Determination of Cd, Pb and Ni in Syrian Cow Milk by Graphite Furnace Atomic Absorption Spectrometry

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Graphite furnace atomic absorption spectrometry method was elaborated and applied for determining Cd, Pb and Ni in Syrian cow milk samples using the transversally heated graphite atomizer with integrated graphite platforms. The effects of several chemical modifiers, such as $\text{NH}_4\text{H}_2\text{PO}_4$, $\text{Mg}(\text{NO}_3)_2$, Zr, W, ascorbic acid and mixture of $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr were studied to obtain optimal pyrolysis and atomization conditions for the studied analytes. The most efficient modifier was proved to be the mixture of $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr, which gave the optimal pyrolysis and atomization temperatures: 900 and 1800 °C, 1000 and 1900 °C, 1500 and 2500 °C respectively for Cd, Pb and Ni. The characteristic masses (m_0) were 1.29 pg Cd, 25.20 pg Pb and 33.13 pg Ni. Limits of detection (LOD, 3s) were 0.044 $\mu\text{g L}^{-1}$ Cd, 0.94 $\mu\text{g L}^{-1}$ Pb and 1.32 $\mu\text{g L}^{-1}$ Ni. Limits of quantification (LOQ, 10s) were 0.146 $\mu\text{g L}^{-1}$ Cd, 3.133 $\mu\text{g L}^{-1}$ Pb and 4.4 $\mu\text{g L}^{-1}$ Ni. The cow milk mean contents were to be 3.78 $\mu\text{g kg}^{-1}$ for Cd, 17.59 $\mu\text{g kg}^{-1}$ for Pb and 0.45 mg kg^{-1} for Ni.

Key Words: Cow milk, Cadmium, Lead, Nickel, Graphite furnace atomic absorption spectrometry, Chemical modifiers.

INTRODUCTION

Milk is an ideal food essential for newborns due to its composition and availability. In addition to its macronutrient, *i.e.* protide, glucide and lipids, milk also contains micronutrients, *i.e.* vitamins (A, D and B groups) and elements that are absolutely essential during the first months of a baby's life since it is the only source of nutrients¹. Milk contains several elements, which are essential or toxic such as cadmium, lead and nickel, due to these elements presence in the biosphere in general². Quantitation of essential and toxic trace elements in milk is an important analytical task, both for human health safety reasons and environmental bio-monitoring purposes. This requires elaboration and application of analytical methods of high sensitivity, selectivity and robustness. But the determination of trace inorganic constituents in milk is not a trivial task because of the complexity of the emulsion. Cow milk contains around 3.4 % of proteins, 2.8 % of casein, 3.7 % of fat and 4.6 % of lactose; additionally, the elements are present as different compounds that can affect both the sample preparation and the measurement strategies³. Direct procedures for milk analysis were proposed using different spectro-analytical techniques. Each one presents some difficulties where Quinaia and Nobrega⁴, demonstrated that the presence of fat compounds affects the performance of the auto-sampler in graphite furnace

