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Evaluation and Comparison between a Conventional Acid Digestion Method and a Microwave Digestion System for Heavy Metals Determination in *Mentha* Samples by ICP-MS

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Abstract

Mentha is the most common plant species grown in Saudi Arabia's Madinah city. Locally, the two popular types of mint are commercially called (Hasawi or Habaq) and (Mograbi). Both species are used in herbal teas, alone or as spice mixtures for many foods to offer aroma and flavour. In this research Hasawi mint samples were collected from three different locations and digested by two methods (acid digestion method and a microwave digestion system). Then, they were analysed for their metal contents by ICP-MS. The results from both digestion methods revealed that the following 10 metals were presented: V, Cr, Mn, Co, Ni, Cu, Zn, As, Cd and Pb. The microwave digestion system was the best for all studied heavy metals in term of sample preparation procedures, linearity (≥ 0.999), recovery (97%- 101%, n=2) and precision (R.S.D% ≈ 1-2 %, n=2). Mint samples from first location contained 7 metals within the permissible limit while the remaining three metals (Cr, Cu and Zn) had higher levels. The second location contained 9 metals within the permissible limit but only one metal (Cu) had a higher level. The third location contained 6 metals within the permissible limit while it had 4 metals (Cr, Ni, Cu, Cd) with higher level. All locations contained As, Cd and Pb within permissible limit except the third location which had a cadmium amount of (0.6 mg/kg) while the cadmium permissible level is (0.3 mg/kg). Some farmers use composts (from inorganic sources), fertilizers and plant nutrients in relatively large amounts that are rich in some metals and this might explain some of the higher value of metals in the mint. Moreover, contamination from industrial activities nearby mint farms might also be expected.

Keywords: Microwave digestion, Method development, Heavy metals, Mentha, Mint, ICP-MS

1. Introduction

Heavy metals, for example, arsenic, lead, chromium and cadmium are naturally present in the earth's crust. As, Pb and Cd are not essential for metabolism and above accepted value they can cause severe side effect and toxicity. In recent years and due to industrial activities, considerable attention has been paid to the effect of heavy metal concentration on human and animal health. Transportation into human body can occur through foodstuff, drink, and air. Heavy metals in soils can be uptake by plants and then consumed by human and animal [1].

A study of different plant types in Poland by Baranowska et al. [2] have shown that Pb in specimens ranged from 5.00 mg/kg to 46.15 mg/kg and Cd from 0.20 mg/kg to 1.28 mg/kg for the multiple specimens of the measured mint's herb. However, Zn was the most prominent heavy metal which ranged from 15.65 mg/kg to 100.5 mg/kg.

Another study conducted by Prkić et al. [3] showed that the mint tea leaves contained approximately the following heavy metals concentrations: ≈ Pb (1.50 mg/kg), Cd (0.35 mg/kg), Zn (20.0 mg/kg), Mn (667 mg/kg), Fe (208 mg/kg), Cr (52.0 mg/kg) and Cu (5.6 mg/kg). The consumption of mint that contaminated with high amount of heavy metals has an adverse effect on the human health. For instance, an acute cadmium dose of 10-30 mg/kg/day was reported to trigger impacts like excessive salivation, diarrhea and vomiting [4]. The cadmium dose of 25 mg per kg body weight had been reported that it may lead to death [5]. When the exposure level is low, its influence causes gastrointestinal, renal, neurological, reproductive, and musculoskeletal

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effects. If individual has a chronic exposure to Cadmium this can target the kidney.

Iron is a vital component for the production of the human body's red blood cells. The findings from Prkić et al. [3] revealed that a large amount of Iron and Manganese were determined in plant samples. The results demonstrated that Fe concentrations were as high as 208 mg/kg. As it is crucial in the diet, Iron's low intake causes diseases like fatigue, anaemia and palsied physique. In the same way, heart infarction, hepatomegaly and nephric malfunction are caused when taken in large amounts. It has been verified that humans have to use iron for adults and infants between 8 and 11 mg/day. However, the study reported that a substantial amount of iron was found, which could be ascribed to contamination or the type of soil in which the mint grew.

