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Quantifying of thallium in Shilajit and its supplements to unveil the potential risk of consumption of this popular traditional medicine

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Abstract
Shilajit, a natural substance used in traditional and modern medicine, has gained prominence as a vital component in dietary supplements. Concerns regarding its use in complementary medicine have arisen due to limited information regarding its composition. Considering thallium's well-known toxicity, this study employs flow - injection differential-pulse anodic stripping voltammetry to accurately quantify thallium in natural Shilajit and Shilajit-based commercial supplements from various regions. The limit of detection and limit of quantification were determined as 6.58×10^{-3} μg.mL^{-1} and 1.98×10^{-2} μg.mL^{-1}, respectively. Thallium contents were detected up to 0.226 μg.g^{-1} in natural Shilajit, but mainly in the range of a few tenths of μg.g^{-1}. Thallium contents were found up to 0.5 μg.g^{-1} in the supplements studied. Consumption of one pill of supplement, introduces up to 0.095 μg of Tl to the body. Notably, the concentration of thallium in some supplements was higher than in crude Shilajit, raising concerns about the potential health risks associated with long-term consumption. This study underscores potential health risks associated with thallium in both Shilajit and supplements. Regular monitoring and standardized testing are essential to ensure compliance with safe thallium limits, protecting consumers against potential thallium poisoning.

Keywords Shilajit, Thallium, Toxicity, Supplementary drugs, Natural substance, Flow-injection differential-pulse anodic stripping voltammetry

Introduction

Shilajit, a natural substance with rich historical usage in both traditional and modern medicine, has been recognized for its significant medicinal properties and dietary supplement value for thousands of years. In traditional Asian herbal medicine, it has been traditionally employed to address a wide range of ailments, including injuries, bone fractures, skin diseases, peripheral nervous system disorders, and as a soothing agent [1]. Shilajit forms within mountainous rocky layers through a natural process influenced by temperature and pressure over an entire season, involving biosynthesis [2]. It exudes as

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a pure and smooth gum from the mountains during the warm season [3].

Interest in alternative medicine has increased in recent years, with a focus on the use of traditional medicine, including natural products, herbs, and "complementary medicine" [4]. Shilajit, a crucial component in many supplements available on the market, has gained popularity. However, limited information is often provided regarding the content of Shilajit. Different companies that manufacture crude Shilajit extracts offer their own recommendations for human application [1].

It has been reported that Shilajit contains significant amounts of heavy metals such as As, Pb, Hg, Ni, and Co [5–10], but there is just a single study by Mishra [7] that provides quantitative analysis of Tl. This scarcity of data presents a challenge when considering Shilajit as a dietary supplement, as heavy metal contamination can cause severe health problems. The health risks associated with heavy metal contamination depend on the specific metal, its dosage, and its chemical form. Among the various heavy metals found in Shilajit, Tl has been confirmed to be present in one study by Mishra [7]. Tl is highly toxic to humans, animals, plants, and microorganisms, presenting a significant health hazard. It can be absorbed through the skin and mucous membranes, quickly spreading throughout the body and accumulating in various tissues, including bones, kidneys, and the central nervous system. The toxicity of Tl stems from its structural resemblance to the potassium (I) ion, leading to the disruption of potassium-associated metabolic processes. In terms of toxicity, Tl surpasses mercury, cadmium, and lead, with a maximum admissible concentration of 0.1 mg.mL^{-1}. It is advised to strictly limit Tl intake to a maximum dose of 15 micrograms, as exceeding this threshold can have severe consequences. The LD$_{50}$ of Tl for humans ranges from 8 to 12 mg.kg^{-1}, indicating its potent toxicity. Ingesting a high dose of Tl over a short period can even result in fatal outcomes [11]. The main source of human intake of Tl is food, and the average intake is estimated to be <2 μg per day [12]. The U.S. Environmental Protection Agency has identified Tl as a priority contaminant because it is mutagenic, cancer-causing, and causes birth defects [13]. Prolonged exposure to Tl has been associated with various side effects such as damage to the liver, testicular tissue, the kidney, hair loss and intestinal damage. Tl is on the US Environmental Protection Agency's list of 129 priority pollutants due to its toxicity [12]. Consequently, it is important to establish safe limits for its content in Shilajit supplements. Therefore, it is of high importance to assay Tl contents of Shilajit and supplements, as it is directly concerning public health.