atomic absorption spectrometry (GFAAS) and the pneumatic nebulization in the inductively coupled plasmas-optical emission spectrometry (ICP-OES)⁵ or inductively coupled plasmas-mass spectrometry (ICP-MS)⁶. The formation of carbon residues causes a gradual deterioration of the graphite tube in GFAAS, affects excitation conditions in ICP-OES and isobaric interferences in ICP-MS. In GFAAS milk samples were diluted with a solution containing ethanol, nitric acid and hydrogen peroxide to remove the organic matter during the heating cycle⁷. This procedure was successfully applied for the determination of Al, Cr, Mn and Mo. Classical wet digestion⁸ and microwave digestion⁹, procedures for milk analysis were proposed using different chemicals for destroying the organic matrix of milk and left the element into a clear solution. Determination of Cd, Pb and Ni by GFAAS has already been found in the literatures with several modifiers¹⁰⁻¹⁵, like $\text{Mg}(\text{NO}_3)_2$, $\text{Pd}(\text{NO}_3)_2$ and $(\text{NH}_4)_2\text{HPO}_4$ ¹⁰ alone or mixtures $(\text{NH}_4)_2\text{HPO}_4$ and $\text{Mg}(\text{NO}_3)_2$ ¹¹, $\text{NH}_4\text{H}_2\text{PO}_4$ and $\text{Mg}(\text{NO}_3)_2$ ¹², $\text{NH}_4\text{H}_2\text{PO}_4$ and $\text{Mg}(\text{NO}_3)_2$ plus Pd^{13} for cadmium, it was also used $\text{Mg}(\text{NO}_3)_2$, $\text{Pd}(\text{NO}_3)_2$ and $(\text{NH}_4)_2\text{HPO}_4$ ¹⁰, EDTA, Pd and NH_4NO_3 ¹⁴, in addition to mixtures such as $(\text{NH}_4)_2\text{HPO}_4$ and $\text{Mg}(\text{NO}_3)_2$ ¹¹, $\text{NH}_4\text{H}_2\text{PO}_4$ and $\text{Mg}(\text{NO}_3)_2$ ¹², $\text{NH}_4\text{H}_2\text{PO}_4$ and $\text{Mg}(\text{NO}_3)_2$ and Pd^{13} , NH_4NO_3 and Pd^{14} for lead and it was also used $\text{Mg}(\text{NO}_3)_2$, $\text{Pd}(\text{NO}_3)_2$ and $(\text{NH}_4)_2\text{HPO}_4$ ¹⁰ individually, in addition to mixtures such as $(\text{NH}_4)_2\text{HPO}_4$ and $\text{Mg}(\text{NO}_3)_2$ ¹¹, Pd

and $\text{Mg}(\text{NO}_3)_2$ ¹⁵ for nickel, with variable optimal conditions. So, we decided to determine the best modifier among five modifiers $\text{NH}_4\text{H}_2\text{PO}_4$, $\text{Mg}(\text{NO}_3)_2$, Zr, W and ascorbic acid (AA), which were chosen depending on its use as a modifier in review and availability in our laboratory, for determining of Cd, Pb and Ni in Syrian cow milk samples.

EXPERIMENTAL

The determination of Cd, Pb and Ni in Syrian cow milk was performed with a Biotech (Phoenix-986) atomic absorption spectrometer, equipped with Self- Reversal (SR) background corrector, pyrolytically coated transverse heated graphite atomizer (THGA), Argon 99.998 % was used as a purge gas. The instrumental conditions adopted for the spectrometer are presented in Table-1.

TABLE-1
INSTRUMENTAL CONDITIONS FOR THE GFAAS

Parameters	Cd	Pb	Ni
Wavelength (nm)	228.8	283.3	232.0
Lamp current (mA)	2.0	2.0	4.0
Slit (nm)	0.2	0.4	0.2
Background corrector	SR	SR	SR
Negative high voltage (V)	377.7	433.5	448.5
Broad pulse current (mA)	1.3	1.1	1.5
Narrow pulse current (mA)	3.0	2.5	3.5

Sample digestion was carried out by using a closed-vessel microwave system, model Ethos D (Milestone, Sorisole, Italy), equipped with 10 TFM[®] vessels of 100 mL and a ceramic vessel jacket.

High purity deionized water obtained from a Milli-Q water purification system was used throughout. Analytical reagent grade HNO_3 65 % ww^{-1} and H_2O_2 30 % ww^{-1} (Merck, Germany) were used for sample digestion. The analytical reference solutions were prepared by successive dilution of 1000 mg L^{-1} of Cd, Pb and Ni standard solutions (Merck, Germany) in 0.2 % HNO_3 and stored at 4 °C in dark glass bottles.

The modifiers stock solution were obtained by weighting an appropriate mass of high purity $\text{NH}_4\text{H}_2\text{PO}_4$, $\text{Mg}(\text{NO}_3)_2$, ZrCl_4 , Na_2WO_4 and ascorbic acid (Merck, Germany), to have a concentration of 1000 mg L^{-1} , then preparing the work solutions by an appropriate dilution for each. All solutions were stored at 4 °C in dark glass bottles.

