irish moss, kelp, sea spaghetti, spirulina, wakame). The samples were decomposed by alkaline carbonate fusion, and the F⁻ concentration was determined by F-ISE.

The F content in eight superfoods (Figure 4.1) was surprisingly high in comparison to the F content in commonly consumed foods ($< 3 \mu\text{g/g}$; see Chapter 1.3.3). The highest F content was found in matcha (373 $\mu\text{g/g}$), followed by gotu kola (111 $\mu\text{g/g}$) and ginkgo biloba (91.0 $\mu\text{g/g}$). The F content in tulsi, neem, brahmi, gynostemma and moringa was lower and ranged between 16.4–68.8 $\mu\text{g/g}$. The simultaneous consumption of superfoods and tea on a normal diet can contribute to the adequate intake of fluorine in non-fluoridated water areas and can easily lead to an intake that exceeds the adequate intake or even reaches the upper limit of fluorine in fluoridated water areas.



Figure 4.1: Superfoods with the highest determined F content.

The F content in these eight superfoods was also surprisingly high in comparison to the usual F content in most plant species ($< 10 \mu\text{g/g}$ [20]), which is worrisome because the reason for such a high F content is unknown.



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Surprisingly high fluorine content in some exotic superfoods

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ABSTRACT

Recent years have seen soaring interest in exotic superfoods, which are believed to have extraordinary health benefits. The purpose of the study was to broaden the current knowledge on exotic superfoods and to analyse them for fluorine (F)¹ since excessive intake during prolonged periods of time can cause adverse effects. The F content was determined in 18 plant-based exotic superfoods available on Slovenian market by fluoride ion selective electrode after prior total decomposition by alkaline carbonate fusion. In 8 of the analysed samples, the F content was surprisingly high in comparison to F content in commonly consumed foods (< 3 µg/g). The highest F content was found in matcha (373 µg/g), followed by gotu kola (111 µg/g) and ginkgo biloba (91.0 µg/g). The F content in tulsi, neem, brahmi, gynostemma and moringa was lower and ranged between 16.4–68.8 µg/g. Despite high F content, these superfoods do not pose a risk to human health in non-fluoridated water areas, because they are usually consumed in low quantities. However, in fluoridated water areas (e.g. some parts of Ireland, United Kingdom, United States, Canada) or in areas with high natural fluoride concentration in drinking water, superfoods should be consumed with caution, since the intake of F can with simultaneous consumption of superfoods and tea on a normal diet easily exceed the adequate intake or even reach the upper limit of fluorine. The F content in these 8 superfoods was also surprisingly high in comparison to the usual F content in most plant species (< 10 µg/g). The high F content in matcha is understandable since it is made of *Camellia sinensis* L. leaves, but the high F content in other superfoods is worrisome because the reason for such high F content is unknown and it is possible that these superfoods are contaminated with fluoride due to industrial or agricultural activities.

1. Introduction

The idea of healthy eating has become increasingly popular in recent years. Many people are making a huge effort to take charge of their food choices and are following at least one of the latest food trends, e.g. eating organic, high-protein, vegan, lactose-free or gluten-free products. A particularly popular current food trend is the consumption of so-called superfoods. The term is widely used to describe foods that are believed to improve overall health and well-being and reduce the risk of certain diseases. Some commonly consumed foods that have gained superfood status include broccoli, blueberries, salmon, avocado and almonds. Moreover, there has been growing interest in foods from remote parts of the world that have no history of consumption in Europe (e.g. acai, maca and moringa from the Amazon rainforest, the Andes and the foothills of the Himalayas, respectively). These exotic foods have been massively imported into Europe and have been marketed as superfoods. They have become an inherent part of product lines in

health food shops and supermarkets. Germany is the biggest market for superfood launches in Europe. In 2017, Germans spent almost €670 million on superfoods and the market is growing by around 12 % annually [1].

But why are superfoods experiencing such rapid growth? The main reason is the perception of consumers that superfoods have extraordinary health benefits and can prevent diseases, replace prescription medicines and compensate for a poor diet. The second reason is the current tendency to idealize primitive foods. Consumers seek foods associated with ancient cultures because they presume that traditional agricultural practices produce healthier foods [2]. The third reason is the common belief that remedies made from plants are safe because they are natural, in contrast to synthetic medications. However, the average consumer generally remains unaware that in addition to the bioactive substances that are responsible for beneficial health effects, superfoods may also contain compounds or elements that are toxic to humans. Therefore, superfoods can cause unexpected side effects when

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consumed in considerable quantities.

Scientific papers dealing with superfoods have generally focused on the identification of bioactive substances (e.g. terpenoids, polyphenols) [3–6] and inorganic elements, including essential elements [7–9]. Amongst possible toxic substances, heavy metals have been well researched [3,10–12], but only a few papers have focused on determining the presence of other toxic substances (e.g. endocrine disruptors, pesticides, mycotoxins) [3,13–15].

