Development of a sample preparation strategy for the determination of tungsten in soil samples by inductively coupled plasma mass spectrometry using a response surface methodology

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Abstract: An alternative rapid digestion method has been developed for the determination of tungsten (W) in soil samples using fractionation studies and response surface methodology. The digestion method using the Kjeldahl instrument was applied to samples, and soluble tungsten was determined via inductively coupled plasma mass spectrometry. Digestion parameters were selected depending on the results of fractionation studies and optimized using a four-factor and five-level central composite design. The contributions of the phosphoric acid concentration (X_1), digestion temperature (X_2), digestion time (X_3), and hydrochloric acid concentration (X_4) were evaluated for the determination of W from samples in which its levels were maximum. Optimum conditions for factors were found to be $X_1 = 9.00$ M, $X_2 = 124$ °C, $X_3 = 45$ min, and $X_4 = 1.52$ M using a predicted W level of 86.82 μg L^{-1}. This yielded an expected level of 1736.40 mg kg^{-1} under the optimized conditions. Experimental W levels were in good agreement with this predicted level and found to be 1682.69 mg kg^{-1}. Thus, measured W levels showed the versatility of the central composite design in such a complex soil matrix for method development.

Key words: Tungsten, tungsten mine, soil, environmental analysis, response surface methodology, central composite design, ICP-MS

1. Introduction

Tungsten (W) is a rare heavy metal that has commonly been used in many household, industrial, scientific, and military applications due to its unique physical and chemical properties,[1−3] such as the highest melting point of any nonalloyed metal and high density.[4] On the other hand, the widespread usages of W-based products and related wastes are potential sources that may contribute to its local accumulation in the environment.[2,3,5,6] In addition to industrialization, many anthropogenic activities related to W, such as mining or the use of fertilizers in agricultural fields, may significantly raise its levels.[2,3,7,8] Thus, it could be transported to biotic systems from terrestrial, atmospheric, or aquatic systems via different mechanisms.[9]

W is not considered an essential mineral nutrient for living organisms. It is naturally present at low concentrations in soils and sediments.[8] The content of W in nonpolluted soils is in the range from 0.4 to 5.0 mg kg^{-1}.[10] Many anthropogenic sources may be a part of the activities responsible for the contamination of ground and surface water.[1] W-containing military munitions reach concentrations of up to 1500 and 3500 mg kg^{-1}. Likewise, concentrations of up to 287 mg L^{-1} and 560 μg L^{-1} W have been reported for pore-water and

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groundwater, respectively.[11] W concentrations can also be high locally due to active and abandoned mining sites.[2] In this regard, mining activities represent a source of pollution[12] and have an important contribution to W accumulation, with a serious potential hazard due to its accessibility from the ecosystem to humans.[2,3,12]

Tungsten is found almost exclusively in the form of tungstate in soils, with the majority of forms being wolframite and stolzite. The oxidation states and coordination numbers of W range from -2 to $+6$ and 5 to 9, respectively, leading to the possibility of soluble complex formation with a variety of inorganic and organic ligands.[13] In contrast, W is a relatively poorly known transition metal,[1] and the biological and biochemical effects of its compounds are not well known. It has been stated that some W compounds may have adverse biological effects on humans and animals.[8] However, the existing knowledge does not provide clear information regarding the behavior of W-based products in the environment.[1] Consequently, many studies have drawn attention to the limitation of environmental regulations for W in comparison with other metals[1,4,8] and the necessity of the reevaluation of W due to the historical view of it as a "nontoxic" and "environmentally inert" metal.[4]

Although the complexity of soil–metal relations makes it difficult to predict metal retention and dynamism in soil,[14] pollution history is one of the important factors for endemically forming W compounds in the environment as a result of biochemical interactions between natural or anthropogenic tungsten and organic compounds or ligands.[1] Thus, a comprehensive understanding of W geochemistry is needed that outputs its mobility, bioavailability, and toxicity as a result of interactions with the soil matrix.[11] One of the significances of the total elemental contents in soils is adding information regarding the level of contamination within this scope.[14] In this context, W quantification from various environmental matrices could be performed based on different techniques. Spectrophotometric[9,15] or spectrofluorometric methods, X-ray fluorescence spectroscopy,[9,16] neutron activation analysis, flame atomic absorption spectrometry, catalytic current polarography,[9] inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS),[9,17,18] and hyphenated liquid chromatography interfaced to ICP-MS techniques[5,19] are some of the chief ones that have been used. Among the many monoelemental or multielemental analytical techniques, such as flame atomic absorption spectroscopy, ICP-OES,[14,20,21] or ICP-MS, outlined for the determination of W, ICP-MS is ideally suited for providing very low detection limits and high sample throughput.[18] Compared to other elemental techniques, it offers excellent sensitivity/selectivity, a wide linear range, multielement determination capability, and the ability to measure isotope ratios.[20]

While it is compulsory to perform partial or total chemical dissolution prior to analysis for the many techniques mentioned above,[22] variable organic, inorganic, and metallic compositions[23] with inherent heterogeneity of soil samples lead to analytical complexity[22] for the analysis. Thus, the determination of some elements in soil is challenging and the importance of alternative and/or complementary methodologies is significant.[22,24] In the literature, W-containing soils have been digested in a microwave oven by using concentrated nitric acid and phosphoric acid to ensure the soluble W and to prevent the formation of tungstates and polytungstates.[25] Even so, it has been stated that traditional acid digestion procedures (using nitric/hydrochloric acids in combination with hydrogen peroxide) are insufficient for tungsten solubilization because of the precipitation of insoluble species (tungstic acid and polytungstic acids). Thus, some strong acid extraction procedures have been modified and used to improve the recovery of W from soil.[5] Nevertheless, these existing methodological approaches are time-consuming and recovery values change depending on the soil components. Additionally, to the best of my knowledge, no report has been previously published that offers new challenges by using fractionation studies

and response surface methodology (RSM) for the optimization of several factors for W solubilization from such complex soil materials depending on ICP-MS measurements with high sensitivity. Thus, the purpose of this work was to evaluate and improve the sample preparation strategy for the determination of W from soil samples of abandoned W mining areas by developing an efficient, simple, less time consuming, and alternative digestion method using fractionation studies and the RSM. Several parameters in the digestion step are optimized using a central composite design (CCD) to propose a sufficient and environmentally friendly procedure as an alternative to conventional acid leaching procedures or complete dissolution of the matrix.

