Atomic Spectroscopy

Article

Indium, Thallium, Antimony, and Bismuth in Antarctic Lichens from the Antarctic Peninsula Region

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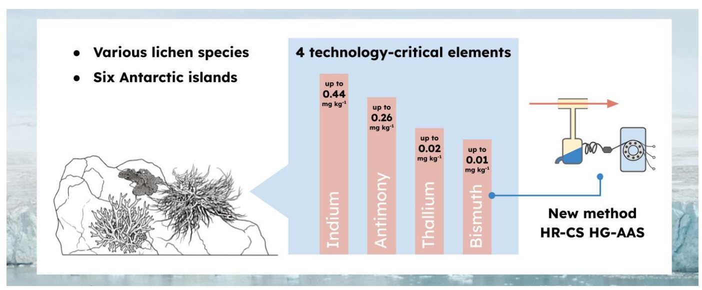
Received: *October 05, 2025;* ***Revised:*** *November 30, 2025;* ***Accepted:*** *December 03, 2025;* ***Available online:*** *December 03, 2025.*

DOI: 10.46770/AS.2025.201

ABSTRACT: The fragile Antarctic ecosystem is now more threatened by anthropogenic impacts than ever before, particularly in maritime Antarctica. Technology-critical elements (TCEs) produced by industrial activities can reach Antarctica by long-range atmospheric transport. This study aimed to determine the contents of indium, thallium, antimony, and bismuth in lichens from Deception Island, King George Island, Livingston Island, Nelson Island, Horseshoe Island, and James Ross Island, in order to identify potential contaminants. An analytical method employing high-resolution continuum source hydride generation atomic absorption spectrometry (HR-CS HGAAS) with a detection limit of 0.002 mg kg^{-1} was developed for the precise determination of bismuth. The highest observed contents of In, Tl, Sb, and Bi were 0.44 mg kg^{-1}, 0.018 mg kg^{-1}, 0.257 mg kg^{-1}, and 0.011 mg kg^{-1}, respectively. Knowing the current state of metal levels in this area is important for further research owing to the minimal clarification of the deposition and fate of TCEs in Antarctica.



INTRODUCTION

The South Shetland Islands, the Antarctic Peninsula and adjacent islands are subject to intensive research into anthropogenic influences on the climate and the pollution of maritime Antarctica.[1-4] Volatile and semi-volatile pollutants enter the Antarctic ecosystem through long-range atmospheric transport from lower latitudes, particularly from South America, South Africa, and Australia.[5-8] Besides long-range atmospheric transport, metals in Antarctica originate from volcanic activity, sea spray, and from local anthropogenic sources, most often scientific stations.[5,8,9,10] The focus of most studies on inorganic pollutants in Antarctica has been on toxic metals;[4] much less research has been done on the presence of technology-critical elements (TCEs).[11]

The ecotoxicological impacts of TCEs are not sufficiently clarified. These elements are currently contained in the ecosystem in ultra-trace amounts; however, they are abundant in electronic waste.[11] The toxicity of these elements varies significantly and is summarized in the monograph.[12-15] Thallium compounds and thallium in the form of aerosol are highly toxic to humans. Thallium poisoning can occur both from food, through accumulation in the food chain, and by inhalation, *e.g.* in the vicinity of smelters.[16] It is also more toxic than Hg, Cd, and Pb.[11] In the past, it was added to rodenticides.[17] Nowadays, the most important anthropogenic sources of these TCEs are the combustion of fossil fuels in cars and coal combustion (Tl, Sb), cement production, metal smelters (Bi, Tl, Sb), and dust from electronic waste processing plants (In, Tl).[11,16,17] Other uses of these TCEs are in semiconductors (Bi, In), energy efficiency (Bi, In), nanofibers (Bi, In), metal alloys (Bi, Sb), the automotive industry (In, Sb), medicine (Bi, Sb), and the chemical industry (Sb).[12-15]

Before the industrial revolution, the main source of Bi was volcanic activity, according to the analysis of ice cores from Greenland.[18] Bi mining has been increasing since 1945.[15] The main sources of anthropogenic Bi emissions are the combustion of fossil fuels, the production of alloys and aluminum. The number of published papers on bismuth and its future industrial uses, such as energy-storage applications, bismuth-based photocatalysts, and nuclear energy, is growing along with importance for current industry.[19-22] Bi, like Tl, Pb, and Cd, is one of the low-boiling point metals that are transported over long distances.[6] Therefore, the increase in the use of this metal means the emergence of a new pollutant with as yet unknown impacts on, and an unknown fate in the environment.