The purpose of the present study was to search for other toxic species present in superfoods in order to gain deeper insight into superfoods given their increasing popularity. Our preliminary tests showed significant fluorine (F)¹ content in ginkgo biloba and matcha, both considered as superfoods. Fluoride can be both beneficial and toxic to humans. At low intake levels it has therapeutic value in the prevention of dental caries. However, slightly higher intake levels in children during the period of enamel formation can lead to dental fluorosis. Prolonged high intake of fluoride at any age can result in several adverse effects, with the development of skeletal fluorosis being the most obvious [16]. Among the diverse variety of exotic superfoods available on the market (i.e. algae, grasses, mushrooms, roots, seeds, fruits), two types were selected for F content analysis: (1) superfoods made from the leaves of different plants, shrubs and trees (alfalfa, barley grass, brahmi, ginkgo biloba, gotu kola, gynostemma, matcha, moringa, neem, stevia, tulsi, wheatgrass) and (2) algae superfoods (chlorella, Irish moss, kelp, sea spaghetti, spirulina, wakame). To the best of our knowledge, only three papers have examined the F content in these plants [17–19].

The F content was determined in 18 exotic superfoods available on Slovenian market by fluoride ion selective electrode after prior total decomposition by alkaline carbonate fusion. The main objectives were to (1) compare the F content in superfoods to the F content in common food, (2) establish whether superfoods can make a significant contribution to the total daily fluoride intake, (3) evaluate the potential health risk resulting from the consumption of superfoods and (4) compare the F content in superfoods to the usual F content in plants.

2. Materials and methods

2.1. Reagents and standards

All reagents were of EMSURE® grade and all solutions were prepared using double-distilled water. A 50 % (w/w) solution of NaOH (Merck Millipore) was prepared by dissolving the reagent pellets in water. For alkaline carbonate fusion, solid Na₂CO₃ (Merck Millipore) was used. For the acidification of the samples, concentrated H₂SO₄ (Merck Millipore) was used. Calibration standards for the calibration of the fluoride ion selective electrode (ISE) were prepared by successive dilution of a certified 100 µg/mL fluoride standard solution (Thermo Fisher Scientific). A citrate buffer solution (CBS buffer) was prepared as reported previously [20]. Certified reference material “Fluoride in Vegetation” (SRM-2695) from the National Institute of Standards and Technology (NIST) consisting of a standard with a high (277 ± 27 µg/g) and low (64.0 ± 8.4 µg/g) level of fluoride was used to check the accuracy of the methods employed.

2.2. Instrumentation

An analytical balance Mettler Toledo model AT201 was used for weighing. An Orion 960 Autochemistry System (Thermo Fisher Scientific) with a temperature sensor and a combination ISE (Orion 96–09) was used for potentiometric determination of fluoride (F[−]).

2.3. Procedures

2.3.1. Sample collection and preparation

Samples were purchased from supplement providers (Nutrisslim and

Table 1

Information on the samples (trade name, binomial nomenclature, country of origin and brand).

Trade name	Binomial nomenclature	Country of origin	Brand
Leafy superfoods			
Alfalfa	<i>Medicago sativa</i>	China	Nutrisslim
Barley grass	<i>Hordeum vulgare</i>	New Zealand	Herbana
Brahmi	<i>Bacopa monnieri</i>	India	Nutrisslim
Ginkgo biloba	<i>Ginkgo biloba</i>	China	Nutrisslim
Gotu kola	<i>Centella asiatica</i>	Sri Lanka	Nutrisslim
Gynostemma	<i>Gynostemma pentaphyllum</i>	China	Nutrisslim
Matcha	<i>Camellia sinensis</i> L.	Japan	dmBio
Moringa	<i>Moringa oleifera</i>	India	Nutrisslim
Neem	<i>Azadirachta indica</i>	India	Herbana
Stevia	<i>Stevia rebaudiana</i>	Brazil	Herbana
Tulsi	<i>Ocimum tenuiflorum</i>	India	Nutrisslim
Wheatgrass	<i>Triticum aestivum</i>	New Zealand	Herbana
Algae superfoods			
Chlorella	<i>Chlorella vulgaris</i>	China	Nutrisslim
Irish moss	<i>Chondrus crispus</i>	Ireland	Herbana
Kelp	<i>Laminariales</i>	Norway	Herbana
Sea spaghetti	<i>Himanthalia elongata</i>	Ireland	Nutrisslim
Spirulina	<i>Arthrospira platensis</i>	China	Nutrisslim
Wakame	<i>Undaria pinnatifida</i>	Ireland	Nutrisslim

Herbana) and the dm-drogerie markt retail store. The characteristics of the analysed samples are listed in Table 1. Samples were dried on polyethylene trays in a laboratory oven Heraeus T 6 (Thermo Fisher Scientific) at 60 °C, then ground and homogenized in an agate ball mill and finally passed through a 0.25 mm sieve (Retsch).

2.3.2. Fluoride determination and measurement uncertainty evaluation

The samples and CRMs were decomposed by alkaline carbonate fusion [21]. Initial weights were approximately 1.0 g. Based on the results of these analyses, the weight of each CRM and sample for subsequent analyses was adjusted so that the potential readout was within the range of the calibration curve.

The content of fluoride (F[−]) in the decomposed samples and CRMs was determined in corresponding aliquots by direct potentiometry using an ISE calibrated at 0.2 µg/mL and 1.0 µg/mL following the previously reported procedure [22]. Each CRM was analysed in triplicate and each sample in triplicate. The content of F[−] in each digestate was measured at least twice.