RESULTS AND DISCUSSION

Pyrolysis and atomization temperature curves: Graphite furnace atomic absorption spectrometry GFAAS is an efficient technique for determining trace elements such as Cd, Pb and Ni. The recommended THGA conditions for the precedent determined elements revealed some differences in the electro-thermal behaviours and chemical modifiers¹⁰⁻¹⁵. Chemical modifiers can alter the vapourization and atomization behaviour of the matrix and analytes, thus they can be used for removing the matrix constituents which may cause interference during measurements and for the thermal stabilization of the analytes. By this importance, standard solutions containing 3 $\mu\text{g L}^{-1}$ Cd, 50 $\mu\text{g L}^{-1}$ Pb and 50 $\mu\text{g L}^{-1}$ Ni in 0.2 % HNO_3 , in absence and presence of 10 μg of several chemical

modifiers, such as $\text{NH}_4\text{H}_2\text{PO}_4$, $\text{Mg}(\text{NO}_3)_2$, Zr, W, ascorbic acid were studied to reach to the best heating programs and analytical conditions for determining Cd, Pb and Ni, depending on their pyrolysis and atomization temperature curves as illustrated in Figs. 1-3, which showed the best modifier for Cd was $\text{NH}_4\text{H}_2\text{PO}_4$ and the best ones for Pb and Ni were $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr. Table-2 present the optimized conditions for the pyrolysis and atomization temperatures of Cd, Pb and Ni in the absence and presence of 10 μg $\text{NH}_4\text{H}_2\text{PO}_4$, $\text{Mg}(\text{NO}_3)_2$, Zr, W and ascorbic acid (AA) as modifiers.

Fig. 1. Modifiers effect on the pyrolysis temperature (a) and atomization temperature (b), for cadmium concentration in aqueous standard solutions 3 $\mu\text{g L}^{-1}$, added modifiers; $\text{NH}_4\text{H}_2\text{PO}_4$ (◆), Zr (■), W (▲), Mg (×), A.A (*), no modifier (●)

Fig. 2. Modifiers effect on the pyrolysis temperature (a) and atomization temperature (b), for lead concentration in aqueous standard solutions $50 \mu\text{g L}^{-1}$, added modifiers; Zr (◆), W (■), $\text{NH}_4\text{H}_2\text{PO}_4$ (▲), Mg (×), A.A (*), no modifier (●)

Fig. 3. Modifiers effect on the pyrolysis temperature (a) and atomization temperature (b) for nickel concentration in aqueous standard solutions $50 \mu\text{g L}^{-1}$, added modifiers; Zr (◆), $\text{NH}_4\text{H}_2\text{PO}_4$ (■), W (▲), Mg (×), A.A (*), no modifier (●)

Concentration effects of $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr modifier:

It is noted (Table-2) that the best modifiers for Cd, Pb and Ni were $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr at constant concentration $10 \mu\text{g}$ to each one. Papers gave variable concentrations for each modifier, for that it is decided to determine the best concentration of the mixture $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr modifiers depending on absorption and pyrolysis temperatures.

TABLE-2
OPTIMIZED PYROLYSIS AND ATOMIZATION TEMPERATURES OF Cd, Pb AND NI IN THE ABSENCE AND PRESENCE OF $\text{NH}_4\text{H}_2\text{PO}_4$, $\text{Mg}(\text{NO}_3)_2$, Zr, W AND ASCORBIC ACID AS MODIFIERS

Element	Modifier	Pyrolysis temperature (°C)	Atomization temperature (°C)
Cd	$\text{NH}_4\text{H}_2\text{PO}_4$	750	1700
	Zr	650	1700
	W	650	1600
	Mg	600	1500
	Ascorbic acid	550	1400
	-	500	1400
Pb	$\text{NH}_4\text{H}_2\text{PO}_4$	750	1900
	Zr	750	1800
	W	650	1700
	Mg	550	1900
	Ascorbic acid	550	1700
	-	450	1700
Ni	$\text{NH}_4\text{H}_2\text{PO}_4$	1300	2400
	Zr	1300	2400
	W	1200	2500
	Mg	1150	2300
	Ascorbic acid	1100	2400
	-	1100	2300