The Antarctic Peninsula is the fastest changing region of Antarctica with an expanding area of deglaciated territories.[4] These areas are the place for the emergence of so-called Antarctic oases, which are inhabited by communities of bryophytes and lichens. Lichens are suitable biological indicators of air pollutants because they accumulate elements from the atmosphere deposited by wet and dry deposition.[9,23,24] According to the published studies related to metal contents in Antarctic lichens, the TCEs studied in this research (In, Tl, Sb, and Bi) are of only marginal interest to the scientific community. The most studied lichens for TCEs contents include the species *Usnea antarctica*, e.g. from James Ross Island,[9] Livingston Island,[25] and King George Island,[26] and *Usnea aurantiaco-atra*, *e.g.* from localities on Livingston Island.[25] The Sb content ranged from 0.005 to 0.019 mg kg^{-1}.[9,25,26] In moss samples from Horseshoe Island,[27] the Tl content ranged from 0.009 to 0.25 mg kg^{-1}, the Sb content was in the range of 0.11-14.8 mg kg^{-1}, and the Bi content was in the range of 0.011-0.6 mg kg^{-1}. Apart from these works dealing with TCEs, the contents of As, Cd, Pb, Zn, Cu, and other elements in the lichens *Usnea antarctica* and *Usnea aurantiaco-atra* are commonly published.[2,3,28-31]

The aim of this research was to identify new contaminants of the Antarctic ecosystem on islands in the Antarctic Peninsula region, which are exposed to increasing anthropogenic impact. The analytes of interest were TCEs, due to their increasing levels in the environment. The main novelty of this study lies in the development of a new method for the accurate determination of trace Bi concentrations using high-resolution continuum source hydride generation atomic absorption spectrometry (HR-CS HGAAS). Quantitative generation of BiH_3 involves the pre-reduction of Bi(V) to Bi(III) using L-cysteine. This proposed method provides accurate and reproducible results. The sensitivity allows the determination of Bi in lichens from pristine environments, and therefore, this method can be used for biomonitoring.

EXPERIMENTAL

Collection of samples. During four polar expeditions, lichens were sampled on islands in the Antarctic Peninsula region. Of all collected samples, samples of the species *Usnea antarctica* (n=16),

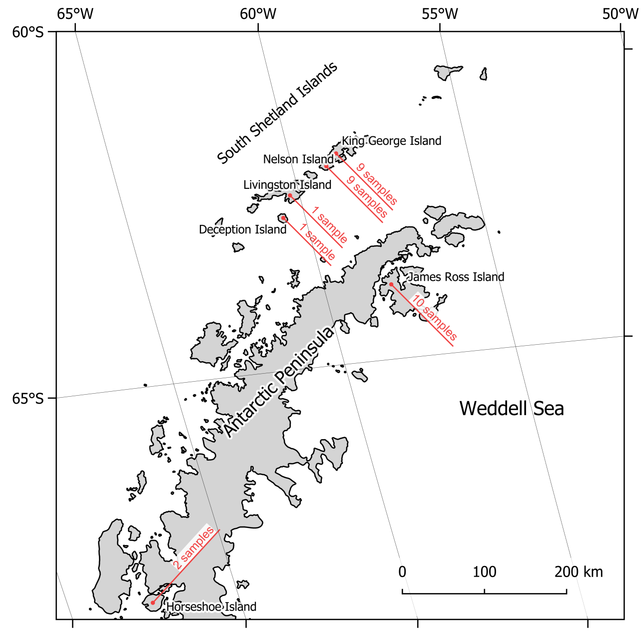


Fig. 1 The Antarctic Peninsula region and sampling sites.