Measurement uncertainty (MU) was evaluated according to the Guide to the Expression of Uncertainty in Measurement (GUM) [23]. Expanded uncertainties (U) were calculated based on a procedure recently suggested by Štepec et al. [22] for MU evaluation according to the GUM for the determination of fluorine in vegetation by ISE after prior total decomposition by alkaline carbonate fusion. The approach is briefly presented in Supplementary data (S1).

3. Results and discussion

3.1. Determination of F content in superfoods

There is practically no information on the F content in superfoods in scientific papers, databases and on superfood packaging labels. Therefore, F content was determined in 18 plant-based exotic superfoods available on Slovenian market by fluoride ion selective electrode (ISE) [24]. Two types of superfoods were selected: (1) superfoods made from the leaves of different plants, shrubs and trees (alfalfa, barley grass, brahmi, ginkgo biloba, gotu kola, gynostemma, matcha, moringa, neem, stevia, tulsi, wheatgrass) and (2) algae superfoods (chlorella, Irish moss, kelp, sea spaghetti, spirulina, wakame). Because superfoods are consumed as actual leaves or in powdered form and not in extract form, total F content was determined after prior total sample decomposition by alkaline carbonate fusion. Expanded uncertainties were

Table 2

The F content determined in superfoods and CRMs (w_F) and associated expanded uncertainties (U).

	w _F [µg/g]	U [µg/g]
Leafy superfoods		
Matcha	373	28
Gotu kola	111	9
Ginkgo biloba	91.0	8.6
Tulsi	68.8	8.7
Neem	67.0	8.9
Brahmi	35.3	8.6
Gynostemma	18.1	8.7
Moringa	16.4	8.6
Alfalfa, barley grass, stevia, wheatgrass	< 10.0 ^a	
Algae superfoods		
Chlorella, Irish moss, kelp, sea spaghetti, spirulina, wakame	< 10.0 ^a	
CRMs		
SRM-2695, high F level	295	
SRM-2695, low F level	68.0	

^a The results of the F determinations were outside the range of the calibration curve.

evaluated according to the GUM. The F content in the superfoods (w_F) and associated expanded uncertainties (U) are listed in Table 2.

The results of the CRM analyses were compared to the certified values using the statistical test recommended by the European Reference Materials [25]. For both CRMs, the results of the determinations agreed well with the certified values, demonstrating that the analytical procedure employed is accurate. The F content in selected superfoods varied from less than 10 $\mu\text{g/g}$ to up to 373 $\mu\text{g/g}$.

3.2. F content in superfoods in comparison to F content in common food

The F content of a substantial number of commonly consumed foods and drinks is included in two fluoride databases [26,27]. The F content is generally low, i.e. below 3 $\mu\text{g/g}$, with the only two exceptions being fish and tea. Fish and shellfish might contain up to 11 $\mu\text{g/g}$ if analysed samples, due to mechanical deboning, contain remains of bones and exoskeleton, where fluoride is accumulated from the sea. Tea (*Camellia sinensis* L.) can contain extremely high F content (up to 800 $\mu\text{g/g}$), given that it is one of the few known plants that can uptake considerable amounts of fluoride from the soil and accumulate it in its leaves [28,29].

In the present study the determined F content in 8 superfoods (out of 18 examined) was surprisingly high in comparison to F content in commonly consumed food. The highest F content was found in matcha (373 $\mu\text{g/g}$), followed by gotu kola (111 $\mu\text{g/g}$) and ginkgo biloba (91.0 $\mu\text{g/g}$). The F content in tulsi, neem, brahmi, gynostemma and moringa was lower and ranged between 16.4–68.8 $\mu\text{g/g}$.

3.3. Estimation of daily fluoride intake with diet, superfoods and/or tea

Fluoride is an important but nonessential nutrient; therefore, no “average requirement” for the performance of essential physiological functions has been established. Nevertheless, the setting of an adequate intake (AI) is appropriate because of the beneficial effects of fluoride in the prevention of dental caries. The Institute of Medicine (IOM) [30] and European Food Safety Authority (EFSA) [31] set an AI of fluoride from all sources (including non-dietary) of 0.05 mg/kg body weight/day for children and adults. This corresponds to 3.0–4.5 mg/day of fluoride for an adult of 60–90 kg body weight. Setting an upper limit (UL) is also necessary because excessive intake during a prolonged period of time can cause the development of dental fluorosis in children and skeletal fluorosis in both children and adults. The IOM [30] concluded that there are no signs of skeletal fluorosis observed unless fluoride intake is greater than 10 mg/day for many years. The EFSA

Table 3

Estimated daily fluoride intake from superfoods in non-fluoridated and fluoridated areas, based on their F content and advised daily doses.

Superfood	F content [$\mu\text{g/g}$]	Daily dose [g]	Daily fluoride intake in non-fluoridated areas ^a [mg]	Daily fluoride intake in fluoridated areas ^b [mg]
Matcha	373	1–2	0.37–0.75	0.87–1.25
Gotu kola	111	1–3	0.11–0.33	0.61–0.83
Ginkgo biloba	91.0	3–6	0.27–0.55	0.77–1.05
Tulsi	68.8	1–3	0.07–0.21	0.57–0.71
Neem	67.0	1–3	0.07–0.20	0.57–0.70
Brahmi	35.3	3–6	0.11–0.21	0.61–0.71
Gynostemma	18.1	1–2	0.02–0.04	0.52–0.54
Moringa	16.4	3–6	0.05–0.10	0.55–0.60

^a Superfoods prepared by mixing with 500 mL of fluoride free water.