2. Results and discussion

2.1. Fractionation studies

Atomic spectrometry techniques commonly require samples in the form of aqueous or acidic solutions; therefore, samples are treated with concentrated acids either individually or in mixtures to solubilize the elements from the sample matrix.[26] Thereby, initial sample preparation methods, such as open-wet digestion, block digestion, or microwave systems, are most commonly used to digest solid sample matrices.[22] In addition to the total dissolution procedures, the geochemistry of metals may also depend on fractionation studies.[5] Because the term fractionation is defined as a process of the classification of an analyte or a group of analytes from a certain sample according to physical or chemical properties and any single and/or sequential extraction procedures yield information about metal fractions, as detailed in Templeton et al.,[27] these studies will inform matrix structures in more detail for any elemental sites.[28] The detailed knowledge based on relatively easy fractionation results may avoid complete dissolution of the matrix for the subject of W analysis. Thus, possible fractionation studies that would provide valuable insights for further digestion steps were preliminarily applied. To select fractionation solvents, conventional W production conditions and requirements on the sampling area were taken into account. W production technologies are generally based on the treatment of low-grade scheelite ($CaWO_4$) or wolframite (($Fe,Mn)WO_4$) with alkaline reagents.[29] Under these alkaline conditions, W has a tendency to dissolve as tungstate (WO_4^{2-}).[1] Therefore, it is more readily mobilized under alkaline conditions.[10] Earlier alkaline processing conditions of the Etibank Wolfram Mine area, with a native pH of 7.1 of soils,[30] support the tungstate form and the importance of using basic reagents instead of acidic media.[5] On the other hand, basic conditions, in which W is more labile and thus commonly most appropriate for fractionation, may interfere with the measurements depending on plasma instabilities that necessitate dilution before analysis.[5] Depending on the results of fractionation, extraction based on ammonia is found to be inappropriate for ICP-MS analysis. Low and nonrepeatable W levels were found; nevertheless, hydrochloric acid treatment was applied to neutralize its effect. Although the elemental contents in these fractions depend on the level of disruption of the tungsten-bound structures through an ultrasonic bath,[31] 0.75 ± 0.33, 104.14 ± 9.70, 47.41 ± 3.45, and 4.96 ± 0.57 mg kg^{-1} W were determined in the phosphoric acid, nitric acid, sodium carbonate, and buffered solutions, respectively. To see the extraction efficiency of temperature, Kjeldahl digestions were also applied using nitric acid or phosphoric acid. Additionally, an acid mixture that was detailed in fractionation studies and proposed by Bednar et al.[5] was also applied for comparison. The tungsten levels in the nitric acid, phosphoric acid, and the mentioned acid mixture were 200.28 ± 16.12, 191.86 ± 20.49, and 1592.24 ± 199.66 mg kg^{-1}, respectively. Phosphoric acid digests were compatible with the nitric acid. On the other hand, higher extraction efficiencies were observed for tungsten by introducing temperature to the digestion procedure using phosphoric acid rather than nitric acid. To reach the higher levels of W with the contribution of temperature, phosphoric acid was selected as an appropriate solvent after fractionation studies. Additionally, as the importance of HCl in the

leaching of scheelite mineral with phosphoric acid was highlighted in recent works,[29] the extraction methodology was extended with the use of hydrochloric acid.[29] Under these conditions, HCl forced tungsten to take a different molecule into its ligand layer other than a water molecule; the following reactions occur when PO_4^{3-} anion is added:[29]

$$12CaWO_4 + 24HCl + PO_4^{3-} \rightarrow [PW_{12}O_{40}]^{3-} + 12CaCl_2 + 12H_2O \tag{1}$$
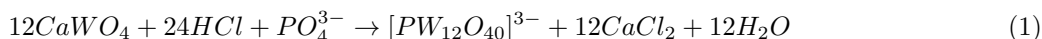
Based on the importance of some excess quantity of HCl in the complexation process,[29] its molarity was designated as the third important variable for further studies. The selected factors were then optimized in the next step using the RSM.

2.2. Optimization using RSM methodology

A total of 30 experiments with all experimental conditions at every step and the corresponding response values are shown in Table 1. The final second-order polynomial equation, in terms of coded factors obtained based on the CCD experimental design,[32] was as follows:

$$\begin{aligned} Y(Wlevel) =\ & +22.19 - 2.94X_1 + 28.44X_2 + 8.43X_3 + 0.91X_4 - 3.54X_1X_2 - 0.98X_1X_3 + 6.83X_1X_4 \\ & +10.66X_2X_3 + 0.54X_2X_4 + 4.61X_3X_4 + 1.46X_1^2 + 7.16X_2^2 + 0.13X_3^2 + 0.23X_4^2, \end{aligned} \tag{2}$$

where Y denotes the response values as W amounts (μg L^{-1}) and X_1, X_2, X_3, and X_4 are the coded values of the molarity of phosphoric acid, temperature, time, and molarity of hydrochloric acid, respectively. Maximum acid molarities in the experimental design depend on their maximum concentrations, and the maximum temperature value was selected based on the boiling point of H_3PO_4. The adequacy and the fitness of the model were tested by using analysis of variance (ANOVA).[32] The ANOVA results are shown in Table 2.

