

## Determination of Selenium in Environmental Samples Using Hydride Generation Coupled to Atomic Absorption Spectroscopy

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### Abstract

A hydride generation coupled to atomic absorption spectroscopy HGAAS method is presented for the determination of Selenium content in soil samples, alfalfa, animal feed, and water samples. The selenium distribution was studied in various locations in Zawia city. The studied areas were divided to seven different zones. The samples were digested in a mixture of mineral acid (HNO<sub>3</sub>, HCL, HClO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>). The Selenium element in acidified sample solution was reduced directly by treating it with Sodium borohydride (NaBH<sub>4</sub>), the metal hydride was introduced to the atomizer of AAS by inert carrier gas (N<sub>2</sub>, He). The instrument used for the generation of hydride was home built in the analytical lab and a batch method was implemented. The analytical parameters were optimized throughout the analytical procedure to give typical sensitivity (0.0055 pg/g). The Atomic Absorbance profile was recorded for all measured samples; the obtained signal profiles show smooth peaks. The Absorption value at the peak height was used as a basis for the analytical calculations. A calibration curve of standard selenium concentrations against absorbance was plotted and used to determine the selenium concentration in each sample. Soil samples were analysed by (HGAAS) for determination of concentration of selenium results are reported for all the studied zones, some zones did not exceeded the reported critical toxicity values (1.0-5.0 pg/g), but another zones are relatively high (5.6-5.9 pg/g). The Selenium content in alfalfa from different zones are relatively low in the range of (0.7 to 2.20 pg/g). Concentrations of selenium in some animal feed samples are relatively low and showing deficiencies (0.62-0.71 pg/g), but other samples are relatively high and were close to the lower limits for high dose (1.41-1.98 ng/g).

**Keywords:** Selenium in environment; HGAAS; Batch method; Selenium in animal feed

### Introduction

Selenium (Se) is now firmly established that it is beneficial or essential in amounts from trace to part-per-billion concentrations for humans and some plants and animals, but toxic at some concentrations present in the environment [1]. This element may favorably or adversely affect growth and survival of plants, fish, birds, and mammals (including humans) [2]. Selenium occurs naturally in the environment. As an element, selenium cannot be created or destroyed, although selenium can change forms in the environment. Weathering of rocks and soils may result in low levels of selenium in water, which may be taken up by plants. Weathering also releases selenium into the air on fine dust-like particles. Volcanic eruptions may release selenium in air. Selenium commonly enters the air from burning coal or oil. Airborne particles of selenium, such as in ash, can settle on soil or surface water. Disposal of selenium in commercial products and waste could also increase the amount of selenium in soil. "The fate of selenium forms in soil depends largely on the acidity of the surroundings and its interaction with oxygen. Elemental selenium that cannot dissolve in water and other insoluble forms of selenium are less mobile and will usually remain in the soil, posing smaller risk of exposure. Selenium compounds that can dissolve in water are sometimes very mobile. Thus, there is an increased chance of exposure to these compounds. Selenium may enter surface water in irrigation drainage water [3].

### Materials and Methods

#### Chemicals

All chemical used were analytical grade and they were: Nitric acid (HNO<sub>3</sub>, 69% w/w) obtained from (BDH), concentrated Perchloric acid (HClO<sub>4</sub>) obtained from Fluka, Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30% w/w) was obtained from BDH, Hydrochloric acid (HCL, 37% w/w), obtained from BDH, Sodium borohydride (NaBH<sub>4</sub>, 1.5% m/v), sodium hydroxide (NaOH, 0.2% w/v), and sodium hydrogen selenite (NaHSeO<sub>3</sub>) were obtained from BDH. High purity water (Double distilled water, DDW)

used throughout this work was produced by double distillation.

#### Instrumentation

Double beam Atomic absorption spectrophotometer model 209 equipped with a high-intensity selenium hollow cathode lamp as the radiation source was used for this work. The operating conditions were; wavelength (196.0, 204 nm); lamp current (6 mA); slit width (1.0, 0.5 nm), and integration time (2.5 sec). Generation and Atomization of the hydride electrically heated a quartz tube cell. A reaction tube was placed on magnetic stirrer. Nitrogen and helium (N<sub>2</sub>-He) carrier gases were use.