^b Superfoods prepared by mixing with 500 mL of fluoridated water (1 mg/L F^-).

[32] set an UL from all sources of 0.12 mg fluoride/kg body weight/day for adults, which corresponds to 7.2–10.8 mg/day of fluoride for an adult of 60–90 kg body weight. Since a relatively small difference in the level of intake can produce these opposing effects, an accurate estimate of fluoride intake from different sources is important in order to resolve potential problems associated with under- or overexposure.

Diet is the major source of fluoride intake in adults. The average total daily fluoride intake from the diet in non-fluoridated water areas was estimated to range between 0.56–1.62 mg, and the estimated average total daily fluoride intake in fluoridated water areas was almost two-fold higher at 0.91–3.78 mg [33].

In the present study, the daily fluoride intake from the consumption of superfoods was estimated for non-fluoridated and fluoridated water areas to ascertain whether superfoods can provide a significant contribution of fluoride to the total daily fluoride intake. Eight superfoods with the highest F content were considered and daily fluoride intake was estimated by taking the advised daily doses into the account (Table 3).

It was estimated that the daily fluoride intake from 8 superfoods with the highest F content ranges between 0.02–0.75 mg in non-fluoridated water areas and between 0.52–1.25 mg in fluoridated water areas (Table 3). Some of these superfoods can substantially contribute to the total daily fluoride intake. Matcha, a superfood with the highest F content, can contribute up to 0.75 mg F and 1.25 mg F in non-fluoridated and fluoridated water areas, respectively. Due to higher advised daily dose, ginkgo biloba and gotu kola can also contribute substantially. Ginkgo biloba can contribute up to 0.55 mg F and 1.05 mg F in non-fluoridated and fluoridated water areas, respectively. Gotu kola can contribute up to 0.33 mg F and 0.83 mg F in non-fluoridated and fluoridated water areas, respectively.

In addition, tea can significantly contribute to the total daily fluoride intake. The fluoride concentration in tea as determined in our previous study ranged between 0.31–3.55 mg/L [34]. If the consumption of tea is 2 cups (500 mL), the daily fluoride intake is 0.16–1.78 mg in non-fluoridated water areas and 0.66–2.28 mg in fluoridated water areas, assuming that water with F^- concentration of 1 mg/L is used for tea preparation.

The daily fluoride intake with diet, superfoods and/or tea in non-fluoridated and fluoridated water areas was estimated based on the data obtained from the current and previous studies [33,34]. Daily fluoride intake on a normal diet and on a normal diet with simultaneous consumption of superfoods and/or tea is illustrated in Fig. 1.

In non-fluoridated water areas, the consumption of 8 superfoods with the highest F content can substantially contribute to the AI of fluoride but does not pose a risk to human health because advised daily doses of superfoods are relatively low. The consumption of one daily dose of superfood on a normal diet amounts to 0.58–2.37 mg F and rises