Any terms in the model with a large F-value and a small P$-$value would indicate a more significant effect on the corresponding response variables.[32−34] While P$-$values ($prob > F$) less than or equal to 0.05 indicate that the term is significant, values greater than 0.05 indicate the insignificance of the term; P $<$ 0.01 indicates that the effect is highly significant.[32,34,35] Among the variables and according to Table 2, temperature is the most significant factor (P $<$ 0.01), while extraction time is significant. Additionally, the individual effects of HCl and phosphoric acid are statistically insignificant. Beyond these linear effects, variables showing insignificant linear effect (X_1 and X_4) have a significant mutual effect on response (P $<$ 0.05). According to the ANOVA results of the model, the factors X_2, X_3, X_1X_4, X_2X_3, and X_2^2 are significant model terms under the studied conditions and have high contribution to the response, showing the linear effects of time and temperature, quadratic effect of temperature, and interactive effects of others on response. The model F-value of 14.93 with a very low P-value ($<$0.0001) indicates that the model is significant. There is only a 0.01% chance that a "Model F-Value" could occur due to noise. The quality of the model is usually evaluated using the determination coefficient (R^2), predicted determination coefficient (R$^2_{pred}$), and adjusted determination coefficient (R$^2_{adj}$).[36] The goodness-of-fit of the model is checked by using an R^2 value of 0.9331 and means that approximately 93% of the data were compatible and 7% of the variance could not be explained by the model.[32,34] R$^2_{adj}$ shows the degree of correlation between the experimental and predicted values[34,37] and also summarizes the model adequacy and fitness and serves to correct R^2 values for the sample size and the number of terms.[32] As values near 1 are acceptable,[32] an R$^2_{adj}$ value of 0.8706 shows the proper significance of the model.[36] Although the R$^2_{pred}$ value of 0.6425 is not very close to the R$^2_{adj}$ value of 0.8706, it may be expected

**Table 1.** Detailed design matrix with experimental responses.

Std	Run	Coded values				Actual values				Response
		X_1	X_2	X_3	X_4	X_1	X_2	X_3	X_4	W content (μg L^{-1})
1	9	−1	−1	−1	−1	9	56	25	1.5	4.412
2	10	1	−1	−1	−1	11	56	25	1.5	3.730
3	6	−1	1	−1	−1	9	124	25	1.5	69.806
4	22	1	1	−1	−1	11	124	25	1.5	36.179
5	18	−1	−1	1	−1	9	56	45	1.5	2.497
6	25	1	−1	1	−1	11	56	45	1.5	0.196
7	14	−1	1	1	−1	9	124	45	1.5	97.965
8	19	1	1	1	−1	11	124	45	1.5	52.596
9	21	−1	−1	−1	1	9	56	25	2	2.395
10	5	1	−1	−1	1	11	56	25	2	3.960
11	3	−1	1	−1	1	9	124	25	2	30.015
12	11	1	1	−1	1	11	124	25	2	43.228
13	23	−1	−1	1	1	9	56	45	2	0.078
14	15	1	−1	1	1	11	56	45	2	2.481
15	13	−1	1	1	1	9	124	45	2	89.950
16	7	1	1	1	1	11	124	45	2	100.054
17	4	−2	0	0	0	8	90	35	1.75	26.863
18	17	2	0	0	0	12	90	35	1.75	18.949
19	16	0	−2	0	0	10	22	35	1.75	0.068
20	27	0	2	0	0	10	158	35	1.75	91.368
21	20	0	0	−2	0	10	90	15	1.75	5.055
22	2	0	0	2	0	10	90	55	1.75	30.122
23	30	0	0	0	−2	10	90	35	1.25	13.741
24	24	0	0	0	2	10	90	35	2.25	22.225
25	12	0	0	0	0	10	90	35	1.75	28.577
26	26	0	0	0	0	10	90	35	1.75	19.523
27	8	0	0	0	0	10	90	35	1.75	18.562
28	29	0	0	0	0	10	90	35	1.75	14.426
29	28	0	0	0	0	10	90	35	1.75	22.180
30	1	0	0	0	0	10	90	35	1.75	29.899

for such a heterogenic matrix because the standard deviation at the center point of the experiments is 6.01 μg L^{-1}, which is nearly high compared with some response values. Nevertheless, the relationships between the experimental and predicted values show an adequate agreement,[32] as shown in Figure 1.

The coefficient of the variation (CV) value of 37.91% shows the deviations between the experimental and predicted values,[32] and its value may be expected for such a heterogenic matrix. The model also shows statistically insignificant lack of fit,[34] confirming the validity of the model.[36] Nonsignificant lack of fit is good and shows that the model equation was sufficient for predicting the responses.[32] The "Lack of Fit F-value" of 4.64 for the regression equation implies that there is a 5.20% chance that this value could occur due to noise. The adequate precision value measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio based on our results indicates an adequate signal, with a ratio of 15.166. Consequently, this model seems adequate and was used to evaluate the response surface plots for optimum conditions.

Table 2. Analysis of variance (ANOVA) results for the polynomial quadratic model of W leaching from soils.