Further research [6] was also carried out on the concentration of lead metal in the different mint samples. The levels were between 4.84 mg/kg and 26.16 mg/kg. Because the lead consumption safety limit has been identified as 1.5 mg/kg, its quantity in the mint sample could be regarded above the limit. The weakness in fingers and wrists as well as brittle bones is a key effect of a massive lead consumption. Then it would be accumulated and results in the sequestration of the bones and teeth [7]. The lead is stored in bones and still re-enters blood streams when bone minerals are recycled at stages like age and pregnancy. The re-deposition of the lead to neurological and renal, developmental, immunological, musculoskeletal, reproductive, and soft tissue effects can also take place within bodies.

Zinc is among the heavy metals which Joy et al. [8] have studied. It is the least toxic and a vital component of the human diet, crucial for the suitable functioning of the immune system. Also, Zinc has been recognized as an indispensable component in the development and growth of normal brain activity and foetus [8]. It can cause a harm effect to human body when it is missed from the diet [9]. It is recommended, according to Bost et al. [6], that men should take 15 mg Zn a day, while women would take 12 mg daily. High zinc levels in the human body have main consequences such as vomiting, renal damage, cramps, and so on. The Bost et al. [6], study showed Lead's concentration of 0.58 mg/kg to 4.50 mg/kg in mint samples. This amount is within the Zn's safe limit.

There is a large volume of broad published studies on the determination of heavy metals in plant and methods of sample preparation. Wong *et al.*, [10] studied the heavy metals (Cd, Cu, Co, Ni and Pb) in 42 Chinese medical plants by dry ash method. Samples were air-dried and the dried samples were supplemented with $Mg(NO_3)_2$ at a ratio of approx. 1:1 as ashing aid. Then charred for 2 hours on a hot plate and ashed for 16 hours at 470°C in a muffle furnace. Extraction from ash samples was done by 20% HNO_3 (volume/volume) and diluted to the volume by 1% HNO_3 for analysis by atomic absorption spectrophotometer equipped with graphite furnace.
Tüzen [11] compared between different sample preparation methods (dry ashing, wet ashing and microwave digestion) for heavy metals determination (Cd, Pb, Mn, Fe, Zn and Cu) in Lichen samples by Graphite furnace atomic absorption spectroscopy (GFAAS). It was concluded that the microwave digestion method was the best in comparison with other sample preparation methods. Besides high recovery and compatible accuracy and precision with the standard reference materials. In addition [12], microwave digestion method was found to be an optimum sample preparation method for determination of trace heavy metals in tea, tobacco and house dust by using a mixture of $HClO_4$/ HNO_3/ H_2SO_4 with different ratio.

Recent studies by Kilic and Soylak [13] determined heavy metals in 28 plant species (green tea, blue tea, rosehip, rosemary etc.) by firstly transferring 0.2 g in a milestone microwave digestion system and then added 6:2 mL HNO_3 and H_2O_2 respectively. Three heating steps were carried from 80°C-150°C for 5min, 150°C -225°C, and cooling to 70°C for 10min. Extracts were then diluted with deionised water to 25mL and determined by ICP-MS.

The validation of several analytical methods for the determination of heavy metals in herbal medicine with variations of oxidator was investigated by Endah and Nofriyaldi [14]. The process of wet destruction was adopted as a sample preparation method using oxidizers of H_2O_2:HNO_3 (1:3), H_2SO_4: HNO_3 (1:3) and HCl:HNO_3 (1:3). After the best destructive conditions and processes were obtained, the analytical method was validated in term of (limit of detection, limit of quantitation, linearity, accuracy, and precision) for the determination of the concentration of Pb and Cd by AAS.

There is a wide choice of sample preparation methods available in the literature. Xia et al., [15] have reviewed recent advantages of sample preparation techniques from over 140 references. The classifications include chemical conversion (chemical derivatization, chemical labelling, enzymatic hydrolysis, etc.); Phase separation (SDME, HF-LPME, DLLME, SPE, SPME, MSPD, etc.); membrane separation (membrane extraction, dialysis,

filtration, etc.); field-assisted extraction (ASE, SWE, SFE, UAE, MAE, etc.).