The aim of this study was to assess the Tl content of various Shilajit and supplement samples and evaluate the potential risk of Tl poisoning associated with the consumption of Shilajit or its supplements to ensure consumer safety. The quantification of Tl was performed using flow-injection differential-pulse anodic stripping voltammetry (FI-DP-ASV), a reliable analytical technique for measuring trace elements. This method offers several advantages, including smaller footprint instrumentation with sub μg.kg^{-1} limit of detection (LOD), cost-effectiveness, and simplicity [14, 15]. This technique was already used for determination of Tl in soil after its mineralization with strong acids [16, 17], water [18, 19], activated sludge [20], and tattoo ink [14]. To prevent interference from other cations, we employed a combination of ascorbic acid and ethylenediaminetetraacetic acid (EDTA) during sample preparation as supporting electrolyte. EDTA forms complexes with metal ions such as bismuth (III), copper (II), iron (II), antimony (III), lead (II) and cadmium (II) at a pH of 4.5, effectively removing them from interfering with Tl during analysis, even when their concentrations were a hundred times higher than Tl. Zinc (II), cobalt (II), nickel (II), arsenic (III), tin (II) and molybdenum (VI) did not interfere because they cannot be electrodeposited on the electrode under the chosen analysis conditions [21]. The method applied in this research for the determination of Tl in shilajit was based on our previous experiences for the Tl determination in complicated matrices [16, 17]. Moreover, we found that its determination is a difficult task since its concentration in natural products is in the range of nanograms per gram. It is possible to choose one of many techniques for determining this element. The selection of the appropriate technique should consider the operational factors related to its application (throughput, complexity, time and consumption of reagents, type of sample matrices analyzed, etc.). In addition to electrochemical techniques, inductively coupled plasma mass spectrometry can be used for thallium determination [22, 23]. While this technique offers a wide range of linearity, high sensitivity and selectivity, and rapid analysis, the high cost of analysis is the main disadvantage of it, especially when a single elemental analysis is desired. Atomic absorption spectroscopy is another elemental analysis technique which can be used for Tl determination [24, 25], which is characterized by low cost and short analysis time; however, it is highly susceptible to the sample's matrix effects [22]. Liquid chromatography techniques provide an alternative to spectroscopic methods for determination of inorganic cations. Ion chromatography (IC) is the most widely used method for this purpose; however, quantifying trace ions in the presence of high concentrations of other ionic species remains to be the most challenging analysis using this technique [26]. The most common interferences in IC are typically other ions that elute within the same retention time of the ion(s) of interest [27].

Mercury film electrodes (MFE), which are metallic Hg electrodes, are typically used in the electrochemical analytical techniques such as ASV. These types of electrodes are usually chosen because of their tiny thickness and volume, which both improve the measurement's sensitivity. Despite the fact that these characteristics make MFEs attractive for use in ASV analyses and lower the amount of mercury required, the downside of MFEs is the inherent toxicity and serious health hazards associated with mercury [28].

Experimental

Apparatus and reagents

The electrochemical analysis was conducted using an Ecochemie MicroAutolab electrochemical analyzer (Utrecht, The Netherlands), equipped with a flow-through cell of the wall-jet type and a flow-injection system for medium exchange and circulation. The working electrode used was a mercury film electrode (MFE) covered with an extremely thin layer of mercury (10–100 µm) which acts as a conductive and inert support. The substrate for MFE is epoxy resin impregnated graphite due to the adhering oxide coatings on metal surfaces and the interaction of metals with mercury., A saturated calomel electrode (SCE) as a reference electrode is connected to the instrument via a high-resistance connection that doesn't draw any current from it, and it is positioned as close to the working electrode as possible. The working electrode's potential is measured against the reference electrode's steady and repeatable potential, which is independent of the sample's composition. Also, a platinum wire acted as the auxiliary electrode, carries the current [29]. All reagents used were of analytical grade (puriss. p.a.) and were provided by Fluka (Switzerland), including ammonia solution (25%), nitric acid (65%), hydrogen peroxide (30%), ethylenediaminetetraacetic acid disodium salt dehydrate, ascorbic acid, hydrofluoric acid (73%), mercury (II) nitrate monohydrate, Tl (I) nitrate standard, and potassium nitrate. All solutions were prepared using reverse osmosis water from a Watek-Demiwa 5 Rosa system (Czech Republic), followed by triple distillation using a quartz apparatus. Only freshly distilled water was employed.