Source	Sum of squares	df	Mean square	F value	P-value Prob > F	
Model	25926.81	14	1851.92	14.93	< 0.0001	significant
X_1	207.22	1	207.22	1.67	0.2157	
X_2	19416.80	1	19416.80	156.59	< 0.0001	
X_3	1703.98	1	1703.98	13.74	0.0021	
X_4	19.71	1	19.71	0.16	0.6958	
X_1X_2	200.67	1	200.67	1.62	0.2227	
X_1X_3	15.27	1	15.27	0.12	0.7305	
X_1X_4	746.17	1	746.17	6.02	0.0269	
X_2X_3	1818.65	1	1818.65	14.67	0.0016	
X_2X_4	4.65	1	4.65	0.04	0.8491	
X_3X_4	340.76	1	340.76	2.75	0.1181	
X_1^2	58.20	1	58.20	0.47	0.5037	
X_2^2	1406.00	1	1406.00	11.34	0.0042	
X_3^2	0.44	1	0.44	0.00	0.9531	
X_4^2	1.40	1	1.40	0.01	0.9168	
Residual	1860.01	15	124.00			
Lack of fit	1679.24	10	167.92	4.64	0.0520	not significant
Pure error	180.78	5	36.16			
Cor total	27786.83	29				
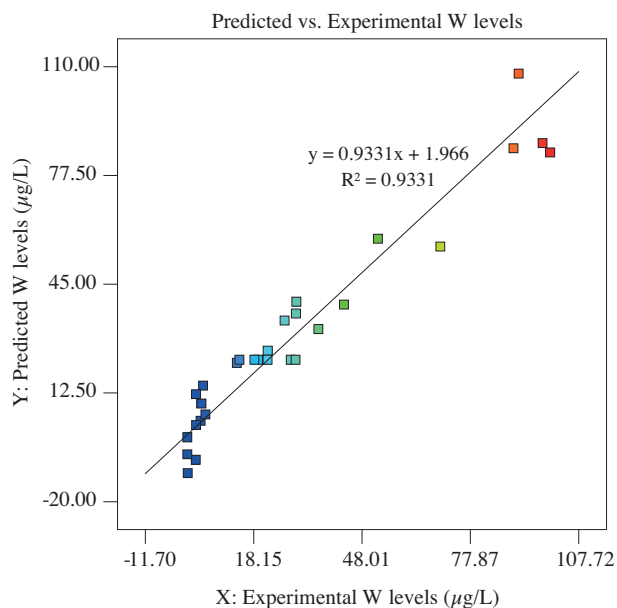
Figure 1. Experimental and predicted W levels based on the model.

2.3. Analysis of response surfaces

Three-dimensional response surface plots show the effects of variables and their interactions[34,38] on W extraction from soil. These contour plots also visually identify the optimal experimental factors.[35] Figure 2 shows the response surface plots of the model, showing the effect of a) temperature-molarity of phosphoric acid, b) time-molarity of phosphoric acid, c) time-temperature, d) molarity of phosphoric acid-molarity of hydrochloric

acid, e) molarity of hydrochloric acid-temperature, and f) molarity of hydrochloric acid-time, while others were maintained at their zero-level and the digestion efficiency in any case was measured by using the W contents of the extracts.
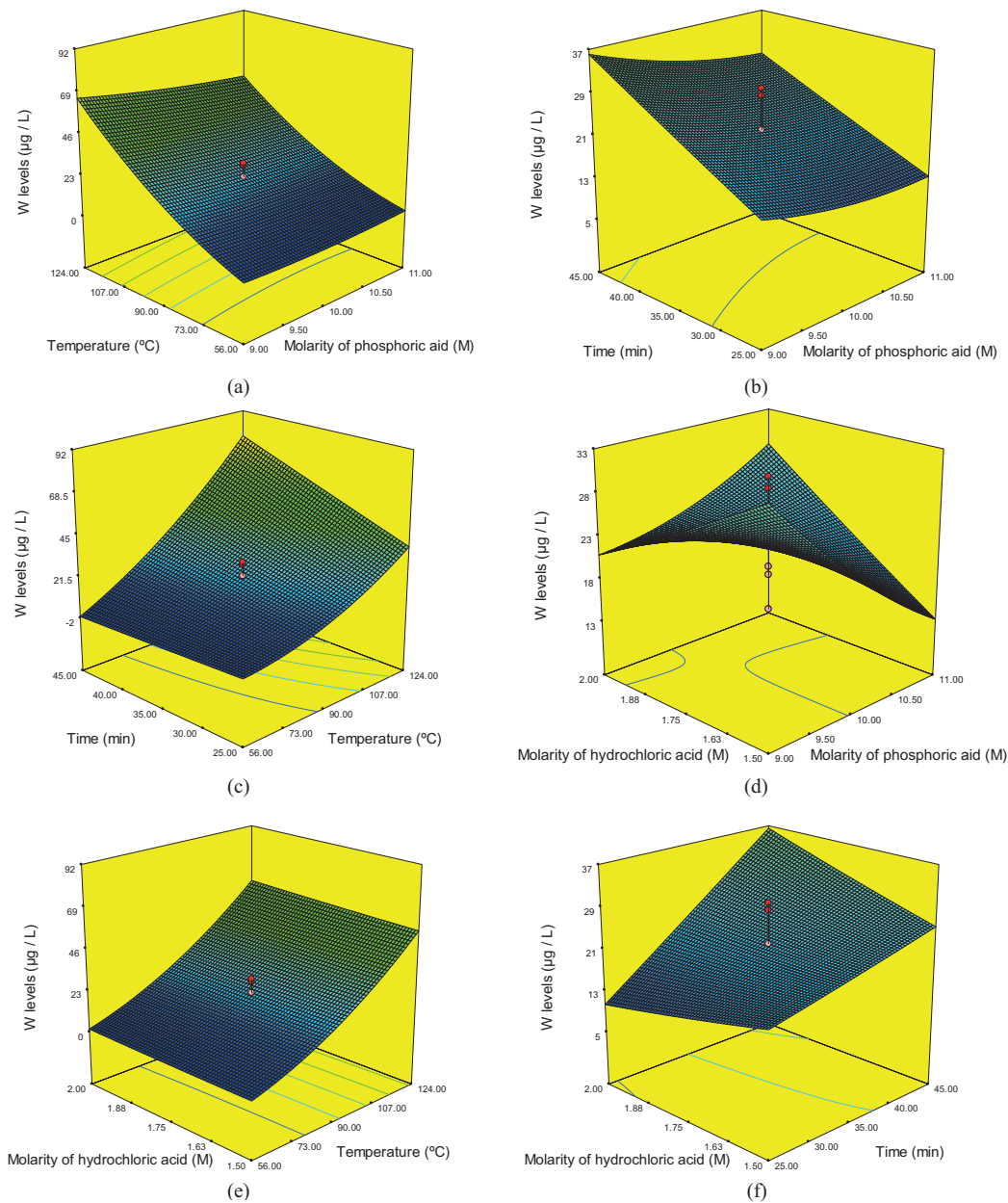