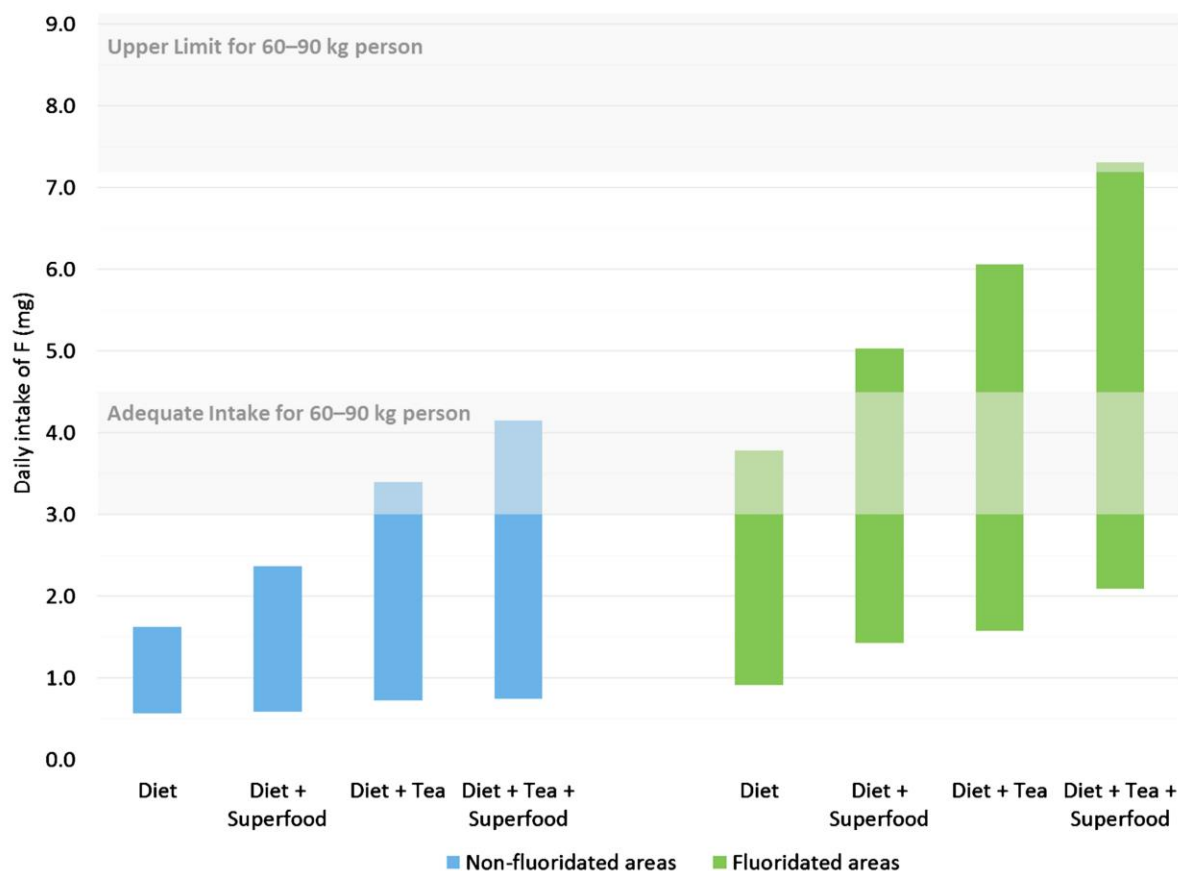


Fig. 1. Estimated ranges of daily fluoride intake from diet, superfoods and/or tea in non-fluoridated and fluoridated water areas.

to 0.74–4.15 mg F with the additional consumption of tea. However, in fluoridated water areas (e.g. some parts of Ireland, United Kingdom, Spain, United States, Canada) or in areas with high natural fluoride concentration in drinking water, superfoods should be consumed with caution, since the intake of F can with simultaneous consumption of superfoods and/or tea on a normal diet easily exceed the AI or even reach the UL. The consumption of one daily dose of superfood on a normal diet amounts to 1.43–5.03 mg F and rises to 2.09–7.31 mg F with the additional consumption of tea. Consumption of more than one daily dose of superfoods with high F content or higher consumption of tea (e.g. in China or England where tea consumption is traditionally high) can result in the UL being exceeded in fluoridated water areas.

Currently, mandatory information on the food label in the European Union includes energy, fat, carbohydrate, protein and salt content, but the declaration of vitamins and minerals is only supplementary information [35]. Therefore, the inclusion of information on the F content is voluntary, and the majority of brands that sell food and drink products with considerable F content do not report its content on the label. However, because it is important to maintain adequate intake, but avoid excessive intake, manufacturers of superfoods should be encouraged to label their products for F content.

3.4. F content in superfoods in comparison to usual F content in plants

The determined F content in 8 (out of 18 examined) plant-based exotic superfoods was also surprisingly high in comparison to usual F content in most plant species. In areas unpolluted by fluoride, where the fluoride concentration in the air is low and plants uptake only a small amount of fluoride from the neutral soil [36], the F content is usually less than 10 µg/g [37]. One of the rare exceptions to this is the tea plant (*Camellia sinensis* L.), which is known to accumulate several

hundred µg F/g even when atmospheric and soil available fluoride contents are low. Therefore, the determined F content in superfoods is worrisome because the reason for such high F content is unknown. The extremely high F content in matcha is understandable since it is made of finely ground and specially grown *Camellia sinensis* L. leaves. But what is the possible reason for the high F content in other superfoods?

The first reason could be that these plants are fluoride accumulators, like *Camellia sinensis* L., however to the best of our knowledge, no study has reported that these plants can accumulate fluoride from the soil. The second reason could be that these plants grow in areas, where soil [38] or air [39] are contaminated with fluoride due to industrial activities (e.g. aluminium smelting, phosphate fertilizer production and glass industry). In fluoride polluted areas, plants can uptake a significant amount of fluoride through the stomata, and the F content in plants near the emission source may exceed 1000 µg/g [40,41]. Moreover, pollution can alter soil pH, potentially changing fluoride solubility and therefore the phytoavailability of fluoride in the soil solution. The third reason could be spraying these plants with fluoride containing irrigation water [39] or use of fluoride containing pesticides [42].

4. Conclusion

Superfoods deserve attention because products bearing this label are increasingly widespread in health food shops and supermarkets. Most studies on superfoods are focused on analysis of active compounds that are responsible for their beneficial health effects, but toxicity studies are scarce. In the present study, 18 plant-based exotic superfoods available on Slovenian market were analysed for their F content, given that excessive intake of fluoride during prolonged periods of time can cause adverse effects. The F content in 8 superfoods was surprisingly

high in comparison to the F content in commonly consumed foods ($< 3 \mu\text{g/g}$). The highest F content was found in matcha ($373 \mu\text{g/g}$), followed by gotu kola ($111 \mu\text{g/g}$) and ginkgo biloba ($91.0 \mu\text{g/g}$). The F content in tulsi, neem, brahmi, gynostemma and moringa was lower and ranged between $16.4\text{--}68.8 \mu\text{g/g}$. Despite high F content, these superfoods do not pose a risk to human health in non-fluoridated water areas, because they are usually consumed in low quantities. However, in fluoridated water areas (e.g. some parts of Ireland, United Kingdom, United States, Canada) or in areas with high natural fluoride concentration in water, superfoods should be consumed with caution, since the intake of F can with simultaneous consumption of superfoods and tea on a normal diet easily exceed the AI or even reach the UL of fluorine.