#### Sampling

Random 27 samples (water, soil, alfalfa, and animal feed) were collected simultaneously (May-Jun-Jul 2007). Soil samples from different agricultural zone around Zawia city. Samples taken, using spade, at a depth of 0-20 cm, kept in a plastic bag and brought to the lab for analysis. Random samples of alfalfa were collected from the same area where soil samples were collected. The whole plants were taken, air-dried and stored in a plastic bag for analysis. A commercial animal feed supplied by local factory were sampled, a quantity of 1.0 Kg were collected and brought to the lab for analysis.

Water samples were collected from different zones. A clean glass

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bottle of (liter) was used for this procedure. Samples are stored and then analyzed.

### Treatment of samples

The soil samples were oven dried (at 100°C) for one hour, and grounded in a ceramic pestle and mortar to pass through a sieve (150 mm) then stored in sealable plastic bags with reference number [4]. The alfalfa samples were cleaned with double distilled water in order to remove the superficial dust particles then dried in an oven for 48 hours at about 30°C. The samples were chopped and grounded in a ceramic pestle and mortar to semi powder then stored in sealable plastic bags with a reference number [5].

### Soil digestion procedure

One gram of soil sample was weighed in 100 ml glass beaker covered with watch glass, then carefully introduced 12 ml of aqua regia solution (1/3 HNO<sub>3</sub> - HCl, v/v). The beaker was heated on hot plate and refluxed for 3 hours at 110°C. After evaporation to near dryness, the sample was diluted with 20 ml of 2% v/v nitric acid and transferred into a 100 ml volumetric flask after filtering through a Whatman filter paper (no.42) and diluted to the mark with double distilled water [6].

### Alfalfa and animal feed digestion procedure

10 ml of conc HNO<sub>3</sub> nitric acid and 5 ml of H<sub>2</sub>O<sub>2</sub> (30% w/v) were added to 1 gram of alfalfa and 2 gram of animal feed material in Teflon beaker and allowed to stand overnight at room temperature. Then, 3 ml conc. HClO<sub>4</sub> was added and the mixture was again maintained at room temperature, for at least 6 hours. Further digestion was conducted at 170°C for 3 hours. The digested solution was diluted with 20 ml of 0.1% (v/v) HNO<sub>3</sub> and transferred into a 50 ml volumetric flask and diluted to the mark with DOW [6].

### Sodium borohydride NaBH<sub>4</sub>

1.5% (m/v) NaBH<sub>4</sub> solution was prepared by dissolving in 0.2% (m/v) NaOH then diluted to 100 ml with DDW. This solution prepared daily.

### Preparation of a series of (0.2, 0.4, 0.6, 0.8) mg/l selenium

1000 mg/l stock Se solution (109118 gm NaHSeO<sub>3</sub> in 1.0 L DDW) was used to prepare a series of standard Se solutions (0.2, 0.4, 0.6, and 0.8 mg/l Se) and used for AA calibration data.

### Optimization of the operating conditions

The effect of Acid concentration was tested using a series of 0.5, 0.75, 1.00, 1.25 and 1.50 molar solution of HCl (the reducing agent) were prepared to be used in studying the effect of acid concentration on the absorption of Se. 10.0 ml of 0.4 mg/l Se solution was mixed with 2.0 ml of each acid solution in reaction flask then 1.0 ml NaBH<sub>4</sub> was injected. Graphical representation of the data shows that the optimum HCl concentration is 1.0 M (total Se is converted to Se<sup>IV</sup>). The influence of the carrier gas (He and N<sub>2</sub>) flow rate on the Se absorption signal was obtained by mixing of 1.0 ml of 0.4 mg/l Se solution with 2.0 ml 1.0M HCl in a reaction flask then 1.0 ml of 1.5% NaBH<sub>4</sub> solution was added and the absorption of the final solution was measured against gas flow rate change. Helium low flow rate did not change did not change absorbance and higher flow rate is required to completely remove Se rapidly (sharp peak and maximum intensity).