Figure 2. Response surface plots of the model, showing the effects of the molarity of phosphoric acid (X_1), temperature (X_2), digestion time (X_3), and molarity of hydrochloric acid (X_4) on W leaching from soil samples.

Figure 2a shows the effect of rising temperature versus phosphoric acid concentration. W contents increased quite sharply with increasing temperature, possibly due to the solubility and the transfer of W from soil structures to the solution. Additionally, temperature may support complexation, and this process seems to be endothermic, depending on the increasing digestibility of W from soil structures. Temperature remained

almost constant after 124 °C. Thus, this variable is found to be more compatible for leaching. On the other hand, W levels slightly decreased with the concentration of phosphoric acid until nearly 10.5 M; its increasing effect was observed after that point, possibly due to the accelerating rate of the complexation process at that condition. The decreasing effect of phosphoric acid may also depend on the interfering ions, which could be complexed by phosphoric acid at lower concentrations. The selectivity of the phosphate anion may be assumed as important in this case. Elemental contents of the studied area were mentioned by Gürmen et al. and Güleryuz et al. [29,30] Additionally, a broad range of elements were quantified by using ICP-MS from the soil of *A. cretica* in our earlier studies, and W, Mo, Zn, Fe, Cu, Co, Bi, Mn, Cd, Cr, and As levels were determined to be in different ranges. Among the quantified elements, one of the important interfering ions may be molybdenum; its antagonist effect on complexation was observed (with violet color) for the soil extracts together with the determined high Mo levels from the uncontaminated soils of the same species in our earlier works. [39] However, in the soils of contaminated sites of the mining area, the molybdenum levels were found to be very low in comparison with the uncontaminated soils. On the other hand, Mn, Cu, Fe, and Zn levels were found at higher concentration levels in the soils among the quantified elements. While a small decreasing effect of the phosphoric acid may be attributed to the existence of these elements, their interfering effects as a result of complexation with phosphate will not be effective, depending on the excessive levels of W in the soils. The same effect of phosphoric acid can be observed from the curves versus digestion time, with the only difference being the more gradated decrement effect of phosphoric acid versus the linear effect of duration (Figure 2b). W levels were slightly decreased initially by the increasing phosphoric acid content, which may be assumed to be possibly due to the mentioned complexation of the PO_4^{3-} anion with other ions at its lower concentrations, decreasing the effectiveness of the phosphoric acid versus W. However, the influence was not notable, as discussed above. The plot illustrates the maximum values of 45 min and 11 M underlying the required extraction time for the complexation process. Increasing temperature slightly was in inverse correlation with the digestion time (Figure 2c), as it may support complex formation of the other possible interfering ions over a long period of time at rising temperatures. This also yielded the importance of the complexation kinetics of the reaction. Figure 2d shows the increasing effect of HCl on W levels, while the phosphoric acid concentration slightly increased the achieved contents of W. This increment was more significant after 10.5 M. As mentioned, HCl was selected as a parameter to increase the solubility for complex formation. [29] Thus, the tendency of the figure may be attributed to the collaboration of HCl and H_3PO_4 for complexation. W levels slightly decreased until near 2 M and then increased with HCl versus temperature (Figure 2e). Consequently, Figure 2f shows the decreasing levels of W until reaching 2 M of HCl, remaining almost constant at that point, while the digestion time reached a maximum value of 45 min. In addition to the evaluation of the plots, analysis of the quadratic model by software offered the optimum digestion conditions of X_1 = 9.00 M, X_2 = 124 °C, X_3 = 45 min, and X_4 = 1.52 M (Table 3) by using a predicted W level of 86.82 μg L^{-1} and a desirability of 0.868.

Table 3. Actual and coded values of the variables/optimized digestion conditions.

Variables	Unit	Symbol	Coded values					Optimum digestion conditions
			−2 (−α)	−1	0	+1	+2 (+α)	
H_3PO_4	M	X_1	8	9	10	11	12	9.00 M
Temperature	°C	X_2	22	56	90	124	158	124 °C
Time	min	X_3	15	25	35	45	55	45 min
HCl	M	X_4	1.25	1.5	1.75	2	2.25	1.52 M