The determined F content in these 8 superfoods was also surprisingly high in comparison to the usual F content in most plant species ($< 10 \mu\text{g/g}$). The high F content in matcha is understandable since it is made of *Camellia sinensis* L. leaves, but the high F content in other superfoods is worrisome because the reason for such high F content is unknown. Superfood packaging labels usually do not include ingredients and quality certificates and no information about their exact origin or conditions in which they were cultivated and processed is available. Based on F determination only, firm conclusions cannot be drawn, but it is possible that these superfoods are contaminated with fluoride due to industrial or agricultural activities.

This leads to the conclusion that exotic superfoods should be consumed wisely because, although remedies made from plants are often promoted as natural and therefore harmless, they are by no means free from harmful elements or compounds. More research should be performed on these so-called superfoods and determination of active compounds as well as possible contaminants should be encouraged to justify the prefix “super”.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: <https://doi.org/10.1016/j.jfluchem.2020.109521>.

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Supplementary data

S1. Measurement uncertainty evaluation

The measurement uncertainty was evaluated according to a "Guide to the expression of uncertainty in measurement" [1] based on a procedure suggested by Štepec et al. [2]. The values of uncertainty components, their standard uncertainties, combined standard uncertainties and the expanded uncertainties are presented in Table S1.

Table S1: Values of uncertainty components and their standard uncertainties, combined and expanded uncertainties.

Value (standard uncertainty)	Matcha	Gotu Kola	Gingko Biloba	Tulsi	Neem	Brahmi	Gynostemma	Moringa
Sample decomposition and determination of F ⁻ by F-ISE								
$W_{F(\text{sam})}$ ($\mu\text{g/g}$)	373 (1.07)	111 (0.315)	91.0 (0.258)	68.8 (0.195)	67.0 (0.191)	35.3 (1.10)	18.1 (0.0516)	16.4 (0.0469)
m_{sam} (g)	0.514 (9.11·10 ⁻⁶)	0.510 (9.11·10 ⁻⁶)	1.02 (9.11·10 ⁻⁶)	1.01 (9.11·10 ⁻⁶)	1.01 (9.11·10 ⁻⁶)	2.03 (9.11·10 ⁻⁶)	3.43 (9.11·10 ⁻⁶)	3.89 (9.11·10 ⁻⁶)
V_{F100} (U_{V100}) (mL)				100 (0.0839)				
V_{F200} (U_{V200}) (mL)				20.0 (0.0212)				
V_{F500} (U_{V500}) (mL)				50.0 (0.0470)				
$\gamma_{F(\text{sam})}$ ($\mu\text{g/mL}$)	0.768 (0.00179)	0.226 (0.000522)	0.371 (0.000856)	0.279 (0.000642)	0.270 (0.000630)	0.287 (0.000672)	0.249 (0.000578)	0.256 (0.000596)
Calibration standards								
$W_{\text{CS-0.2}}$ ($U_{W\text{CS-0.2}}$) ($\mu\text{g/g}$)				0.201 (0.00210)				
$W_{\text{CS-1.0}}$ ($U_{W\text{CS-1.0}}$) ($\mu\text{g/g}$)				1.00 (0.00890)				
γ_{100} ($U_{\gamma 100}$) ($\mu\text{g/mL}$)				100 (0.289)				
V_{F10} (U_{V10}) (mL)				10.0 (0.0131)				
V_{F100} (U_{V100}) (mL)				100 (0.0839)				
V_{F500} (U_{V500}) (mL)				50.0 (0.0470)				
V_{F1} (U_{V1}) (mL)				1.00 (0.00818)				
V_{F5} (U_{V5}) (mL)				5.00 (0.0300)				
ρ_{100} ($U_{\rho 100}$) (g/mL)				0.996 (0.00553)				
Precision ($N_{\text{sam}} = 3$)								
$U_{F(\text{sam})}$ ($\mu\text{g/g}$)	1.87	1.37	0.651	0.835	1.23	0.648	0.859	0.715
$S_{W F(\text{sam})}$ ($\mu\text{g/g}$)	3.23	2.38	1.13	1.45	2.13	1.12	1.49	1.24
Bias ($N_{\text{CRM}} = 5$)								
$U_{B\text{CRM}}$ ($\mu\text{g/g}$)	14.0	4.35	4.35	4.35	4.35	4.35	4.35	4.35
$S_{W F(\text{CRM})}$ ($\mu\text{g/g}$)	5.72	1.65	1.65	1.65	1.65	1.65	1.65	1.65
$U_{W F(\text{CRM-cer})}$ ($\mu\text{g/g}$)	13.8	4.29	4.29	4.29	4.29	4.29	4.29	4.29
Combined standard uncertainty								
$U_{c(\text{sam})}$ ($\mu\text{g/g}$)	14.2	4.57	4.41	4.43	4.52	4.40	4.43	4.41
Expanded uncertainty ($k = 1.96$)								
U_{sam} ($\mu\text{g/g}$)	27.8	8.96	8.63	8.69	8.87	8.62	8.69	8.64