### HGAAS operating conditions

Operating conditions for HGAAS measurements were;

Wavelength=190.0 nm, Lamp current=6.0 mA, slit width=1.0 nm, integration time=2.5 sec. flow rate=750 ml/min, carrier gas=He and Se standard solutions where 0.2, 0.4, 0.6, and 0.8 mg/l. The standard curves were obtained by mixing 1.0 ml of each standard solution with 2.0 ml of 1.0 M HCl in a reaction flask then 1.0 ml NaBH<sub>4</sub> and the mixture is inject and absorbance is recorded and plotted against concentration.

## Results and Discussion

### Determination of selenium by HGAAS

**Soil sample:** Results of selenium in soil samples are presented in Table 1. Calibration curve for the determination of selenium using this method showed very good linearity for the used standards solution. For zone 1, selenium concentrations in Site 1 were in normal levels (4.1 to 4.5 µg/g), in Site 2 (5.9 to 6.2 µg/g) which is relatively high and in Site 3 (0.3 to 3.4 µg/g), a lower than the concentrations in site 1 (the lowest). In zone 2, selenium concentrations in Site 1 were relatively high (5.7 to 5.9 µg/g) while in Site 2 and Site 3 were low (2.5 to 3.0 µg/g). In zone 3, selenium concentrations in site 1 were high (5.1 to 6.2 µg/g) (the highest), in Site 2 (2.1 to 2.4 µg/g) were low (the lowest) and in Site 3 (3.9 to 4.0 µg/g) were in normal levels Selenium concentrations in each site of zone 4 were low and relatively constant (3.9 to 4.4 µg/g) in Site 1, (3.3 to 4.4 µg/g) in site 2 and in Site 3 (2.7 to 3.3 µg/g) (the lowest).

Selenium concentrations in zone 5, site 1 were in normal levels (4.4 to 4.5 µg/g), while the concentration in sites 2 and 3 were lower (2.9 to 3.9 µg/g), while in zone 6 the Selenium concentrations in site 1 were relatively high in range of 5.4 to 5.6 µg/g, in site 2 (3.4 to 3.7 µg/g) were low and in site 3 (1.4 to 1.5 µg/g) were lower than the concentrations in site 2 (the lowest). The data for Selenium concentrations in each site of zone 7 were low and relatively constant (2.7 to 3.0 µg/g) in site 1, (2.0 to 2.1 µg/g) in site 2 and in site 3 (1.7 to 1.9 µg/g) (the lowest).

Selenium mean concentrations in site 1 for zones (1, 4, 5 and 7) were relatively low and in normal range, while that in site 1 for zones (2, 3 and 6) were relatively high and were even close to the lower limits of toxicity range. Selenium mean concentrations in site 2 for zones (2, 3, 4, 5, 6 and 7) were low and in normal range, but the mean concentration in zone 1 (5.9 µg/g) which is relatively high. The mean Selenium concentrations in site 3 for all of the zones are relatively low and within the normal range. On the other hand, Selenium concentration in soil the reported critical toxicity values (5-10 mg Se Kg<sup>-1</sup>) but ranges in the normal levels (0.1-5 mg Se Kg<sup>-1</sup>).

From the tabulated data (Table 2), we can conclude that selenium mean concentrations in the studied soils sites for different zones are as follow.

In zone 1; site 2 >> site 1 > site 3.....Zone 2; site 1 > site 2 ~ site 3  
Zone 3; site 1 > site 3 > site 2.....Zone 4; site 1 > site 2 > site 3  
Zone 5; site 1 > site 2 ~ site 3.....Zone 6; site 1 »site 2 > site 1  
Zone 7; site 1 ~ site 2 > site 3

From the information obtained before, the mean concentration of selenium in some sampling sites from same zones are similar. Selenium levels in soils increases due to the larger numbers of factors, such as human and industrial activities. Products of coal or oil combustion that can settle on soil, commercial products and waste disposals, and the use of selenium pesticides in agriculture. Accumulation of pollutants in soil may lead to a high or low Se concentration. The mean concentration of selenium in zone 1, Site 2 is relatively high because of its being very near to the city waste dumping sites, as shown in the map. This is due