2.4. Comparison of the optimized method

The same soil sample was used for final quantification once the digestion method was optimized. Separate digestions (n = 5) and recovery studies were performed under optimum conditions, achieved via CCD. The results were then compared to the reference material analysis to evaluate the accuracy of the method. According to the optimized procedure, a W concentration of 1682.69 ± 212.10 mg kg^{-1} was calculated by using a sample amount, dilution factor, and the remaining final volume of digested samples in the Kjeldahl system. This level is compatible with the predicted W level of 1736.40 mg kg^{-1} found by using the CCD, with a calculated relative error of 3%. The proposed method was also tested on the existing digestion procedure proposed by Bernard et al., which is a modification of US EPA 3050B that uses phosphoric acid in digestion.[5] This procedure was applied in the fractionation part of the study and a 1592.24 ± 199.66 mg kg^{-1} W level was found with the same soil mentioned earlier. Indeed, one of the variant procedures was also applied by Clausen and Korte earlier,[25] and nitric acid–phosphoric acid digestion has also been mentioned by Clausen et al. as a simpler and safer alternative to hydrofluoric acid for the near-quantitative digestion of W from soil.[18] Additionally, five different soil samples were subjected to the method of Bernard et al.,[5] and a 1313.331 ± 158.62 mg kg^{-1} W concentration was found, showing the heterogeneity of the distribution of W around the waste removal pool even for the same species. The difference between the optimized and existing methods regarding W contents of soils was tested by using one-way ANOVA. All of the statistical tests were performed at a significance level of 0.05. As a result, there were no significant differences between the two methods in terms of determined W concentrations (F = 0.564, P = 0.474). The results of the optimized and proposed procedure of this study are consistent with those obtained by using the existing methodology; thus, the optimized method may be used as an alternative for W leaching from soil samples. Relatively high standard deviations in the two methods are acceptable, depending on the heterogeneous nature of the soil structure for W distribution, which is proven by applying the same method proposed by Bednar et al.[5] to different contaminated soil samples of the same species. Additionally, spiked samples were prepared at two concentration levels for recovery analysis to see the interference effects originating from the inherent heterogeneity of the matrix; the proposed method yielded a high recovery value. W recovery of 82% was achieved by the optimized method. This recovery also matched well the value of 86% achieved by Bednar et al.,[5] who mentioned that tungsten may be present in silicate form of the matrices; thus, total dissolution may not be expected in this case and the recoveries are meaningful.[5] The relationship with the silicate structure may be verified by using the approximate results of W recovery after using hydrofluoric acid for soil digestion.[5] Therefore, recoveries may depend on the form of W, although a certified reference material was used. On the other hand, depending on the wolframite form of W in the abandoned mining area, as detailed above, recovery studies after the addition of a standard tungstate solution seem more meaningful and appropriate when applied in our study versus reference material analysis, in which the form of W is not known. Nevertheless, the reliability of the method was evaluated through standard reference material analysis in addition to the recovery studies, and the measured value of 19.1 ± 3.0 μg g^{-1} W was in good agreement with the certified value (23.0 ± 1.0 μg g^{-1}) at the 95% confidence level. Regarding measurement, it is stated that tungsten isotopes (especially 182 and 184) can potentially be affected by various oxides of holmium, dysprosium, erbium, and ytterbium. However, the low concentrations of the mentioned elements in most samples, as well as the low oxide values (less than 3%) of the parent ion of properly tuned ICP-MS, prevent this type of interference in general.[19] Ce/CeO level was smaller than 0.03 before all analyses. Even so, any isobaric or matrix interferences can be reduced by diluting the samples and also by using correction equations from the software, with possible interferences originating from the matrix being evaluated via analysis

of the four tungsten isotopes with ICP-MS. In this work, no significant variation was found among the isotopes, and the most abundant one (W-184) was selected for quantification. The detection limit of W was 0.26 μg L^{-1}. The calibration equation was found as y = 700.69 x for the W-184 isotope, and the r^2 value of 0.99968 indicated the high linearity of this curve. The interday and intraday repeatability were found to be 3.2% and 6.9%, respectively. Method validation parameters are outlined in Table 4. One of the important problems of dealing with the carry-over and memory effect of W in sample introduction systems of ICP-MS[18] was screened by analyzing the same W standard solution prepared in ultrapure water and a blank solution of digests after every third run systematically, minimized by using an appropriate volume of washing solution (1% HNO_3) after every run depending on the background levels of W before every analysis.

3. Experimental

3.1. Chemicals and reagents

A single-element standard solution of W at a concentration of 1000 μg mL^{-1} was purchased from PerkinElmer (Product number: N9303809, PerkinElmer Sciex, Shelton, CT, USA). Nitric acid (65%, suprapure), hydrochloric acid (30%, 1 L = 1.15 kg, suprapure), ortho-phosphoric acid (H_3PO_4, 85%, 1 L = 1.71 kg, Merck 100573), buffer solution (Merck 109461, (boric acid/potassium chloride/sodium hydroxide), pH 9.00 $\pm$ 0.01 (20 °C) Certipur), and all other reagents were obtained from the local suppliers of Merck KGaA (Darmstadt, Germany). NCS DC73034 "soil-trace elements and oxides" certified reference material was obtained from LGC Standards (Teddington, UK). The water used in all experimental parts was an ultrapure grade (18.3 MΩ.cm^{-1}, Zeneer Power I, Human Corporation, Seoul, Korea). Argon (99.999% purity) gas was purchased from Asalgaz (Bursa, Turkey). Polyvinylidene fluoride (PVDF) hydrophilic syringe filters (0.45-μm pore size, Millex-HV, Millipore Corporation, Bedford, MA, USA) were used for filtration purposes.