There have been numerous studies to investigate the heavy metals and largely focused on soils and sediments. For instance, bioaccumulation modelling in soil-ecosystem [16–18], associated risks for heavy metals in soil and grown vegetables [19–22], accumulation and speciation of heavy metals from soil to rice at various steps of growth [23] and a recent review [24–26] in remediation technology for contaminated soil. Several instrument techniques have been used to assess the level of heavy metals after sample preparation amongst these were ICP-MS with time of flight, laser ablation and other interfaces [27–30]. Also other techniques have been applied such as GF-AAS [31,32], ICP-AES [33], PIXE [34], CV-AFS [35].

Although there are many studies on heavy metals in plants, the research in mint samples remains limited. To the best of our knowledge no study has been conducted to measure heavy metals in Madinah's (city in Saudi Arabia) Hasawi mint using a microwave system. The objective of this paper was to compare between a microwave digestion system and an acid digestion method for analysis of heavy metals in Hasawi mint samples. This has been accomplished by first developing and optimized condition for both methods and then applied it to the samples prior to ICP- MS analysis.

2. Materials and Methods

2.1. Reagents

Deionized water produced by a Milli-Q Plus with a resistivity of 18.2 MΩ cm was applied for sample preparation and standards. Solutions of 30% hydrogen peroxide and 65% nitric acid (Trace analytical grade) were used to digest samples. Standards calibrations for ICP-MS were prepared by dilution of certified element stock solutions with deionized water.

2.2. Sample preparation

Mint (trade name: Hasawi mint) from three different locations in Madinah were collected and firstly washed to remove external contamination. Then oven-dried for 12 hours at 110 °C. After that, they were left to cool down to room temperature and then grinded by using a coffee grinder. In order to measure the whole experimental repeatability and variation, each mint from specific location was divided to two portions. All portions went through the entire sample preparation and measurement including standards and blanks. In this case the results for each location were repeated twice from the beginning using two digestion methods (which make six portions with replications in total).

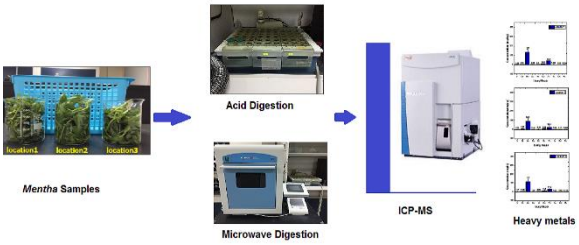

Figure 1. Sample preparation for Hasawi's mint from location1, location2 and location3.

2.3. Open vessel acid digestion

0.5 g mint from each location (n=2) was weighted in Teflon tubes with (8 ml of 65% nitric acid and 2 ml of 30% hydrogen peroxide). The samples including blanks and standards were heated on the graphite block at 110 °C for 3-4 hours (or until the samples reduced in volume to 3 ml). After cooling, each extract was delivered with deionized water to a 50 ml volumetric flask and made up to the volume with deionized water. Because the extract was slightly colored, 1 ml was taken and diluted to 25 ml with deionized water and appeared less colored. Then filtered and sent to ICP-MS for analysis. To assess the efficiency of the method, mint samples were spiked (n=2) with certified reference materials at a concentration of 10 mg/kg and treated as samples.

2.4. Microwave-assisted digestion

The microwave instrument (Milestone Ethos 1) with closed microwave vessels was employed for sample digestion. Table 1 shows the optimized operating conditions.

About 0.5 g of mint from each location (n=2) was weighed using an analytical balance and placed in a microwave vessel/container. The mixture of (2 ml of 30% hydrogen peroxide and 8 ml of 65% nitric acid) was cautiously added. The vessels were sealed, gently shaken and positioned in the microwave oven following the optimized instrumental conditions (Table 1). The same proportion of HNO_3& H_2O_2 alone was used as blank solution (n=2). Standards were also included and compared with untreated standards to see if the method had an effect on standards.

Table 1. Optimized operating condition for microwave digestion system.