Usnea aurantiaco-atra (n=11), *Umbilicaria decussata* (n=2), and *Ramalina terebrata* (n=3) from the South Shetland Islands (Deception Island, King George Island, Livingston Island, Nelson Island), Horseshoe Island (west coast of Graham Land), and James Ross Island on the northeast coast of the Antarctic Peninsula were selected for analysis (Fig. 1). These localities vary considerably in their historical and current anthropogenic influence. On King George Island, samples were collected in the wider vicinity of the Base Profesor Julio Escudero (Chile), Bellingshausen Station (Russia), and Great Wall Station (China), which represent potential local sources of contaminants. Deception Island is visited by tourist and scientific expeditions. On Nelson Island, there is only a temporary station CZ*ECO-Nelson (Czech Republic) with occasional stays of scientists in a summer camp. Turkish Antarctic Scientific Research Base is located on Horseshoe Island. The Johann Gregor Mendel Czech Antarctic Station on James Ross Island is a modern scientific summer station with minimal impact on its surroundings.

The 3-5 lichen thalli of the selected species were collected at each sampling site (Table S1). The analyzed samples were selected from collected transects with regard to the coverage of this region in a pilot study. The lichen thalli were rinsed with deionized water, dried, and then frozen at -18 °C until analysis.

Digestion of samples. Lichen samples were processed in the trace laboratory in a laminar flow box (class 100). The mineralization was performed in quartz tubes with subboiled distilled HNO_3 using an UltraWAVE microwave mineralizer (Milestone, Italy) at 250 °C for 30 min. Samples were digested in a ratio of 3 mL HNO_3

Table 1. Analytical parameters of ETAAS and HR-CS ETAAS methods

Parameters	ETAAS			HR-CS ETAAS
	In	Tl	Sb	Bi
Wavelength	325.6 nm	276.8 nm	217.6 nm	223.061
Slit	0.7 nm	0.7 nm	0.7 nm	central pixel±2
Source/Current	HCl/5 mA	EDL/340 mA	EDL/410 mA	Xe lamp
Pyrolysis temperature	1200 °C	700 °C	1200 °C	900 °C
Atomization temperature	2100 °C	1600 °C	2000 °C	1900 °C
Sample volume	20 µL	20 µL	20 µL	20 µL
Pd/Mg(NO_3)$_2$ modifier	10 µg/6 µg	5 µg /3 µg	5 µg /3 µg	5 µg /3 µg

per 0.2 g dry weight. A set of blank solutions was prepared together with the samples. Similarly, lichen samples and certified reference materials (CRMs) spiked with standard solutions of Bi, In, and Sb (see chapter Analytical methods) were prepared to calculate the recovery of these elements. Digests were diluted with ultrapure water and quantitatively transferred into polypropylene vials.

Reagents and standards. Subboiled distilled HNO_3 was used for the mineralization of samples. In addition, subboiled distilled HCl in a PFA distillation apparatus (Savillex DST 1000, USA) was used for Bi determination. Pd modifier solution (10 g L^{-1}, Merck,Germany), and $Mg(NO_3)_2.6H_2O$ (Suprapure, 99.99%, Merck, Germany) were used for the preparation of mixed matrix modifier. For Bi determination, L-cysteine hydrochloride (Lachema, Czech Republic), $NaBH_4$ (Merck, Germany), and NaOH (Suprapur, Merck, Germany) were used. Standard solutions of In, Tl, Sb, and Bi for AAS (1 g L^{-1}, Sigma-Aldrich, Switzerland) were used for calibration purposes. Standard solutions of As, Cu, Hg, Ge, Pb, Sb, Se, Sn, and Te (1 g L^{-1}, Sigma-Aldrich, Switzerland) were used for interference tests during the development of HR-CS HGAAS method for Bi determination.