3.2. Apparatus and instrumentation

Samples were digested using the DK 20 model Kjeldahl digestion unit (VELP Scientifica, Milan, Italy) with 20 borosilicate digestion vessels. An Elma LC-30H model ultrasonic bath (Elma Hans Schmidbauer GmbH & Co. KG, Singen, Germany), operated at an ultrasonic frequency of 35 kHz and a power of 240 W, was used for fractionation studies. A MSE Mistral 2000 centrifuge (MSE Scientific Instruments, UK) was used for the sample preparation. W levels in the samples and fractions were measured using an Elan 9000 ICP-MS (PerkinElmer SCIEX, Shelton, CT, USA). The components of the ICP-MS equipment used were as follows: PerkinElmer Ryton cross-flow nebulizer, a Scott-type double-pass spray chamber, a standard glass torch, nickel sampler, and skimmer cones (i.d.: 1.1 mm and 0.9 mm, respectively). Additionally, the operating conditions are summarized as follows: RF power, 1000 W; plasma argon flow rate, 17.0 L min^{-1}; auxiliary argon flow rate, 1.2 L min^{-1}; nebulizer flow rate, 0.85 L min^{-1}; sample uptake rate, 1.5 mL min^{-1}; dwell time, 50 ms; dead time, 60 ns; scanning mode, peak hopping; detector mode, dual. Additionally, the measured isotopes of W, together with their isotopic abundancies, were as follows: ^{182}W (26.50%), ^{183}W (14.31%), ^{184}W (30.64%), and ^{186}W (28.43%).

3.3. Sampling area

Sampling was performed around the abandoned Etibank Tungsten Mine Work at Mount Uludağ in Bursa, Turkey. This mine area is located at an altitude between 2100 and 2487 m, lying at the intersection of 40° N

Table 4. Analytical performance of the proposed method.

Isotope	Instrumental limits of detection (μg L^{-1})	Working Range (μg L^{-1})	Calibration equation	Correlation Coefficient (r^2)	Intraday precision (%)	Interday precision (%)	Measured W according to developed method (mg kg^{-1})	Measured W according to existing methodology (Ref.5) (mg kg^{-1})	Certified value[a] (μg g^{-1})	Measured[a] value (μg g^{-1})	W recovery (%)
^{184}W	0.26	10–2000	y = 700.69x	0.99968	3.2	6.9	1682.69 ± 212.10	1313.33 ± 158.62	23.0 ± 1.0	19.1 ± 3.0	82

[a] Represents the values of W from NCS DC73034 "soil-trace elements and oxides" certified reference material.

latitude and 29° E longitude in a national park. Mining activity in this area started in 1969 and continued until 1989. In this mine, the ore has a content of 40% WO_3, with the by-products of concentrated magnetite and pyrite. The geological structure of the area was reported as granitic and calcareous rocks in the north and south slopes, while the soil structure is granite around the mine work area at lower altitudes and calcareous at the upper altitudes.[30] Sampling was done around the waste removal pools of this abandoned mine area. The substrata of the waste removal pools and waste canals were on the granite structure.[30]

3.4. Sample collection

Soils samples that belong to *Anthemis cretica* L. surrounding the contaminated waste removal pools were taken from a 0–5 cm depth and a 10 cm cap of the base of the plant using vinyl gloves (n = 5). The samples were then placed in a polyethylene bag and transported to the laboratory. Afterwards, they were mixed, sieved with a standard plastic sieve that was 0.5 mm in diameter, dried in air, and transferred to closed paper bags. The samples were mixed separately by hand in their original packages immediately before any experimental procedure. A portion of one homogenized sample was used for fractionation and optimization studies, while the others were used for checking the responses based on the heterogeneity of the soil matrix in any specified method.

3.5. Sample preparation

First, 0.5 g of soil samples was weighed into clear glass vials, and a total volume of 20 mL of the solvents, including 10% (v/v) HNO_3, H_3PO_4, sodium carbonate (1 mol L^{-1}), ammonia solution (25%), buffer solution or a mixture of nitric–phosphoric acids, hydrogen peroxide, and ultrapure water (12.5 mL, 1 mL, 5 mL, and 2.5 mL, respectively), was added separately for fractionation studies. After manual agitation of the soil samples, extractions were performed in vials, with the cap closed or open, for 2.0 h at room temperature in an ultrasonic bath. The samples were then transferred to polypropylene centrifuge tubes, held overnight, and centrifuged at 5000 rpm for 5 min. The supernatants of the samples were diluted 10-fold prior to ICP-MS analysis and filtrated through 0.45-μm PVDF syringe filters (n = 3). Digestion of the samples was performed using Kjeldahl digestion equipment. A total of 0.5 g of homogenized samples was digested with 10% (v/v) HNO_3, H_3PO_4, or acid mixtures as detailed above. After cooling to room temperature, the same procedure was applied to the samples as described for the fractions. A triplicate of replicates in any of the fractionation or digestion procedures was prepared for each of the samples and reagent blanks, except for the digestion with the acid mixture (n = 5). The overall extraction/digestion scheme is shown in Figure 3.