For $\text{NH}_4\text{H}_2\text{PO}_4$: different concentrations from $10 \mu\text{g}$ to $200 \mu\text{g}$ were used as a modifier in aqueous standard solutions, containing for each element a constant concentration as follows: $3 \mu\text{g L}^{-1}$ Cd, $50 \mu\text{g L}^{-1}$ Pb and $50 \mu\text{g L}^{-1}$ Ni. The results showed that the best concentrations were at the range $10\text{-}50 \mu\text{g}$ for Cd, $25\text{-}50 \mu\text{g}$ for Pb and $25\text{-}100 \mu\text{g}$ for Ni (Fig. 4).

The same study was done for Zr modifier at different concentrations from $0.2 \mu\text{g}$ to $15 \mu\text{g}$ in aqueous standard solutions containing $3 \mu\text{g L}^{-1}$ Cd, $50 \mu\text{g L}^{-1}$ Pb and $50 \mu\text{g L}^{-1}$ Ni. The results showed that the best concentration was at the range $1\text{-}3 \mu\text{g}$ for Cd, $1\text{-}15 \mu\text{g}$ for Pb and Ni (Fig. 5).

Fig. 4. Effects of concentration $\text{NH}_4\text{H}_2\text{PO}_4$ modifier on absorption (◆) and pyrolysis temperatures (■), for Cd (a), Pb (b) and Ni (c)

Fig. 5. Effects of concentration Zr modifier on absorption (◆) and pyrolysis temperatures (■), for Cd (a), Pb (b) and Ni (c)

After determining the best concentration of $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr modifiers for determination of Cd, Pb and Ni in aqueous solutions. It is decided to use a mixture containing $25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$ plus $1 \mu\text{g Zr}$ as a modifier for determining above elements.

The mixture showed much better results than using each one individually, depending on thermal stabilization of studied elements and atomic absorption (Table-3).

TABLE-3
OPTIMIZED PYROLYSIS AND ATOMIZATION TEMPERATURES FOR Cd, Pb AND Ni IN THE ABSENCE AND PRESENCE OF $25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$ AND $1 \mu\text{g Zr}$, INDIVIDUALLY AND MIXTURE OF $25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$ AND $1 \mu\text{g Zr}$, AS MODIFIERS

Element	Modifier	Pyrolysis temp. (°C)	Atomization temp. (°C)
Cd	-	500	1400
	$25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$	750	1700
	$1 \mu\text{g Zr}$	650	1700
	$25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$ and $1 \mu\text{g Zr}$	900	1800
Pb	-	450	1700
	$25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$	750	1900
	$1 \mu\text{g Zr}$	750	1800
	$25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$ and $1 \mu\text{g Zr}$	1000	1900
Ni	-	1100	2300
	$25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$	1300	2400
	$1 \mu\text{g Zr}$	1300	2400
	$25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$ and $1 \mu\text{g Zr}$	1500	2500

By conclusion, the results presented in Table-3 proves that the using mixture of $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr as modifiers are better than using each one individually. So, we proposed a mixture containing $25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$ and $1 \mu\text{g Zr}$ as modifiers, for determining Cd, Pb and Ni and optimized heating program for precedent elements as demonstrated in Table-4.