Step	Time	E (W)	T1 (°C)	T2 (°C)	P (bar)
1	00:10:00	1000	200	100	45.0
2	00:10:00	1000	200	100	45.0
3	00:20:00	0	30	30	1.0

As caution the digests had been allowed to cool at atmospheric pressure for approximately 180 minutes prior to the opening of the reaction chamber. Extracts were individually transferred to a 50 ml volumetric flask and made up to the volume with deionized water. After that, it was filtered using 0.45µm PTFE membrane filter which attached to a plastic syringe and then samples were sent to ICP-MS for analysis. Concentration was measured using the following formula [36]:

$$\text{Conc. of metals (µg/g)} = \frac{\text{Final conc.} \left(\frac{\mu g}{ml}\right) \times 50}{\text{sample weigh (g)}} \quad (1)$$

To assess the efficiency/recovery of the method, mint samples were spiked (n=2) with certified reference materials at a concentration of 10 mg/kg and treated as samples.

2.5. Instrumentation

Agilent ICP-MS-7500 instrument was used for analysis. Efficient low flow nebulizers and Peltier-cooled Scott spray tanks are used. The optional Integrated Sample Input System (ISIS) is supported by all applications for additional efficiency and flexibility. The system was calibrated and tuned. To test for accuracy and contamination, certified reference materials and blanks were added with each analysis.

3. Results and Discussion

3.1 Calibration curves

The ICP-MS system was calibrated (≥ 0.999) for all heavy metals. Figure 2 shows an example of calibration curve for V, Ni and As.

The results of the levels of heavy metal present in the mint (10 metals) will be discussed in the following sections. The concentration of V, Cr, Mn, Co, Ni, Cu, Zn, As, Cd and Pb was determined after using two types of digestion: Open-vessel digestion and Closed- vessel microwave digestion. All mint portions were blank corrected. Each location repeated twice (n=2) with interval plots displayed the 95% confidence intervals. The source of variation from one location replicates can be attributed to several factors such as: weighing, handling of solutions, homogeneity, and random error during analysis. Blanks were kept and treated with samples from the beginning to assess the possible contamination.

3.2 Open vessel acid digestion

It can be seen from (Figure 3) that the results of open-vessel digestion were two to eight times higher than the results from the microwave digestion in next section (Figure 4). This was probably because the open vessel digestion method was not highly efficient in removing the organic matter although hydrogen peroxide which is an oxidizing agent ($2H_2O_2 \rightarrow 2H_2O + O_2$) was used. Adding this to nitric acid can reduce the nitrous vapours and it accelerates the digestion of organic samples [36,37]. However, after open vessel digestion finished, the solution still contained some organic matter (slightly colored) and even after dilution; the solution seemed to have an effect upon ICP-MS analysis (High signal error, signal suppression, interferences, etc.) and it was difficult to control the precision with this condition. The recovery by spiking reference material at a concentration of 10 mg/L showed a very poor recovery (30%-51%) and the variation between analysis (n=2) was very high. It seems that the condition resulted in an incomplete digestion and for this reason, results from an open vessel digestion were excluded.

3.3. Microwave-assisted digestion

The safety guideline accompanied with the Milestone's microwave digestion system Ethos 1 was strictly followed. The Milestone Microwave Patented Rotor (MDR) technology offers unprecedented efficiency and the highest level of safety to analysts when performing closed vessels digestion. The MDR rotors have no molded or machined threads that can be chemically assaulted and destroyed by hot vapors during the ventilation and opening of the vessel. The durability and protection of milestone vessels is an essential factor in this specific function. A reclosing (vent and reseal) valve mechanism protects all vessels in MDR rotors. A reclosing (vent and reseal) valve mechanism protects all vessels in MDR rotors. The vessels mounted in an MDR rotor body are screwed and the relief valve operates with a calibrated torque wrench. The torque wrench assures that vessels are sealed for optimum pressure and tightly vents if gases reach the maximum operating pressure. The advantages of using the microwave digestion method can be summarized in the following:

1) High Speed: the speed of digestion can be achieved within minutes or less in comparison with conventional methods.
2) Economic benefit: the lower use of solvent eliminates the purchasing and disposal costs of solvents.
3) High Efficiency: Higher analyte recoveries are obtained from microwave method than older methods.

4) High consistency: Precise monitoring of all reaction parameters by software guarantees the reproductivity of the methods.