Zone	Site	Mean of conc. (µg/g)	SD	%RSD	Sensitivity	DL (mg/g)
1	Site 1	4.16	0.249	5.98	$5.09 \times 10^{-3}$	0.492
	Site 2	5.9	0.244	4.1	$5.12 \times 10^{-3}$	0.489
	Site 3	3.16	0.054	1.7	$5.29 \times 10^{-3}$	0.108
2	Site 1	5.8	0.081	1.407	$5.10 \times 10^{-3}$	0.163
	Site 2	2.55	0.05	1.96	$5.02 \times 10^{-3}$	0.1
	Site 3	2.85	0.15	5.263	$4.50 \times 10^{-3}$	0.3
3	Site 1	5.65	0.55	9.7	$5.12 \times 10^{-3}$	1.1
	Site 2	2.233	0.1247	2.089	$5.16 \times 10^{-3}$	0.249
	Site 3	3.766	0.2624	6.96	$5.17 \times 10^{-3}$	0.524
4	Site 1	4.15	0.25	6.024	$5.29 \times 10^{-3}$	0.5
	Site 2	3.16	0.1247	3.946	$5.16 \times 10^{-3}$	0.249
	Site 3	2.7	0.0816	3.024	$5.16 \times 10^{-3}$	0.163
5	Site 1	4.666	0.309	6.62	$5.09 \times 10^{-3}$	0.618
	Site 2	3.266	0.2624	6.32	$5.07 \times 10^{-3}$	0.524
	Site 3	3.966	0.1247	3.144	$5.13 \times 10^{-3}$	0.249
6	Site 1	5.4	0.1632	3.02	$5.12 \times 10^{-3}$	0.326
	Site 2	3.7	0.2449	6.62	$5.14 \times 10^{-3}$	0.489
	Site 3	1.45	0.05	3.44	$4.73 \times 10^{-3}$	0.1
7	Site 1	2.85	0.15	5.26	$5.11 \times 10^{-3}$	0.3
	Site 2	2	0.081	4.08	$5.17 \times 10^{-3}$	0.163
	Site 3	1.8	0.1	5.55	$5.10 \times 10^{-3}$	0.2

Table 1: The mean Concentration of Se, SD, % RSD, DL and sensitivity.

to the weathering condition responsible for the transfer of traces of this element to the surrounding sites.

The mean concentration of selenium in zone 3, site 1 is relatively high and were even close to the lower limits of toxicity range because the location of this site is very close to a commercial district (oil refining factory) and to a lot of human activation such as motor vehicle, small industries, etc. while the mean concentration of selenium is high in zone 6, site 1 which is located within an agricultural area where the soils are treated with fertilizers, but zones 4,7,5,2 which are in the rural areas with low human activities, the Se levels are within the normal range.

### Determination of selenium in Alfalfa

Alfalfa samples were collected from the cultivated zones. These samples were brought to the lab, dried, digested and their solutions analyzed for the determination of selenium. Results were presented in Table 2. The Table shows that the Selenium concentrations in alfalfa from different studied zones were relatively low and constant in the range of 0.7 to 2.2 µg/g. The concentrations included in the table above were reported as average Se content of alfalfa: (0.03- 0.88 mg Kg<sup>-1</sup>) [7], and were even close to the lower limits Selenium toxicity for consumed livestock which can only occur at concentrations as high as (3-4 mgKg<sup>-1</sup>) in material consumed as food [8]. From the data shown in Table 2, no general correlation between soil and alfalfa Se content. This is because of the larger numbers of factors that influences the availability of selenium from soil to alfalfa. From the tabulated data, we can conclude that the selenium mean concentrations in studied sites alfalfa for different zones follows the order; zone 6 > zone 7 » zone 4 > zone 1 — zone 2 > »zone 5 > zone 3. These results allow us to suggest that if the alfalfa cultivated in this region is the forage availability for the livestock [9].