The certified reference materials (CRMs) BCR-482 Lichen (IRMM, Belgium), INCT-TL-1 Tea Leaves (INCT, Poland), and Metranal AN-BM02 Green Tea (ANALYTIKA, Czech Republic) were analyzed to verify the accuracy of the analytical methods.

Analytical methods. The concentrations of In, Tl, and Sb were determined by electrothermal atomization atomic absorption spectrometry (ETAAS) using an Analyst 600 (PerkinElmer, USA) spectrometer. The determination was performed in an advanced platform graphite tube with an End-Cap (PerkinElmer, USA). The optimized analytical parameters for the determination of these elements are listed in Table 1. Three parallel analyses were performed for each sample. Calibrations by the external calibration method were performed for all elements using standard solutions. (Calibrations were performed up to 20 µg L^{-1} due to low expected metal contents in the samples.) Standard additions of In and Sb into the samples (spikes) were performed for the recovery calculation due to verification purposes. Spiked samples of 10 µg L^{-1} In and 10 µg L^{-1} Sb were prepared in triplicates.

The following method was developed for the determination of Bi using HR-CS HGAAS. The analysis was performed using an HS 60 modular hydride generator and a ContrAA 300 atomic absorption spectrometer (Analytik Jena GmbH, Germany). The commercial quartz tube atomizer was replaced by a T-shaped quartz tube atomizer with an inner diameter of 5 mm, expanding to 15 mm at the quartz windows, due to the reduction of detection limit. The length of the atomizer was 145 mm. Bi determination was performed according to the following program: 950 °C cell temperature, 6 L h^{-1} gas flow, 3 pump speed level, 6 s load time, 20 s AZ wait time, 15 s reaction time, 25 s wash time, and 40 s read time. The carrier solution was 3% HCl. A solution of 0.5% $NaBH_4$ in 0.4% NaOH was used as a reducing agent. The Bi determination was performed at three wavelengths: 222.825 nm (7-pixel evaluation), 223.061 nm (7-pixel evaluation), and 306.772 nm (9-pixel evaluation). For the determination of Bi in samples, the following procedure for Bi pre-reduction was used. A solution of L-cysteine was added in the ratio of 1 mL of 5% L-cysteine in 3% HCl for every 5 mL of sample and left to stand at laboratory temperature for 30 min. The Bi content in Antarctic lichen samples was determined at the wavelength of 223.061 nm.

Three parallel analyses for each calibration standard, sample, blank, and CRM were performed. Calibrations at three wavelengths were performed in the concentration range of 0.1-3 µg L^{-1}. The three-day variability of the calibration slopes were observed. The multi-level spike recovery of CRM BCR-482 (using a Bi standard solution) was also checked. Spikes of 0.25 µg L^{-1}, 0.5 µg L^{-1}, and 1 µg L^{-1} were performed, and the Bi contents were determined. Furthermore, the determination of Bi in CRM BCR-482 without pre-reduction using L-Cysteine were evaluated. Interference tests for the Bi determination by the developed method were performed using spikes of metal standards to CRM BCR-482. The additions of mixed solutions of As, Cu, Hg, Ge, Pb, Sb, Se, Sn, and Te were made for two concentration levels - 10 µg L^{-1} and 100 µg L^{-1}. Higher concentrations of interferents are unsuitable due to enormous contamination of the apparatus and due to inadequate metal content in lichens.

To verify the hydride generation method for Bi determination, a comparative analysis of CRM BCR-482 was performed using high-resolution continuum source electrothermal atomic absorption spectrometry (HR-CS ETAAS). A ContrAA 800G (Analytik Jena GmbH, Germany) high-resolution spectrometer was used for these analyses; the analytical parameters are summarized in Table 1.