3.6. ICP-MS analysis

To minimize contamination, all of the glassware and polypropylene centrifuge tubes were soaked in 10% (v/v) nitric acid solution overnight and then rinsed several times with ultrapure water. Working solutions of W were prepared by diluting the single-element standard solution for external calibration prior to use. The calibration curve was constructed from six levels ranging from 10 to 2000 μg L^{-1} W. Certified reference material analysis was applied to evaluate the accuracy of the optimized method. Additionally, recovery studies were performed and spiked samples (at two levels, 5 and 10 μg L^{-1}) were prepared by adding W standard solutions to soil samples prior to the optimized digestion procedure. The supernatants, obtained similarly to that detailed in the fractionation/digestion procedures, were diluted, filtrated, and analyzed. The instrumental detection and quantification limits were calculated as being three and ten times the standard deviations at the
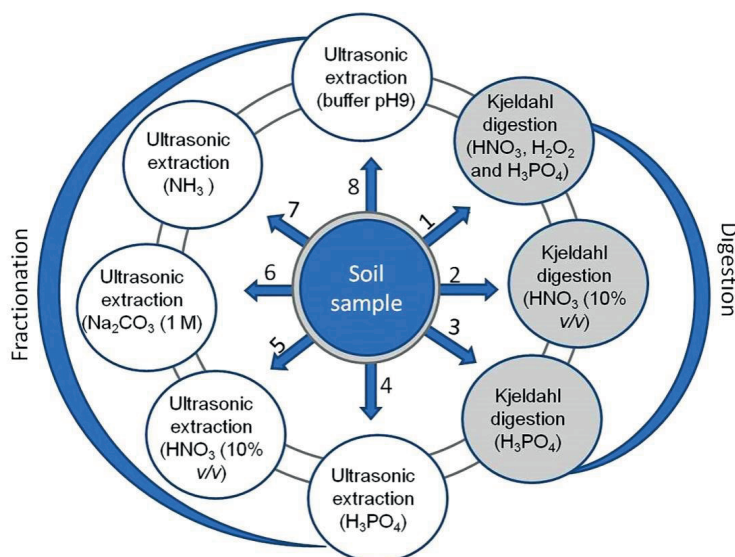
Figure 3. The fractionation and digestion steps applied for the soil samples.

lowest concentration of W. Intraday and interday precisions were calculated from the percent relative standard deviation of five separately prepared samples at the lowest concentration of W, which were analyzed on the same day and over a period of one week, respectively. [40]

3.7. Optimization of the extraction conditions with experimental design

First, 0.2000 ± 0.0010-g soil samples were weighed into the Kjeldahl tubes. A total volume of 20 mL of solvent was selected, as in the fractionation studies. The selected variables for optimization were molarity of phosphoric acid, temperature, extraction time, and molarity of hydrochloric acid. For all experiments 0.2 g of the sample mass was kept constant. As contaminated soil samples were selected for optimization strategies, 200-fold dilution with ultrapure water was applied to samples prior to analysis, depending on the determined high W levels from soils earlier.

When a combination of several independent variables and their interactions affect responses, RSM is used[41] as a chemometric approach for designing experiments, obtaining models, evaluating the effects of factors, and finding optimum conditions,[42] leading to a time-saving process, as well as a decrease in the use of reagents and materials.[34] CCD, on the other hand, is one of the most commonly used RSM designs[35] and all the other basic terms of the chemometric techniques were outlined elsewhere.[43,44] In the present study, CCD was used to optimize the extraction parameters for W solubilization with the Kjeldahl instrument from soil samples.

The main areas of designing an experiment are screening the factors (factor is defined as any aspect of the experimental conditions that affects the result obtained from an experiment), optimization (i.e. the process of finding optimum factor levels), saving time, and quantitative modelling.[43,44] A five-level, four-factor CCD, consisting of 30 runs with six replicates of the central points, was applied[33,42,45,46] to optimize and evaluate all possible linear, quadratic, and interaction effects of the selected factors.[32,33] The low (coded value = –2), middle (coded value = 0), and high (coded value = +2) levels of each variable are shown in Table 3. Moreover, the design matrix, with coded and actual values, is shown in Table 1. The behavior of the system was fitted to

a second-degree (quadratic) polynomial model with a response function, as follows: [32,33]

$$Y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{i=j}^{k} b_{ij} x_i x_j + e, \qquad (3)$$

where Y represents the response and corresponds to the W content of samples (μg L^{-1}), b$_0$ is the constant term, and b$_i$, b$_{ii}$, and b$_{ij}$ are the regression coefficients obtained for linear, quadratic, and interaction effects, respectively. Additionally, x$_i$ and x$_j$ are independent variables, where i and j represent the linear and quadratic coefficients, respectively. b, k, and e are the regression coefficient, number of factors studied, and random error, respectively. [32,33,42] The data obtained from the CCD were analyzed in terms of tungsten levels in the solutions using the Design Expert (version 7.0.0, STAT-EASE Inc., Minneapolis, MN, USA) software package, and all runs were analyzed in triplicate using the method of ICP-MS.

3.8. Statistical analysis

The data obtained from the two procedures for W analysis were evaluated via one-way ANOVA using STATISTICA for Windows (SAS/STATISTICA Version 6.0 SAS Institute Inc., Cary, NC, USA. 1984-199).

4. Conclusions

This study describes the utility of the fractionation approach with the application of an optimization strategy combined with ICP-MS detection for W analysis from a soil matrix. In this regard, RSM facilitates finding the optimum conditions for the digestion of soil samples in proper W leaching by assigning the individual and/or interactional effects of all selected factors instead of evaluating them separately, which is also time-saving. The total sample preparation was achieved within 45 min, and 1117.48, 1262.56, 1557.68, and 1315.61 mg kg^{-1} W levels were found by existing methodology. Additionally, 1428.16, 1531.24, 1820.60, 1953.21, and 1680.23 mg kg^{-1} W levels were found by the developed method. Two measured levels were good agreement at the 95% confidence level according to Student's t-test (t = 2.78). Thus, it was regarded as compatible and effective compared with the existing methodology. Finally, the optimized digestion method could be used as a fast and efficient alternative to the proposed methods, which take a long time. It may be useful for a large number of samples in routine analysis by avoiding complete digestion and/or tedious sample preparation steps. This approach may also extend the use of a minimal sample amount with detectable/quantifiable levels of an element for analysis. Furthermore, because the W content of soils could be an indicator of accumulation in the ecosystem, this study will provide good experimental foresight for many similarly difficult environmental matrices instead of total digestion and contribute to further research on phytoremediation and biogeochemical exploration studies in terms of its sample preparation step. It is projected that the proposed methodology may be used with many other elements such as uranium and molybdenum depending on their complexation capability with phosphoric acid for future leaching studies from soil matrices.

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