### Determination of selenium in animal feed samples produced by local factories

The concentration of selenium were calculated from the calibration curve obtain for each run. Results are presented in Table 3. The ratio between the required and the toxic levels, shows a big safety factor as

it is pointed to that the critical level for dietary Selenium, below which deficiency symptoms are observed, is apparently about (0.02 ppm) for ruminants, and (0.03 to 0.05 ppm) for poultry.

This indicates that Se in animal feed is above the deficiency level and lower than the toxic level, but the levels are different from one producer to the other owing to the blending program they utilized. This signifies concentrations of selenium in samples 1 and 4 were relatively deficient, and sample 5 can be used as forage for ruminants but not be for poultry. Toxic effects on animals can occur when selenium concentration in animal feed reaches 2 to 5 mg/Kg range. The concentration of selenium in samples 2, 3 relatively high and were even close to the lower limits for high dose. The minimum lethal dose of selenium administered orally (mg/Kg) ranged from 1.2 to 2 mg Se/Kg body weight; given subcutaneously killed swine in 4 hours and 5 days, (1.5-6 mg Se /Kg body) intravenously to rats and rabbits were fatal, and cattle fed 0.5 mg Se/Kg body weight 3 times weekly lost their appetite.

### Determination of selenium in water

The concentrations of selenium were calculated from the calibration curve obtain for each run was shown in Table 4.

Selenium concentrations in water from different zones are relatively low in the range of (0.001 to 0.005 ppin) for water from all zones. Under natural conditions, the concentration of selenium in water is usually range from few tenths to (2 or 3 pg/l).

### Quality control

Determination of trace elements in environmental samples requires strict quality control of the analysis. The application of quality assurance requires the analysis of certified reference materials (CRM) that match as closely as possible the matrix type and the element concentration level of the real samples. In this case the accuracy of the analytical methodologies applied tor soils were assessed analyzing different (CRM) as shown in in Table 5.

### Data quality control assessment

Zones	Mean of con. (pg/g)	SD	%RSD	Sensitivity	DL
Zone 1	1.05	0.0408	3.88	$4.62 \times 10^{-3}$	0.0816
Zone 2	1.046	0.0776	7.41	$5.13 \times 10^{-3}$	0.155
Zone 3	0.65	0.0408	6.28	$5.12 \times 10^{-3}$	0.0816
Zone 4	1.066	0.0623	5.84	$5.13 \times 10^{-3}$	0.1247
Zone 5	0.85	0.0816	9.6	$5.08 \times 10^{-3}$	0.1632
Zone 6	2.066	0.1433	6.93	$5.12 \times 10^{-3}$	0.2867
Zone 7	1.866	0.0623	3.34	$5.10 \times 10^{-3}$	0.1247

Table 2: The mean of concentration of Se, SD, % RSD, DL, and sensitivity in studied zones.

Samples	Mean of con. (pg/g)	SD	% RSD	Sensitivity	DL
Sample(1)	0.71	0.0513	7.17	$5.41 \times 10^{-3}$	0.102
Sample(2)	1.41	0.0625	4.42	$6.12 \times 10^{-3}$	0.125
Sample(3)	1.98	0.1190	6.03	$6.12 \times 10^{-3}$	0.239
Sample(4)	0.62	0.0250	4.76	$6.10 \times 10^{-3}$	0.050
Sample(5)	1.19	0.1040	8.79	$6.12 \times 10^{-3}$	0.200

Table 3: The concentration of Se (pg/g), SD, % USD, DL and sensitivity in different animal feed samples.

Zones	Mean of con. ppm
Zone (1)	<0.005
zone (2)	<0.005
Zone (3)	<0.005
Zone (4)	<0.005
Zone(5)	<0.005

Table 4: The concentration of Se (ppm) in Water from different Zones.

The first step in data quality control is the rejection of the suspected data if any exist, then for the retained data "good data" we calculated the mean, then percentage relative standard deviation for further data assessment and reliability assurance.