5) Easy to use and simplicity in applying different conditions.

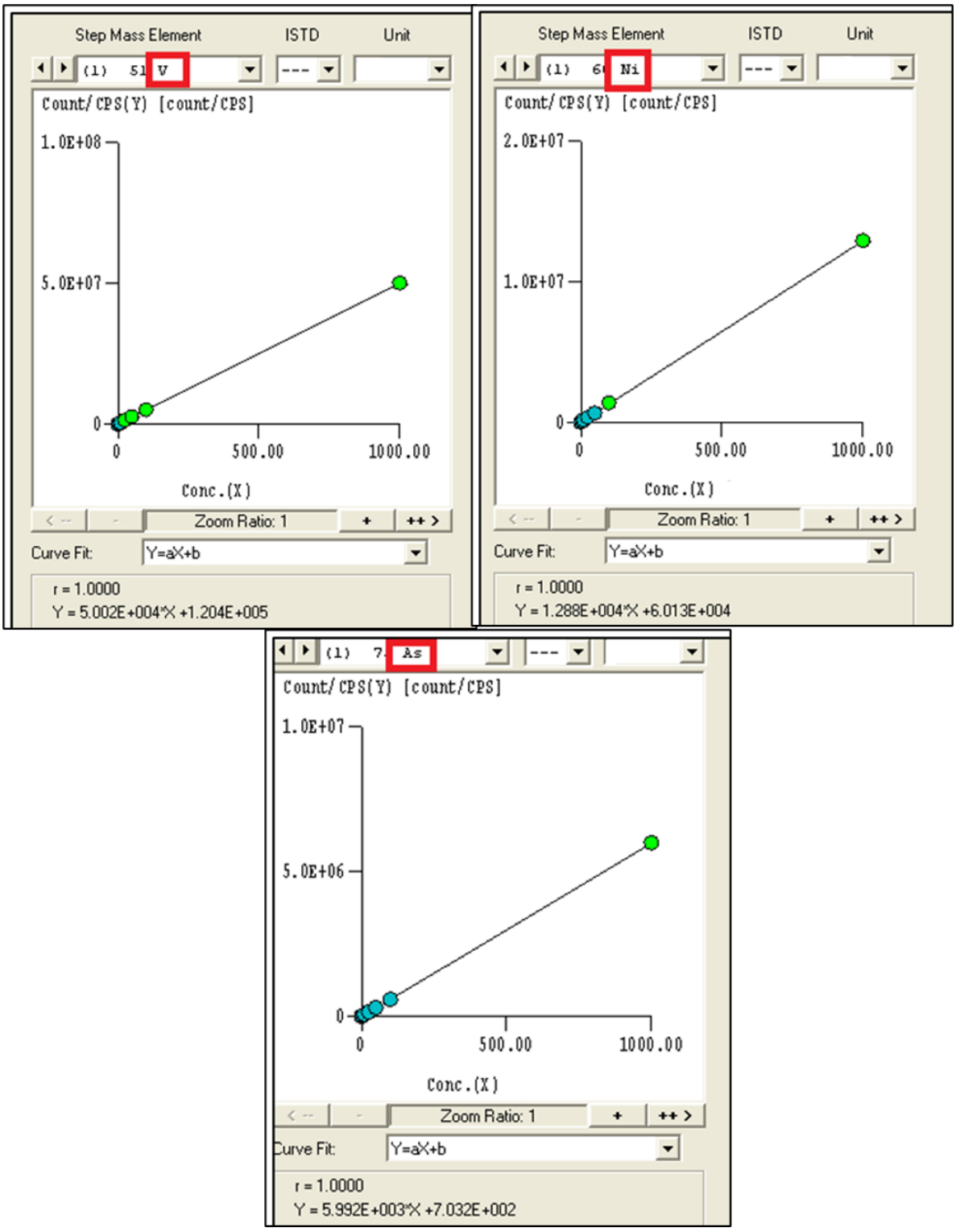

Figure 2. Representative calibration curves for V, Ni and As

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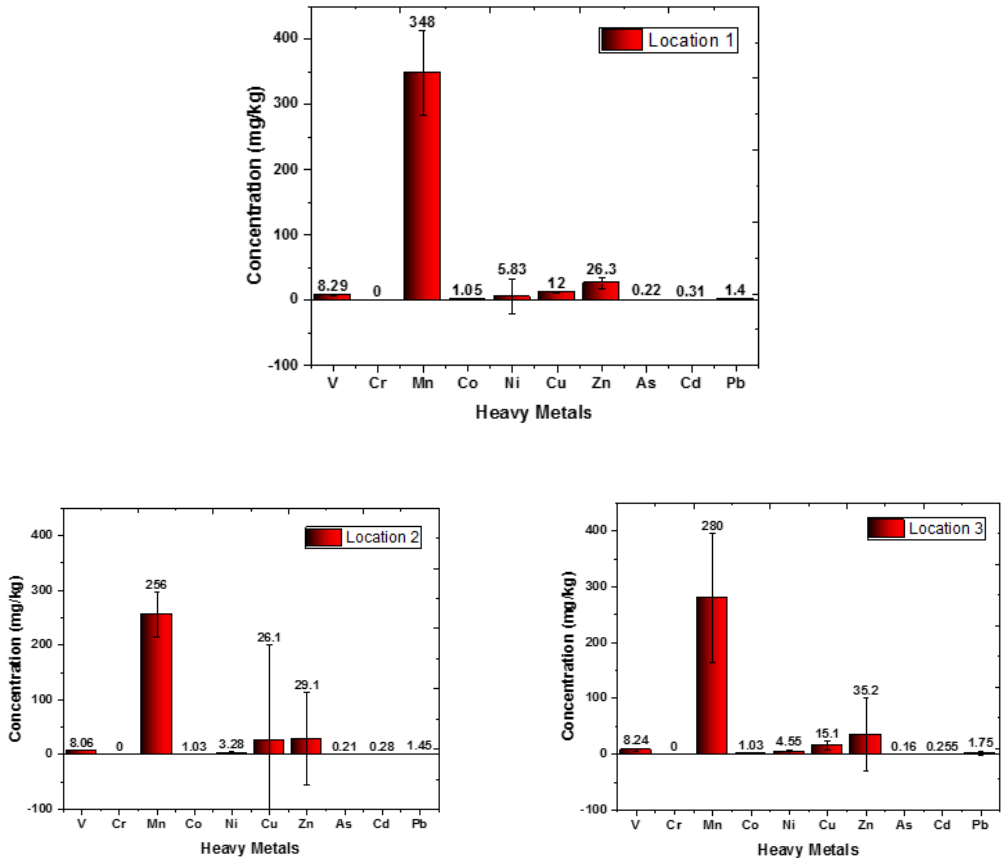

Figure 3. ICP-MS results for Hasawi mint taken from Location 1,2,3 and digested by Open-vessel digestion, mg/kg. Interval plots display 95% confidence intervals for the mean (two samples from each location, n=2).

To assess the efficiency of the microwave method, mint samples were spiked (n=2) with certified reference materials at a concentration of 10 mg/kg. So, as can be seen from Table 2, the recovery for all metals ranged from ≈ 97- 101% with R.SD% from ≈ 1-2%.

The systematic microwave digestion method (Figure 4) work better than the open vessel digestion and the final solutions were colorless. In all figures, the (mean ± 95% confidence intervals, n=2) were reported to evaluate the uncertainty of each location. Also, for comparison purposes results in Figure 4 were clustered together (see, Figure 5). Table 3 also summarizes the highest and lowest range values for each heavy metal in all three locations (n=6 in total).

Based on the summarized results of Table 3 from the microwave digestion system, the following will describe the level of each metal and its permissible level according to several regulatory agencies:

Table 2. Recovery of spiked standard reference material in mint samples with microwave digestion system, n=2.

Heavy metal	Added (mg/kg)	Recovery%	R.S.D%
V	10	101.21	1.46
Cr	10	99.7	1.73
Mn	10	98.92	2.31
Co	10	99.1	1.36
Ni	10	97.86	1.87
Cu	10	99.23	1.34
Zn	10	98.23	2.01
As	10	100.32	1.23
Cd	10	97.92	1.53
Pb	10	98.65	2.43

Vanadium: The concentrations of Vanadium in all samples were ranged from (≈ 1.22- 1.48 mg/kg). When the literature search was conducted for vanadium, its concentration within the leaves of plants

was mostly between 8 and 13 mg/kg and this indicates that the concentration of vanadium is acceptable in mint and that it did not exceed the natural rate [38–40].