Abbreviations

B_{CRM}^a

bias determined by CRM analysis ($\mu\text{g/g}$)

m_{sam}^b	sample weight (g)
N_{CRM}	number of replicate analyses of CRM
N_{sam}^b	number of replicate analyses of sample
P_{sam}^b	precision of sample analysis ($\mu\text{g/g}$)
$S_{wF(\text{CRM})}$	standard deviation of replicate analyses of CRM ($\mu\text{g/g}$)
$S_{wF(\text{sam})}^b$	standard deviation of replicate analyses of sample ($\mu\text{g/g}$)
U^c	standard uncertainty
$U_{c(\text{sam})}^b$	combined standard uncertainty ($\mu\text{g/g}$)
U_m	standard uncertainty of weighting operations (g)
U_{sam}^b	expanded uncertainty ($\mu\text{g/g}$)
V_{f50}	volume of 50 ml volumetric flask (ml)
V_{f100}	volume of 100 ml volumetric flask (ml)
V_{p1}	volume of 1 ml pipette (ml)
V_{p5}	volume of 5 ml pipette (ml)
V_{p10}	volume of 10 ml pipette (ml)
V_{p20}	volume of 20 ml pipette (ml)
$W_{\text{CS-0.2}}$	mass fraction of 0.2 $\mu\text{g/ml}$ calibration standard ($\mu\text{g/g}$)
$W_{\text{CS-1.0}}$	mass fraction of 1.0 $\mu\text{g/ml}$ calibration standard ($\mu\text{g/g}$)
$W_{F(\text{CRM-cer})}$	certified mass fraction of F in CRM ($\mu\text{g/g}$)
$W_{F(\text{sam})}^b$	mass fraction of F in sample ($\mu\text{g/g}$)
Y_{100}	mass concentration of certified 100 $\mu\text{g/ml}$ F ⁻ standard solution ($\mu\text{g/ml}$)
$Y_{F(\text{sam})}^b$	mass concentration of F ⁻ in sample solution ($\mu\text{g/ml}$)
ρ_{100}	density of certified 100 $\mu\text{g/ml}$ F ⁻ standard solution (g/ml)

^a CRM with high F level was employed for estimation of $u_{B(\text{CRM})}$ for the sample with high F content (matcha) and CRM with low F level was used for estimation of $u_{B(\text{CRM})}$ for the samples with low F content (gotu kola, ginkgo biloba, tulsi, neem, brahmi, gynostemma and moringa)

^b subscript sam refers to the samples of matcha, gotu kola, ginkgo biloba, tulsi, neem, brahmi, gynostemma and moringa

^c standard uncertainties of each uncertainty component are not listed separately

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Chapter 5

Conclusions

In the first part of the thesis (Chapters 4.1, 4.2 and 4.3), we broadened the knowledge on some aspects of fluorine analysis, i.e. on the choice of a total sample decomposition method and on the evaluation of measurement uncertainty.

In our laboratory alkaline carbonate fusion is used for the decomposition of combustible (plants) and non-combustible (soil) samples, and pyrohydrolysis is used for the decomposition of non-combustible samples. Because alkaline carbonate fusion is very time-consuming and needs constant operator supervision, oxygen bomb combustion has been introduced for the decomposition of combustible samples. Analyses of CRMs with matrix similar to analysed samples (BCR-461 "Fluorine in clay" and SRM-2695 "Fluoride in vegetation") proved that alkaline carbonate fusion and pyrohydrolysis assure accurate results within the certified intervals for soil samples and that alkaline carbonate fusion and oxygen bomb combustion assure accurate results for plant samples. However, lower results have been observed in the case of oxygen bomb combustion in comparison to alkaline carbonate fusion (69–89 %). In search for a probable explanation for this observation, possible reactions of selected metal fluorides (AgF , BaF_2 , CaF_2 , CdF_2 , CoF_2 , CrF_3 , CuF_2 , FeF_2 , FeF_3 , MgF_2 , MnF_2 , NiF_2 , PbF_2 , ZnF_2) proceeding during all three total decomposition methods were suggested and Gibbs energies were calculated. It was demonstrated that reactions proceeding during alkaline carbonate fusion and pyrohydrolysis are thermodynamically favourable, while reactions proceeding during oxygen bomb combustion are not and fluoride remains bound. Therefore, the results are probably lower in the case of oxygen bomb combustion because only the content of soluble fluorides is determined by F-ISE. Insoluble fluorides, present in the leaves (CaF_2 , MgF_2) or on the leaves as a result of deposition of particulate fluorides from industrial emissions (AlF_3 , cryolite, fluorapatite) are not decomposed during the combustion and their content cannot be determined. This leads to the conclusion that alkaline carbonate fusion is applicable for the decomposition of soil and plant samples, pyrohydrolysis is applicable for the decomposition of soil samples, while oxygen bomb combustion gives unsatisfactory results for decomposition of plant samples. However, before concluding that this method is not applicable for the decomposition of plant samples, higher recovery should be attempted to be achieved by mixing the sample with different catalysts (WO_3+Sn , MoO_3+Sn , $\text{V}_2\text{O}_5+\text{Sn}$).