### Precision

Decision is the reproducibility of multiple measurements and is usually described by the standard deviation, standard error. The reliability of data was justified by the statistical treatment, which showed in general acceptable values of RSD% as measure of precision as shown in Table 6. The precision verification was also checked by duplicating some sample, at the same conditions, which gave equivalent results. The

Reference Material	Element	Certified value	Experimental value
Stream sediment GBW07306	Se(pg/g)	0.3	0.425

Table 5: The results of obtained for the reference material which analysed.

results are in Tables 7 and 8.

### Analysis of spiked sample

Two grams of animal feed samples were spiked by adding 0.2 ppm content of Se, then the sample was digested using the same procedure.

### Conclusion

Although the results reported here should be considered practical and limited to the zone studied and they contribute to the scientific knowledge and data available in Zawia city about essential selenium element, present in the different components of agriculture ecosystem, information that is not reasonably well established in our Zones before this study.

In this work, Hydride generation-atomic absorption spectroscopy technique (HGAAS) was used for determination of low levels of selenium in environmental samples, the advantages of this technique are more sensitive for detection of selenium than classical technique (FAAS), separating selenium from the matrix, volume of samples are less, thus offering more potential for obtaining low detection limit, with good sensitivity when simple instrumentations are applied.

The results obtained by this technique can be determined, the concentration of selenium in soil samples when accurate sampling in the studied zones. Selenium concentrations in some zones did not exceeded the reported critical toxicity values (5-10 mg SeKg) and

Samples	SD	%RSD	Sensitivity	DL(pg/g)
Soil	0.05-0.24	1.40-4.08	$(5.09-5.29) \times 10^{-3}$	0.10 - 0.24
Alfalfa	0.04-0.62	3.88 - 5.84	$(4.62-5.13) \times 10^{-3}$	0.08 - 0.12
Animal feed	0.025-0.05	4.42 - 6.03	$(5.41-6.10) \times 10^{-3}$	0.05-0.10

**Table 6:** The typical values of {SD, % RSD, DL, and sensitivity} for selenium in studied samples

Type	Sample	Mean of con.(pg/g)	SO	RSD%	sensitivity	DL
Soil	Zone 4 Site 3	2.712	0.081	3.024	$5.16 \times 10^{-3}$	0.160
Alfalfa	Zone 7	1.866	0.0623	3.340	$5.10 \times 10^{-3}$	0.124
Animal feed	Sample2	1.410	0.0625	4.420	$6.12 \times 10^{-3}$	0.125

**Table 7:** The results of typical duplicated measurements.

Method of digestion	Con. (pg/ml)	Recovery
(Sample+HNO <sub>3</sub> +H <sub>2</sub> O <sub>2</sub> + HClO <sub>4</sub> )	0.049	
(Sample +HNO <sub>3</sub> +H <sub>2</sub> O <sub>2</sub> +HClO <sub>4</sub> )+0.2ppm	0.19	76.3%
(Sample KHNO <sub>3</sub> +H <sub>2</sub> O <sub>2</sub> +HClO <sub>4</sub> )	0.042	
(Sample+TINCh+fTCA+HClO <sub>4</sub> +	0.19	78.5%
(Sample+HNO <sub>3</sub> +H <sub>2</sub> O <sub>2</sub> +HClO <sub>4</sub> )	0.047	
(Sample+ITNO <sub>3</sub> +H <sub>2</sub> O <sub>2</sub> +HClO <sub>4</sub> )+0.2ppm	0.2	80.9%

**Table 8:** The result of recovery test of selenium in animal feed sample.

there are at normal ranges. Some results obtained are below value, but another zone is relatively high.

Selenium concentrations in alfalfa from different zones are relatively low and con (0.7 to 2.2 pg/g) for alfalfa from all zones. These results permit to suggest that if the alfalfa cultivated in this region is the forage availability for the livestock.

Selenium toxicities and deficiencies have been known to cause endemic diseases in animals in many parts of the world. The occurrence depends, among other factors, on the availability of the selenium to plants and animals. Concentrations of selenium in some animal feed. Samples are relatively low and show deficiencies, but some animal feed stock are relatively high the toxic level and were close to the lower limit for high dose.

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