TABLE-4
OPTIMAL GRAPHITE FURNACE TEMPERATURE PROGRAM FOR DETERMINING Cd, Pb AND Ni IN PRESENCE OF A MIXTURE $25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$ AND $1 \mu\text{g Zr}$ AS MODIFIER

Step	Temperature (°C)	Ramp time, (°C s ⁻¹)	Hold time (s)	Argon flow rate (mL min ⁻¹)
Drying 1	75	4	5	250
Drying 2	85	3	5	250
Drying 3	95	3	3	250
Drying 4	120	3	8	250
Pyrolysis	900^a 1000^b 1500^c	10	10	250
Atomization	1800^a 1900^b 2500^c	0	5	0
Cleaning	2000^a 2200^b 2600^c	0	1	250

^acadmium, ^blead, ^cnickel

Validation: Limits of detection (LOD), limit of quantification (LOQ) were calculated, by considering the variability of 10 consecutive measurements of blank solution, according to $\text{LOD} = 3 S_b/a$ and $\text{LOQ} = 10 S_b/a$ (S_b = standard deviation of the blank and a = calibration curve slope), characteristic masses (m_0) from the calibration curves were based on the integrated absorbance, linear range and linearity (R^2), were determined, All results were measured in absence and presence of the best modifiers, such as $25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$ and $1 \mu\text{g Zr}$ individually and a mixture of $25 \mu\text{g NH}_4\text{H}_2\text{PO}_4$ plus $1 \mu\text{g Zr}$ (Table-5). The analytical calibration curves of Cd, Pb and Ni are given in Fig. 6.

The results presented in Fig. 6 and Table-5, showed that the best analytical results for the three elements Cd, Pb and Ni

TABLE-5
ANALYTICAL CHARACTERISTICS FOR Cd, Pb AND Ni WITHOUT AND WITH DEFERENT MODIFIERS

Element	Modifier	Linear rang ($\mu\text{g L}^{-1}$)	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)	m_0 pg	R^2
Cd	$\text{NH}_4\text{H}_2\text{PO}_4$ and Zr	0.25 - 5	0.044	0.148	1.29	0.9836
	$\text{NH}_4\text{H}_2\text{PO}_4$	0.25 - 5	0.051	0.171	1.35	0.9879
	Zr	0.25 - 5	0.052	0.176	1.38	0.9884
	-	1 - 3	0.061	0.205	1.58	0.9840
Pb	$\text{NH}_4\text{H}_2\text{PO}_4$ and Zr	1 - 80	0.942	3.142	25.20	0.9961
	$\text{NH}_4\text{H}_2\text{PO}_4$	5 - 80	1.161	3.870	28.14	0.9948
	Zr	2 - 80	0.970	3.235	25.91	0.9964
	-	10 - 80	1.624	5.416	37.12	0.9955
Ni	$\text{NH}_4\text{H}_2\text{PO}_4$ and Zr	15 - 100	1.320	4.400	33.13	0.9974
	$\text{NH}_4\text{H}_2\text{PO}_4$	15 - 100	1.374	4.583	34.76	0.9973
	Zr	25 - 100	1.571	5.238	36.94	0.9986
	-	25 - 100	2.842	9.473	42.18	0.9992

Fig. 6. Analytical calibration curves of Cd (a), Pb (b) and Ni (c), in aqueous standard solutions; Zr ($\blacktriangle$), $\text{NH}_4\text{H}_2\text{PO}_4$ ($\blacksquare$), $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr ($\blacklozenge$), no modifier ($\times$)

was the using of a mixture modifiers $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr, therefore this mixture modifiers were adopted in order to determine Cd, Pb and Ni in Syrian fresh cow milk samples. Cd, Pb and Ni recovery with $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr as modifiers were studied.

About 1 g fresh cow milk sample purchased from local super market (Azaz village) was put in microwave vessel and added an oxidant mixture 8 mL HNO_3 + 2 mL H_2O_2 , without and with three different added concentrations of Cd, Pb and Ni (Table-6), then digestion performed according to a microwave heating program (Table-7). After digestion, samples were transferred to beaker capacity of 50 mL and then vapourized on electric heater, at relatively low temperature, to near drought, then transferred to the 10 mL flask and added 250 μL $\text{NH}_4\text{H}_2\text{PO}_4$ modifier 1 mg L^{-1} and 100 μL of Zr modifier 0.1 mg L^{-1} to each samples then completed up to 10 mL with deionized water. After that applied optimized conditions and measurements were made by injection of 20 μL from the sample inside the graphite furnace. The results obtained are presented in Table-6.