In analytical chemistry, MU has generally been reported as one standard deviation, thus including only random effects. We suggested a procedure for MU evaluation based on the GUM for the determination of total fluorine in plant samples, where in addition to random effects, also systematic effects and all possible sources of uncertainty (weighing, volumetric glassware, cell potential of F-ISE, concentration and density of standard F^- solution) are included. The expanded uncertainties were estimated and represented 7–15 % of the result for different types of tea (black tea, matcha, green, oolong, Pu-erh). The largest contribution to the expanded uncertainty was the uncertainty of bias (systematic effects), which represented 76–98 %, the contribution of the uncertainty of precision (random effects) was 2–23 % and other uncertainties were negligible. The evaluation of MU according to the GUM is a challenging mathematical task; however, the developed spreadsheet can be used for MU evaluation for fluorine determination in different samples analysed in our laboratory.

In the second part of the thesis (Chapters 4.4 and 4.5), we determined the fluorine content in some samples with plant matrix, i.e. in plants contaminated with fluorides in the vicinity of a glassworks and in plant-based superfoods.

Chlorotic and necrotic lesions were observed on some plant species in the vicinity of the Rogaška glassworks, which uses hydrofluoric acid for polishing and etching of the glass. Five plant species were collected in the vicinity of the glassworks (Norway spruce, peach, common hornbeam, common bean and common grape vine). The samples were decomposed by alkaline carbonate fusion, and the F^- concentration was determined by F-ISE. High F content determined in all five plant species (87–676 $\mu\text{g/g}$) in comparison to the F content in an area unpolluted by fluoride (10 $\mu\text{g/g}$) confirmed the uncontrolled release of fluorides from the glassworks. The F content in peach, common hornbeam, common bean and common grape vine was above the maximum permissible F content in food for humans (21 $\mu\text{g/g}$ for an adult of 70 kg body weight) and animal feedstuffs (50 $\mu\text{g/g}$). It was estimated that the F content in the Norway spruce would decrease to 50 $\mu\text{g/g}$ and 21 $\mu\text{g/g}$ at a distance of approximately 380 m and 570 m from the emitter, respectively. Forage and crops were not sampled because their growth was suppressed or their growth started later than the release occurred. However, on the basis of the determined F content in all five sampled plant species, it can be assumed that forage and crops contained similar amounts of F and were not suitable for consumption by animals or humans at distances of at least a couple of hundred meters from the emitter. Therefore, more emphasis should be put on identifying possible accidental releases of fluorides from different industries, since they can represent a threat to plants and subsequently the entire food chain.

Over the past few years, there has been soaring interest in so-called superfoods. These foods from remote parts of the world are imported into Europe and promoted to provide extraordinary benefits to human health. Since there is practically no information on the F content in superfoods, the F content was determined in 18 plant-based superfoods available on the Slovenian market (alfalfa, barley grass, brahmi, ginkgo biloba, gotu kola, gynostemma, matcha, moringa, neem, stevia, tulsi, wheatgrass, chlorella, Irish moss, kelp, sea spaghetti, spirulina, wakame) by F-ISE after prior total decomposition by alkaline carbonate fusion. The F content in eight superfoods (matcha, gotu kola, ginkgo biloba, tulsi, neem, brahmi, gynostemma, moringa) was 16–373 $\mu\text{g/g}$, which is surprisingly high in comparison to the F content in commonly consumed foods (< 3 $\mu\text{g/g}$). Although the F content is high, these superfoods are usually consumed in small quantities and therefore do not pose a risk to human health in non-fluoridated water areas. However, in fluoridated water areas, the simultaneous consumption of superfoods and tea on a normal diet can lead to an F intake that easily exceeds the AI or even reaches the UL of fluorine. The F content in these eight superfoods was also surprisingly high in comparison to the usual F content in most plant species (< 10 $\mu\text{g/g}$). This is worrisome because the reason for such high F content is unknown. Superfood packaging labels usually do not include proper documentation, and no information about their exact origin or cultivation conditions is available. It is possible that these superfoods are contaminated with fluoride due to industrial or agricultural activities. This leads to the conclusion that superfoods should be consumed wisely, and the determination of beneficial active compounds as well as possible toxic elements or compounds should be performed in order to justify the prefix "super".

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Publications Related to the Thesis

Journal Articles

- D. Štepec, G. Tavčar and M. Ponikvar-Svet, "Surprisingly high fluorine content in some exotic superfoods," *Journal of Fluorine Chemistry*, vol. 234, 109521, 2020.
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Biography

Dona Štepec, the author of this thesis, was born on November 22, 1989 in Jesenice. She finished secondary education in 2008 at Gimnazija Jesenice. She then enrolled in a university study programme in Chemistry at the Faculty of Chemistry and Chemical Technology at the University of Ljubljana. She obtained a Bachelor of Science degree (equivalent to a Master of Science degree after the Bologna reform) in 2013 under the supervision of Prof. Dr. Darko Dolenc.

After the graduation, she was employed as a researcher at the Faculty of Chemistry and Chemical Technology (University of Ljubljana) on a project in collaboration with BIA Separations d.o.o. Her research activities were focused on the introduction of reactive functional groups on CIM® monoliths produced by BIA Separations with the purpose of improving the separation of biological macromolecules.

In 2015, she enrolled in the Ecotechnology postgraduate study at the Jožef Stefan International Postgraduate School. She became a young researcher at the Department of Inorganic Chemistry and Technology at the Jožef Stefan Institute under the supervision of Prof. Dr. Maja Ponikvar-Svet. Her research activities were focused on the determination of fluorine in environmental and food samples with the purpose of establishing potential contamination or excessive daily intake with diet.