RESULTS AND DISCUSSION

Bi determination by HR-CS HGAAS and HR-CS ETAAS. The developed method for BiH_3 generation was used for the determination of Bi at all three wavelengths. The calibration lines (Fig. 2) were linear in the selected concentration range. The highest sensitivity was observed at the wavelength of 223.061 nm, which was subsequently used for the determination of Bi in the samples. A characteristic doublet was observed at the 306.772 nm line (Fig. 3), which is integrated using a wider spectral interval (9-pixel evaluation in this case). Analytical characteristics of Bi determination are summarized in Table 2. The three-day variability of the calibration slopes were 5%. None of the CRMs used had a certified Bi content (Table 3). The wavelenght-resolved spectra of CRM BCR-482 are shown in Fig. 3. The Bi content determined in CRM BCR-482 was 0.092±0.003 mg kg^{-1}, which corresponds to the reference measurement (HR-CS ETAAS) and the value published in the thesis by Tucker.[32] Multi-level spike recovery tests were performed for this CRM with recoveries in the range of 97.3-104% and a relative standard deviations (RSD) of up to 4%. Interference tests with mixed metal solutions did not show a reduction in analytical signal for CRM BCR-482.

The method for volatile BiH_3 generation includes the pre-reduction of Bi(V) to Bi(III) using L-cysteine, which is crucial for the quantitative determination in digests containing oxidizing agents such as HNO_3. The Bi content of 0.006 mg kg^{-1} in CRM BCR-482 was determined without the pre-reduction by L-cysteine,

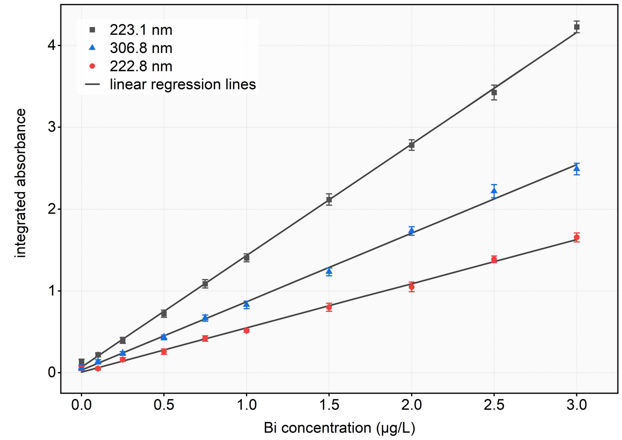


Fig. 2 Calibration lines for Bi at 222.825 nm, 223.061 nm, and 306.772 nm.

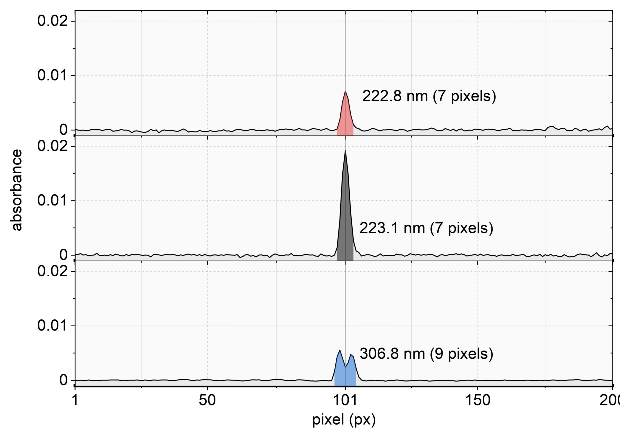


Fig. 3 Wavelenght-resolved spectra of Bi at three examined absorption lines observed for CRM BCR-482 (1.9 µg L^{-1} Bi).