Sample collection and preparation

Sample collection for this study involved collecting, purchasing from local markets, and ordering online of 13 crude Shilajit samples. Samples were from different regions in Iran, India, Nepal, Russia, and Kyrgyzstan. Figure 1 shows a sample of Shilajit collected from the southeast of Iran. Moreover, 5 Shilajit-containing supplements were ordered online. Since the crude Shilajit samples are sticky in nature, to facilitate their analysis, lyophilization was employed to dry them. The dried Shilajit samples were then carefully pulverised in an agate mortar. The powdered samples were homogenized to ensure uniformity and consistency within each sample. The samples were placed in polyethylene bottles in a desiccator at room temperature. For sample preparation, 0.250 g of dried Shilajit or 1 tablet of supplement was weighed and placed in a tapered Teflon beaker and treated with a mixture of 2.0 mL of HNO_3 (65%) and 1.0 mL of H_2O_2 (30%). The mixture was subjected to microwave-assisted digestion for 5 min. After that, the sample was evaporated to dryness, 1.0 mL of 73% HF was then added and the sample was allowed to rest for 1 h. Subsequently, it was heated to dryness and the residue was dissolved in 1.0 mL of HNO_3 and heated for 2 h on a graphite heater. This step ensures complete digestion of the sample and assists in the formation of a homogeneous solution. 5.0 mL of 1.0 M ascorbic acid and 12.5 mL of 0.2 M EDTA were then added to digest the sample. The pH of the solution was lowered to 4.5 by adding 25% ammonia solution drop by drop. Finally, a 50.0 mL aliquot of this sample was transferred to the electrochemical cell to determine its Tl content by FI-DP-ASV. The same digestion process was applied to all samples in three separate experiments. The same procedure was followed to prepare reagent blanks for each experiment.

Determination of total content of Tl

Determination of Tl content of samples was performed by the Lukaszewski method [30] with small modifications. This is the same analysis and instrumental conditions which was applied in several previous research works for Tl determination in a variety of samples [11, 17, 20, 30]. A solution with 0.05 mM mercury (II) nitrate and 0.1 M potassium nitrate was used to make a thin layer of mercury film which took 10 min to form. A single mercury film was sufficient for a full day's measurements. The electrochemical instrument was set to work under the following conditions: a differential pulse amplitude of 50 mV, a step potential of 2 mV, preconcentration at -1.2 V vs. SCE. Before measurement, each sample was degassed by a nitrogen gas flow for 10 min. The pre-concentration of Tl in the samples was carried out over 300–900 s, depending on the Tl concentration. Tl preconcentration was performed at a potential of -900 mV vs. SCE. (Fig. 2). Voltammograms were recorded after the supporting electrolyte medium was changed to pure 0.05 M EDTA. Due to the application of this electrolyte, the method could tolerate a 1000-fold excess of lead, which is the most interfering element. A three-point standard addition method was used to determine the concentration of Tl in the samples. The accuracy of the method was verified by analysing a soil NCS DC 73,382 reference material (Chinese National Standard Reference

Fig. 1 Black Shilajit, collected from the south-east part of Iran by one of the authors

Materials, Beijing), certified for a total Tl content of 1.0 ± 0.2 μg.g^{-1}.

An example of a voltammogram of one of the crude Shilajit samples together with voltammograms recorded after a sequential standard addition is presented in Fig. 3.