TABLE-6
RECOVERY OF SPIKED MILK SAMPLE WITH CADMIUM, LEAD AND NICKEL ANALYZED BY GFAAS WITH $\text{NH}_4\text{H}_2\text{PO}_4$ PLUS Zr AS MODIFIER (N = 3)

Element	Add	Determined	Recovery (%)
Cd	0 $\mu\text{g kg}^{-1}$	$2.75 \pm 0.16 \mu\text{g kg}^{-1}$	-
	1 $\mu\text{g kg}^{-1}$	$3.79 \pm 0.15 \mu\text{g kg}^{-1}$	101
	2 $\mu\text{g kg}^{-1}$	$4.81 \pm 0.19 \mu\text{g kg}^{-1}$	101
	4 $\mu\text{g kg}^{-1}$	$6.89 \pm 0.21 \mu\text{g kg}^{-1}$	102
Pb	0 $\mu\text{g kg}^{-1}$	$15.87 \pm 0.90 \mu\text{g kg}^{-1}$	-
	5 $\mu\text{g kg}^{-1}$	$21.12 \pm 1.31 \mu\text{g kg}^{-1}$	101
	10 $\mu\text{g kg}^{-1}$	$26.77 \pm 1.35 \mu\text{g kg}^{-1}$	103
	20 $\mu\text{g kg}^{-1}$	$36.40 \pm 1.47 \mu\text{g kg}^{-1}$	101
Ni	0 mg kg^{-1}	$0.22 \pm 0.02 \text{mg kg}^{-1}$	-
	0.1 mg kg^{-1}	$0.31 \pm 0.02 \text{mg kg}^{-1}$	96
	0.2 mg kg^{-1}	$0.41 \pm 0.03 \text{mg kg}^{-1}$	97
	0.4 mg kg^{-1}	$0.59 \pm 0.03 \text{mg kg}^{-1}$	95

From Tables 5 and 6, it was observed that the optimum analytical method for determining Cd, Pb and Ni was in presence a mixture modifiers of $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr. The obtained results have a good accuracy, sensitivity and recovery.

Determination of cadmium, lead and nickel in some Syrian fresh cow milk: A microwave-assisted wet decomposition

of 15 fresh cow milk samples, which purchased from local supermarkets in four villages (Azaz, Afrin, Zorba and Mansoura) was performed according to a microwave heating program (Table-7). And followed by the procedures which stated in the top of the vapourization and the addition of the mixture modifier from $\text{NH}_4\text{H}_2\text{PO}_4$ plus Zr and then measurements by injection of 20 μL from the sample inside the graphite furnace. The results obtained are presented in Table-8.

TABLE-7
MICROWAVE HEATING PROGRAM FOR SAMPLE DIGESTION

Step	Time (min)	Power (W)	T (°C)
1	1	250	180
2	1	0	180
3	6	250	200
4	5	400	210
5	5	650	220
6	Vent: 5 min		