Table 2. Analytical characteristics of Bi determination at selected wavelengths

Wavelengths (nm)	Slope	Intercept	Correlation coefficient (R^2)	LOD (mg kg^{-1})	LOQ (mg kg^{-1})
222.825	0.5405	0.0080	0.9975	0.006	0.020
223.061	1.3631	0.0699	0.9993	0.002	0.008
306.772	0.8367	0.0331	0.9973	0.004	0.013

Table 3. Certified, indicative, and determined TCEs contents in CRMs (mg kg^{-1})

CRMs		In	Tl	Sb	Bi
BCR-482 Lichen	Certified value	-	0.061±0.003[b]	0.35±0.09[c]	-
	Determined value	0.173[a]	0.044±0.002	0.25±0.03	0.092±0.003
INCT-TL-1 Tea Leaves	Certified value	-	0.063±0.005	0.050[c]	-
	Determined value	<LOD	0.060±0.001	<LOD	0.012±0.001
Metranal AN-BM02 Green Tea	Certified value	-	0.200±0.030[c]	-	-
	Determined value	<LOD	0.179±0.003	<LOD	0.026±0.002

[a] The value lies between LOD and LOQ; [b] Other data; [c] Indicative value.

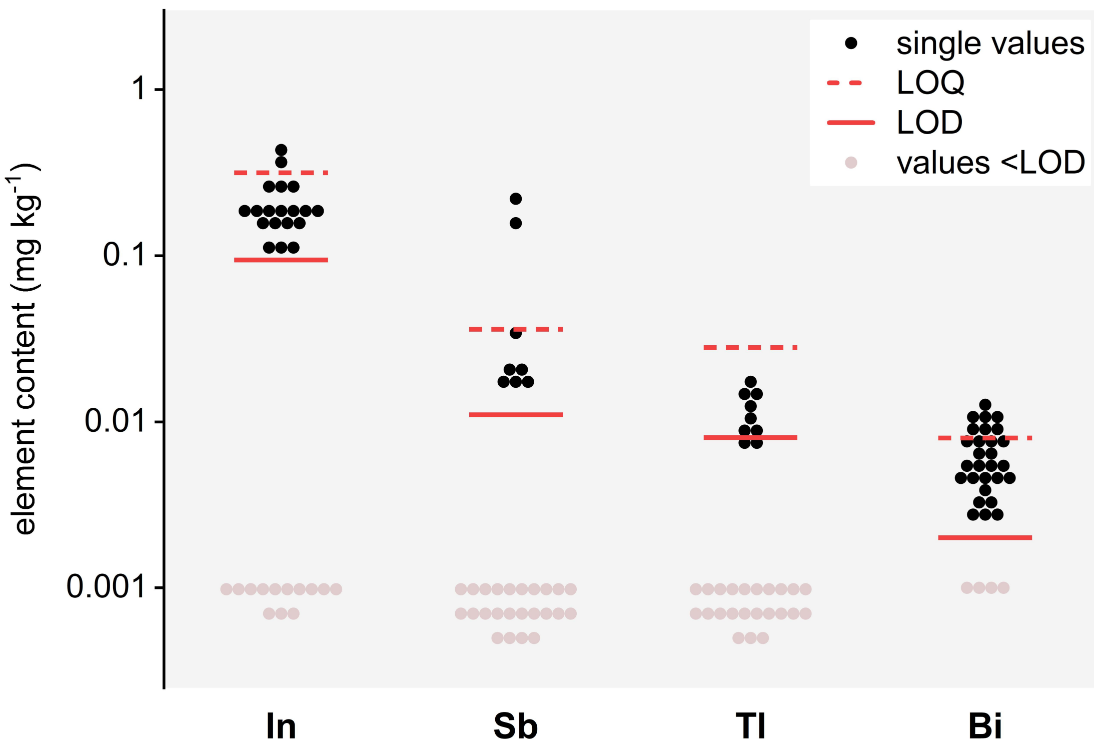


Fig. 4 Contents of In, Sb, Tl, and Bi determined in Antarctic lichens.

which was a 6.5% yield. The method of pre-reduction using L-cysteine was proposed by Welz and Šucmanová,[33] but only for arsenic and antimony. This procedure was modified in our study for the quantitative determination of Bi. An advantage is that the use of L-cysteine for the pre-reduction of Bi(V) does not affect the conditions for bismuthane generation.[34]

A comparative analysis to developed method was performed by means of HR-CS ETAAS. The Bi content of 0.085±0.008 mg kg^{-1} in CRM BCR-482 was determined. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.004 mg kg^{-1} and 0.013 mg kg^{-1}, respectively.