Results and discussion

Method validation

The linearity range of the method was found to be in the concentration range of 0.02 to 0.10 μg.mL^{-1} of Tl and could be well-described by the equation y = 40.3215x + 0.7245, where 'y' represents the instrument's response, and 'x' corresponds to the Tl concentration. The resulting calibration curve was of high quality, confirmed by a correlation coefficient (R^2) of 0.9964. This exceptional R^2 value underscores the robustness and reliability of the FI-DP-ASV method in accurately quantifying Tl concentrations within the specified range.

The calculated LODs, based on standard deviation of the free term (s_a=0.0597) and the residual standard deviation (s_{xy}=0.1012), were 4.89×10^{-3} μg.mL^{-1} and 8.28×10^{-3} μg.mL^{-1}, respectively. Averaging was adopted, and the value of LOD was calculated as 6.58×10^{-3} μg.mL^{-1}. Considering three times the concentration of LOD, the limit of quantification (LOQ) was obtained as 1.98×10^{-2} μg.mL^{-1}. The correctness of the determination of LOD was verified according to the protocol suggested by Mazurek, et al. [31].

Method control

Calibration tests were performed with each series of the experiments. Moreover, as many as 9 independent tests were conducted for the reference material to determine its Tl content. The result obtained for Tl concentration in the soil reference material was 0.847 ± 0.079 μg.g^{-1}, which certified the method.

Thallium content of the crude shilajits

The analysis of Tl concentrations in crude Shilajit samples revealed a wide range of values, from close to zero to 0.226 μg.g^{-1}. A previous report by Mishra [7] shows that the Tl concentrations in Shilajits of Sirmaur region