TABLE-8
ELEMENTS CONCENTRATIONS IN SOME
FRESH SYRIAN COW'S MILK

Village	No.	Cd ($\mu\text{g kg}^{-1}$ $\pm$ SD)	Pb ($\mu\text{g kg}^{-1}$ $\pm$ SD)	Ni (mg kg^{-1} $\pm$ SD)
Azaz	1	2.73 $\pm$ 0.12	15.53 $\pm$ 0.90	0.21 $\pm$ 0.02
	2	3.33 $\pm$ 0.05	15.40 $\pm$ 0.55	0.37 $\pm$ 0.02
	3	2.81 $\pm$ 0.07	14.76 $\pm$ 0.73	0.26 $\pm$ 0.01
	4	3.10 $\pm$ 0.10	16.60 $\pm$ 0.50	0.30 $\pm$ 0.02
Afrin	1	3.03 $\pm$ 0.11	11.36 $\pm$ 1.06	0.33 $\pm$ 0.01
	2	2.55 $\pm$ 0.13	12.38 $\pm$ 0.77	0.36 $\pm$ 0.03
	3	3.27 $\pm$ 0.06	13.52 $\pm$ 1.02	0.24 $\pm$ 0.02
Zorba	1	4.40 $\pm$ 0.05	20.04 $\pm$ 0.56	0.69 $\pm$ 0.05
	2	5.41 $\pm$ 0.07	26.86 $\pm$ 1.20	0.72 $\pm$ 0.05
	3	5.46 $\pm$ 0.08	18.73 $\pm$ 0.38	0.86 $\pm$ 0.05
	4	4.74 $\pm$ 0.11	19.56 $\pm$ 0.68	0.65 $\pm$ 0.04
Mansoura	1	3.53 $\pm$ 0.11	24.43 $\pm$ 0.76	0.40 $\pm$ 0.02
	2	4.56 $\pm$ 0.03	18.54 $\pm$ 0.78	0.54 $\pm$ 0.03
	3	4.01 $\pm$ 0.36	16.74 $\pm$ 2.12	0.35 $\pm$ 0.02
	4	3.91 $\pm$ 0.06	19.44 $\pm$ 0.98	0.49 $\pm$ 0.03
Mean		3.78	17.59	0.45

The lowest concentration of Cd was found as 2.55 $\mu\text{g kg}^{-1}$ for Afrin (sample no. 2) and the highest one 5.46 $\mu\text{g kg}^{-1}$ for Zorba (sample no. 3), with average 3.78 $\mu\text{g kg}^{-1}$ and relative standard deviations less than 9.1 %. The lowest concentration of Pb was found as 11.36 $\mu\text{g kg}^{-1}$ for Afrin (sample no. 1) and the highest 26.86 $\mu\text{g kg}^{-1}$ for Zorba (sample no. 2), with average 17.59 $\mu\text{g kg}^{-1}$ and relative standard deviations less than 12.7 %. The lowest content of Ni was found as 0.21 mg kg^{-1} for Azaz (sample no. 1) and the highest 0.86 mg kg^{-1} for Zorba (sample no. 2), with average 0.45 $\mu\text{g kg}^{-1}$ and relative standard deviations less than 10.6 %.

From the results shown in Table-8, the minimum concentration of the most elements was in the village Afrin, which is a touristic area and a little industrial pollution. The highest

concentrations of all elements were in the village Zorba, which is an industrial area (dye house, paper, detergent factories *etc.* in addition to be beside high way.

The average concentrations of elements cadmium, lead and nickel, in analyzed samples were found to be under permissible limits compared with world health organization¹⁶.

Conclusion

In the present work, the effects of several chemical modifiers, such as $\text{NH}_4\text{H}_2\text{PO}_4$, $\text{Mg}(\text{NO}_3)_2$, Zr, W and ascorbic acid were studied to obtain optimal pyrolysis and atomization conditions for three elements cadmium, lead and nickel. It was found that $\text{NH}_4\text{H}_2\text{PO}_4$ modifier is the best for Cd and Zr or $\text{NH}_4\text{H}_2\text{PO}_4$ is the best for Pb and Ni. The mixture $\text{NH}_4\text{H}_2\text{PO}_4$ and Zr were the best of all at the optimized modifiers concentrations in relation to atomic absorbance values and pyrolysis temperatures. Individually $\text{NH}_4\text{H}_2\text{PO}_4$ modifier concentration was from 10 to 50 μg for Cd, 25 to 50 μg for Pb and 25 to 100 μg for Ni and Zr modifier concentration was from 1 to 3 μg for Cd and 1 to 15 μg for Pb and Ni, where the mixture modifier concentrations 25 μg $\text{NH}_4\text{H}_2\text{PO}_4$ and 1 μg Zr gave much better results compared with each one individually. Validation of the proposed method was good and suitable, for that it was applied to determine a cadmium, lead and nickel in Syria fresh cow milk samples and to estimate the risk of contamination with these toxic heavy metals, compared with the obtained results, in published data and world health organization.

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