The main advantage of Bi determination by hydride generation (compared to HR-CS ETAAS) is the separation of the analyte from the matrix and the elimination of interference from environmental matrices, which is obvious from the very low background on the spectra (Fig. 3). The achieved LOD for Bi using the developed analytical method (at the wavelength of 223.061 nm) was 0.002 mg kg^{-1} (0.04 µg L^{-1} in sample digests). This LOD is one order of magnitude higher than the LOD for Bi determination by electrothermal vaporization atomic fluorescence spectrometry (ETV-AFS) after hydride trapping.[35] The hydride trapping in a graphite furnace of ETAAS can be used for Bi preconcentration.[36] However, omitting the trapping step in the proposed method greatly simplifies the analysis. The achieved LOD was one order of magnitude lower than for Bi determination by hydride generation inductively coupled plasma atomic emission spectrometry (HG-ICP-AES).[37,38] Photo-induced chemical-vapor generation (Photo-CVG)[39] can be used for the determination of Hg, As, Bi, Sb, Se, and Te by means of various detection techniques. Using UV-CVG-AFS,[39] LOD of 0.2 µg L^{-1} Bi in water samples was achieved, which is higher than our LOD for sample digests.

In, Tl, and Sb determination by ETAAS. The contents of In, Tl, and Sb in the samples were determined using optimized temperature programs for the graphite furnace (Table 1). The LODs for these elements were 0.094 mg kg^{-1}, 0.008 mg kg^{-1}, and 0.011 mg kg^{-1}, respectively. Determined TCEs contents in CRMs are listed in Table 3. The determination of analytes using CRMs was verified only for Tl by means of CRM INCT-TL-1, since the other metals were not certified. The recovery of In in spiked samples was 93.7-101.5% with an RSD of up to 5%; the recovery of Sb was 98.3-101.9% with an RSD of up to 3%.

Trace element contents in lichens. The contents of In, Tl, Sb, and Bi determined in Antarctic lichens (Fig. 4) are close to the LODs for all samples; some samples had analyte contents below the LODs. However, these seemingly "negative" results should be

Table 4. Reported TCEs contents in the lichen and moss samples

Location, specie	In ($mg\ kg^{-1}$)	Tl ($mg\ kg^{-1}$)	Sb ($mg\ kg^{-1}$)	Bi ($mg\ kg^{-1}$)	Ref.
King George Island, *Usnea aurantiaco-atra*	<LOD-0.196	<LOD-0.010	<LOD-0.021	<LOD-0.011	This work
King George Island, *Usnea sp.*	-	-	0.007-0.019	-	26
Livingston Island, *Usnea aurantiaco-atra*	-	-	0.01	-	25
Livingston Island, *Usnea antarctica*	-	-	0.01	-	25
Nelson Island, *Usnea aurantiaco-atra*	<LOD-0.115	<LOD	0.037-0.257	0.003-0.006	This work
Nelson Island, *Usnea antarctica*	0.218-0.302	<LOD	<LOD-0.020	0.005-0.006	This work
James Ross Island, *Usnea antarctica*	<LOD-0.437	<LOD-0.018	<LOD	0.003-0.013	This work
James Ross Island, *Usnea antarctica*	-	-	0.005-0.010	-	9
Horseshoe Island, moss	-	0.010-0.25	0.11-4.82	0.011-0.17	27
Norway, *Hylocomium splendens*	-	0.0022-1.4	0.0083-4.3	0.0012-0.8	40
Turkey, *Xanthoria parietina*	-	0.3	-	-	43

understood as confirmation of the occurrence of these TCEs in the monitored region, which is the main result of this preliminary study aimed at revealing the potential risks of anthropogenic pollution in maritime Antarctica.