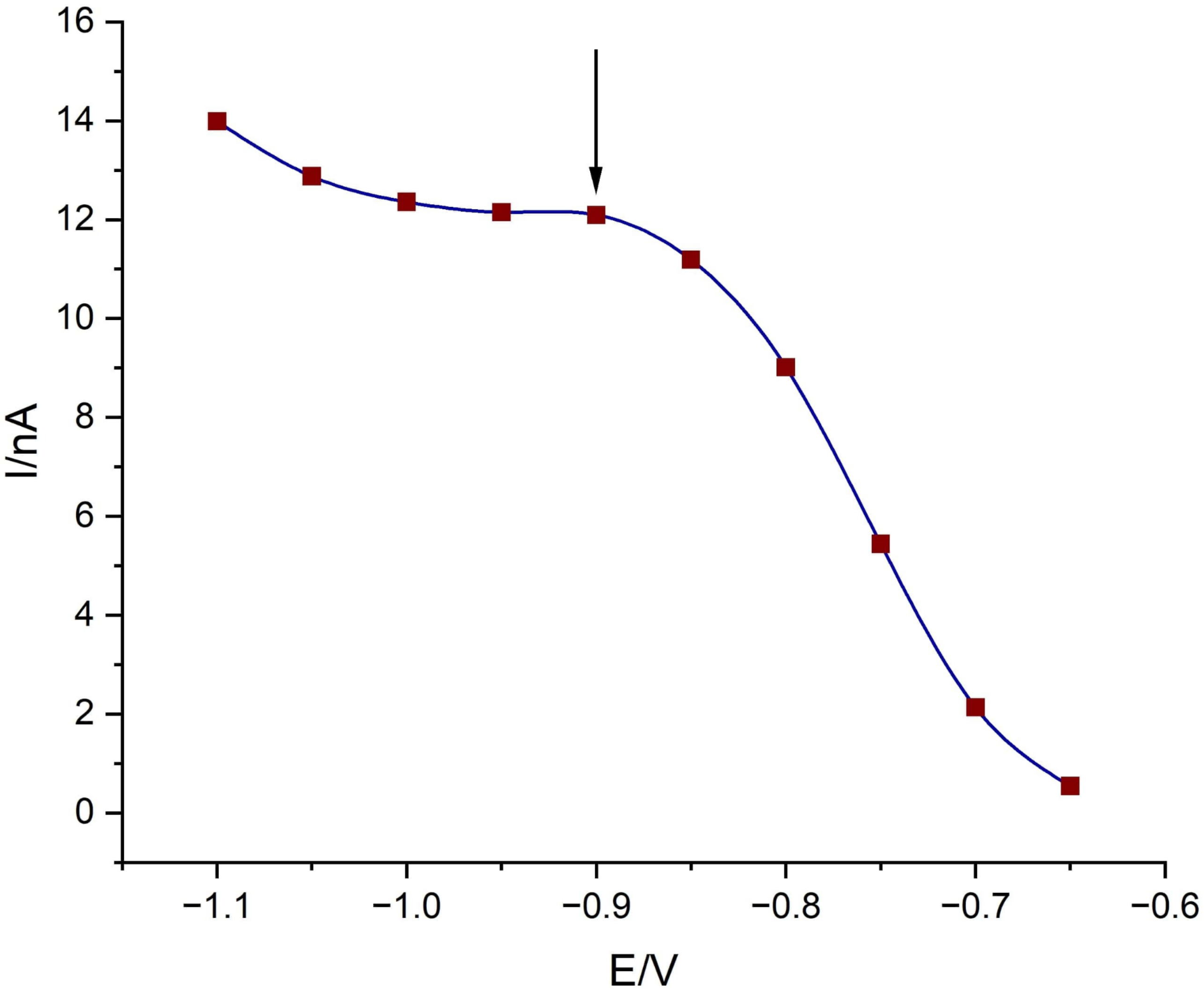

Fig. 2 Dependence of the peak current on the deposition potential. Base electrolyte: 0.05 M EDTA (pH 4.4), concentration of thallium:1 μg.mL^{-1}, preconcentration time: 300 s

in India range from 0.015 to 0.023 μg.g^{-1}. Notably, the Shilajit samples from Iran exhibited the highest Tl concentrations among all the other samples. A Shilajit from Iran/Saravan has been found to contain the highest concentration of Tl, quantified at 0.226 μg.g^{-1}. A sample from Kyrgyzstan has almost the same Tl concentration as the Bijar (Iran) sample, and a sample from India has the lowest amount of Tl with 0.007 μg.g^{-1}. This significant variation in Tl content among different samples from different countries and regions can be attributed to the diverse geology and soil composition from which Shilajit is derived. While crude Shilajit is widely consumed, there are no specific guidelines regarding daily intake or duration of consumption. Given that Tl can accumulate in various organs of the body, prolonged consumption of Shilajit with high Tl content may pose health risks. It should be mentioned that apart from Tl, other heavy metals have been reported in Shilajit [10]. The obtained results for Tl content in crude Shilajits are presented in Table 1.

Thallium content of the supplements

In order to dissolve the supplements, three different weighing methods were employed. Firstly, a single tablet or capsule of the supplement was weighed then digested in a tapered Teflon beaker for three replicates. Secondly, eleven capsules were crushed and mixed thoroughly, and weighed. Subsequently, approximately 0.25 g of the mixture was weighed and digested for three replicates. Thirdly, three tablets of S2 were individually weighed in Teflon beakers, digested, and the contents were combined. Each supplement underwent identical digestion and sample preparation processes, as outlined in Sect. 2.4.