Tl, Sb, and Bi, metals subject to long-range atmospheric transport,[40] are most often determined in biomonitors in industrial areas of Europe (mostly without In). Tl contents in uncontaminated plants in Poland ranged from 0.008 to 0.05 $mg\ kg^{-1}$; the Tl content in vegetables can reach up to 0.3 $mg\ kg^{-1}$. The highest Tl concentrations in plants in Poland are found in the vicinity of Zn and Pb smelters – up to tens of $mg\ kg^{-1}$.[16] Atmospheric Bi deposition in Turkey has been confirmed by the analysis of wood and tree bark, with contents reaching tens of $mg\ kg^{-1}$.[41] In general, Bi contents in terrestrial plants are below 0.02 $mg\ kg^{-1}$, and below 0.06 $mg\ kg^{-1}$ in vegetables. Bi tends to be concentrated in plants only in industrial areas, mainly due to coal combustion.[42] (The Bi content in coal is around 5 $mg\ kg^{-1}$.)

In Antarctic lichen and moss samples, metal contents tend to be lower. A comparison of published and determined values of TCEs contents in moss and lichen samples is presented in Table 4. In this study, the highest Sb contents were determined in samples from Nelson Island (up to 0.257 $mg\ kg^{-1}$). On James Ross Island, the Sb content was below the LOD (0.011 $mg\ kg^{-1}$). The In contents of lichen samples were around hundreds of $\mu g\ kg^{-1}$, with maxima occurring on James Ross Island (up to 0.44 $mg\ kg^{-1}$), where the highest Tl (up to 0.018 $mg\ kg^{-1}$) and Bi (up to 0.011 $mg\ kg^{-1}$) contents were determined. In general, detected Tl and Bi contents in this research were very low compared to those in European lichens (Table 4).

Previous studies from James Ross Island[44,45] confirmed the bioaccumulation of mercury and cadmium in cyanobacterial mat. Highly volatile, long-range transported pollutants such as Bi and Tl[6] can also be expected to accumulate in Antarctic biota. Thus, the future research will involve the determination of Bi and Tl in *Usnea antarctica* lichens in the northern deglaciated part of James Ross Island in order to map the occurrence of these metals in dependence on terrain geography.

CONCLUSION

In this study, a new method for the accurate determination of trace Bi concentrations using high-resolution continuum source hydride generation atomic absorption spectrometry was proposed. The pre-reduction of Bi(V) to Bi(III) using L-cysteine was suggested for the quantitative generation of BiH_3. The linearity of the calibration line was observed in the concentration range of 0.1-3 $\mu g\ L^{-1}$; the limit of detection was 0.04 $\mu g\ L^{-1}$ in samples mineralized using HNO_3, which corresponded to 0.002 $mg\ kg^{-1}$ of the dry weight of lichen sample. Therefore, the developed method is suitable for biomonitoring of Bi at trace levels.

The technology-critical elements thallium, antimony, and bismuth were detected mostly in the order of $\mu g\ kg^{-1}$ in Antarctic lichens from the Antarctic Peninsula region. However, despite the low contents compared to those in European lichens, the results are valuable due to limited knowledge about the occurrence of TCEs in the Antarctic ecosystem.

ASSOCIATED CONTENT

The supporting information (Table S1) is available at https://www.at-spectrosc.com.

AUTHOR INFORMATION

Pavel Coufalík, an Assoc. Prof. at Institute of Analytical Chemistry of the Czech Academy of Sciences, Czech Republic, is concerned with trace and ultratrace analyses of metals in the environment using atomic absorption spectrometry methods including hydride generation, solid sampling, and speciation

analysis. Particular emphasis in his research is placed on atmospherically transported metals to polar regions.

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Notes

The authors declare no competing financial interest. The data are available in the ASEP data repository at 10.57680/asep.0639477.

ACKNOWLEDGMENTS

The research was supported by the Czech Science Foundation (25-17229S) and the Institute of Analytical Chemistry of the CAS under the Institutional Research Plan RVO: 68081715. The work of OZ was supported by Masaryk University (project MUNI/A/1545/2024). The authors are grateful to the J.G. Mendel Czech Antarctic Station for use its facilities.

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