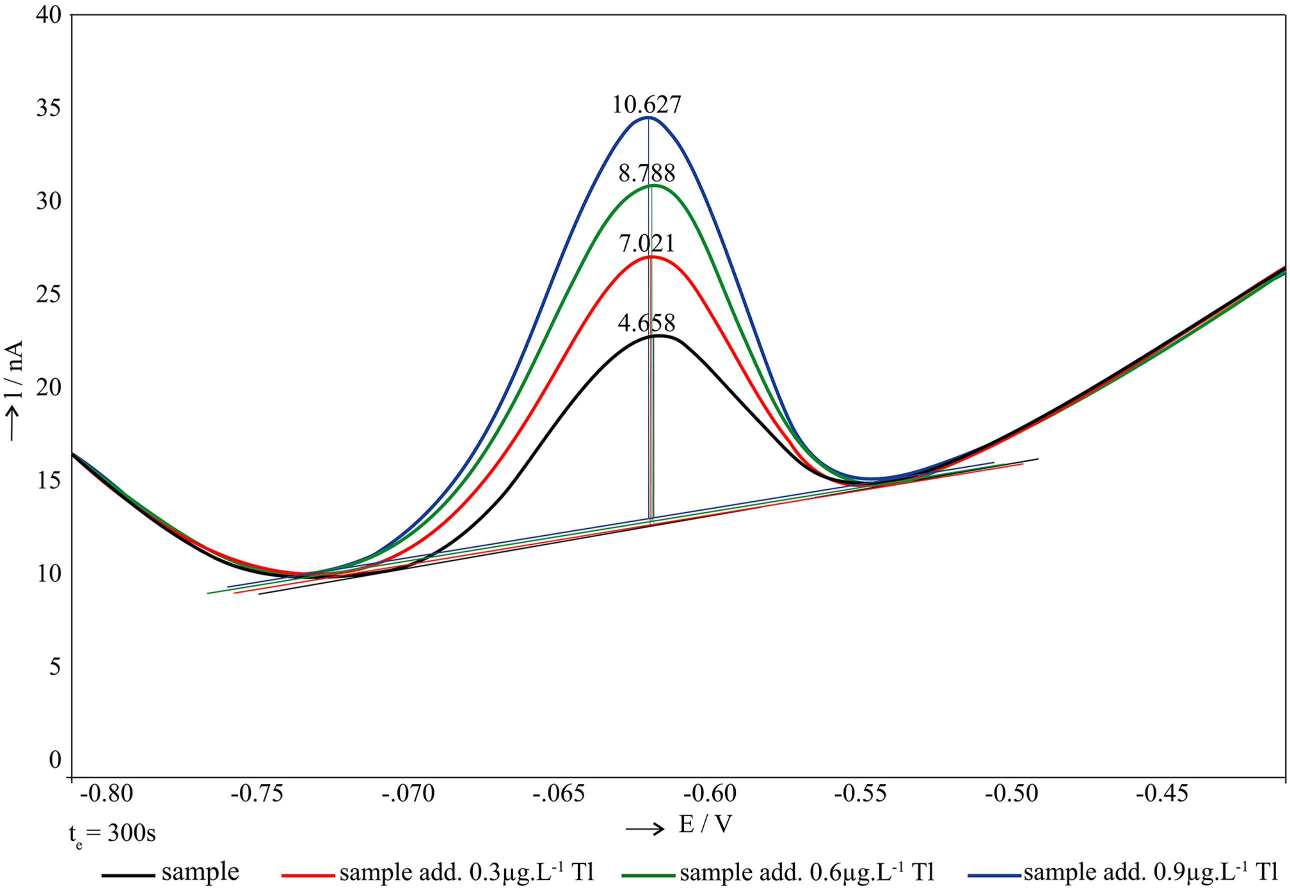

Fig. 3 Shilajit voltammogram with and without standard additions

Table 1 Thallium content in crude shilajit samples measured by FI-DP-ASV

Sample No.	Country/ region	Average Tl found (μg. g^{-1})	SD (μg. g^{-1})/RSD% ($n = 3$)
1	Iran / Unknown	0.153	0.006/4.098
2	Iran / Lorestan	< LOD	-
3	Nepal / Unknown	0.143	0.005/3.169
4	Iran / Kurdistan	< LOD	-
5	Iran / Ravar	0.077	0.006/8.285
6	Iran / Bijar	0.031	0.002/8.022
7	India / Unknown	0.007	0.001/13.384
8	India/ Unknown	0.021	0.003/13.125
9	India / Unknown	0.063	0.007/11.125
10	Russia /Unknown	0.010	0.0002/2.04
11	Iran / Kahnuj	0.210	0.007/7.479
12	Kyrgyzstan/Unknown	0.033	0.002/7.49
13	Iran / Saravan	0.226	0.020/9.017

The amount of Tl in the six commercial supplements was analysed by the same method (Sect. 2.5), and the results are presented in Table 2.

A matched concentration of Tl was obtained between the analysis of one capsule of supplement 1 (S1) and the mixing of 11 capsules of S1, followed by taking an equivalent weight of one capsule (~0.25 mg). Moreover, by digesting three tablets and mixing the digested samples together before analysis, Tl concentration was consistent with the amount of Tl measured in one tablet which was selected randomly from the package. This consistency in results indicates that the analytical method used for Tl determination is reliable and robust. The method showed a high reproducibility since the relative standard deviations for triplicate measurements of each sample were less than 22.519%.

Surprisingly, for all of the commercial supplements purchased, their packages did not provide any information about their chemical composition, including heavy metals content. Since there is a lack of available data regarding Tl content in Shilajit supplements on the market, the findings from our study hold crucial importance for healthcare-related organizations and can serve as valuable information for consumers. The highest concentration of Tl was quantified in S4 with 0.095 μg in one tablet. Considering the daily consumption of two tablets, the individual would intake 0.19 μg of Tl daily. The duration of supplement use should also be considered. It is significant that the concentration of Tl in some

Table 2 Thallium content of supplements measured by FI-DP-ASV

Supplement	Country	Average Tl content of one tablet or capsule (μg)	SD (μg.tablet^{-1}) / RSD% (*n* = 3)	Average Tl concentration in one gram of the supplement (μg.g^{-1})	SD (μg.g^{-1}) / RSD% (*n* = 3)
S1(capsule)	Poland	0.052	0.003/4.816	0.19	0.009/4.839
S2 (tablet)	Russia	0.09	0.009/10.341	0.38	0.040/10.340
S3 (tablet)	Kyrgyzstan	0.062	0.006/10.134	0.31	0.030/9.790
S4 (tablet)	Russia	0.095	0.007/6.997	0.5	0.040/7.020
S5 (tablet)	Russia	0.028	0.001/3.948	0.15	0.010/4.490
S6 (tablet)	Russia	Not detected	Not detected	Not detected	Not detected
Mixture of 11 capsules of S1	Poland	0.052	0.006/11.484	0.192	0.022/11.484
Mixture of 3 tablets of S2	Russia	0.096	0.022/22.519	0.408	0.034/8.224

supplements, such as S2, S3 and S4, is higher than that in crude Shilajits.

Conclusion

In this study, we performed quantification analyses on various crude Shilajit from different regions and Shilajit-based supplements to quantify thallium (Tl), one of the most toxic elements for the human body, using DP-FI-ASV, a reliable and accurate method for Tl quantification. Our findings revealed that crude Shilajit and supplements contain Tl. The highest amount of measured Tl was found in crude Shilajit from Iran and supplement S4, with concentrations of 0.226 and 0.5 μg.g^{-1}, respectively.

Remarkably, the Tl content in some supplements was higher than in some raw Shilajits. This highlights the need to pay attention to the content of supplement-based Shilajit products, as long-term consumption may lead to the accumulation of Tl in kidneys and bones, potentially harming different organs such as the liver. Another important point is that there is a lack of information on the supplement packaging regarding the chemical ingredients. This underscores the importance of standard safety tests to establish safe limits for trace elements in products.

Overall, this research emphasises the importance of quality control and safety testing for Tl content in Shilajit and Shilajit-based supplements to protect public health, given the widespread use of Shilajit supplements worldwide. More research on Shilajit is essential to mitigate the risk of heavy metal contamination and ensure consumer health. It should be noted that sampling of Shilajit as a natural product is a challenging step. A sample of Shilajit taken from one mountain has a different composition compared to that from another mountain or even from a different rock within the same region, due to the variation in the materials involved in its formation and the geological conditions in which Shilajit has remained over time.

Based on our research, both raw Shilajit and Shilajit-based supplements contain thallium. This highlights the importance of quantifying and qualifying heavy and toxic metals in Shilajit, as they can directly impact human health. Our future research will focus on quantifying other heavy metals in Shilajit and its supplements.

Abbreviations

EDTA	Ethylenediaminetetraacetic acid
FI-DP-ASV	Flow injection differential pulse anodic stripping voltammetry
IC	Ion chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MFE	Mercury film electrode
RSD	Relative standard deviation
SCE	Saturated calomel electrode
SD	Standard deviation

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Author contributions

EK: investigation and writing original draft. JZ and MK: supervision, writing, review and editing. WZ: data curation and methodology. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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