Chromium: The concentration of Chromium was found to be on the range (≈ 4.22-7.09 mg/kg). The WHO chromium limits have not yet been established for medicinal plants. However, Canada's allowable chromium limit was 2 mg/kg. Chronic chromium exposure can damage the lung, liver and kidney [38,40,41].

Manganese: The results showed that the concentration of manganese is high in all the studied samples. The concentrations ranged from (≈ 85.85-134.95 mg/kg). Nevertheless, WHO limits for manganese for medicinal herbs are not yet established. However after research in other organizations [38,40] found that the concentration of manganese in leafy plants such as mint is about 20 to 300 mg/kg [38,40].

Cobalt: The concentrations levels of cobalt in the selected mints ranged (≈ 0.45-2.26 mg/kg). But WHO / FAO does not have any cobalt regulatory limits [38,40].

Nickel: Results obtained showed that maximum concentration ranged (≈ 2.41-5.03 mg/kg). The allowable limit for edible plants set by FAO / WHO was 1.63 mg/kg [40,42].

Copper: Results obtained showed that maximum concentration ranged (≈ 5.82-14.32 mg/kg). The allowable limit for edible plants set by FAO / WHO was 3.00 mg/kg [40,42].

Zinc: Results obtained showed that maximum concentration ranged (≈ 25.41-39.93 mg/kg). The allowable limit for edible plants set by FAO / WHO was 27.4 mg/kg [40,42].

Arsenic: Results obtained showed that maximum concentration ranged (≈ 0.23- 0.95 mg/kg). The allowable limit for edible plants set by FAO / WHO was 0.9 to 1.1 mg/kg. Accordingly, it was within the permissible limit [40,42].

Cadmium: Results obtained showed that maximum concentration ranged (≈ 0.04- 0.62 mg/kg). The allowable limit for edible plants set by FAO / WHO was 0.21 mg/kg. For medicinal herbs, however, WHO, China and Thailand set an acceptable limit of 0.3 mg/kg for cadmium. Likewise, the allowable limited in raw plant materials for cadmium established by Canada was 0.3 mg/kg and the permissible limits in finished WHO products were 0.006 mg / day. It was found that all studied samples (the three mint locations) have cadmium below the permissible limit set by WHO, Canada, China and Thailand except the third location (≈ 0.6 mg/kg) which exceeded the allowable limit by two folds. Cadmium tends to cause both chronic and acute poisoning, adverse effects to the liver, vascular, renal and immune systems [40,42].

Lead: Results obtained showed that maximum concentration ranged (≈ 0.14-0.59 mg/kg). The limit for lead content prescribed by the WHO in herbal medicine is 10 mg/kg, while the dietary intake limit for lead is 3 mg/week. Also, China, Malaysia, Thailand and FAO set a limit of 10 mg/kg for medicinal herbs. Lead cause both chronic and acute poisoning and adverse reactions to the kidney, liver, vascular and immune systems. Lead is non-essential trace elements with neither human body nor plant functions. It induces different toxic effects at low doses in humans. Colic, headache, convulsions, chronic kidney nephritis, brain damage and central nervous system disorders are all typical symptoms of lead poisoning. All mint samples studied showed lead concentration below WHO, China, Malaysia and Thailand permissible limits [40,42].

Some farmers use composts (from inorganic sources), fertilizers and plant nutrients in relatively large amounts that are rich in some metals and this might explain some of the higher value of metals in the mint. Besides, contamination from industrial activities nearby mint farms might also expected [1,4,9].

Statistically, the three location means for each metal were compared to see if they differ significantly from each other. One-Way ANOVA for location 1, location 2 and location 3 were depicted in Figure 6. Difference among the means are significant ($p<0.05$) except for Vanadium, Nickle and Arsenic which are not significant ($p>0.05$) in all three locations.

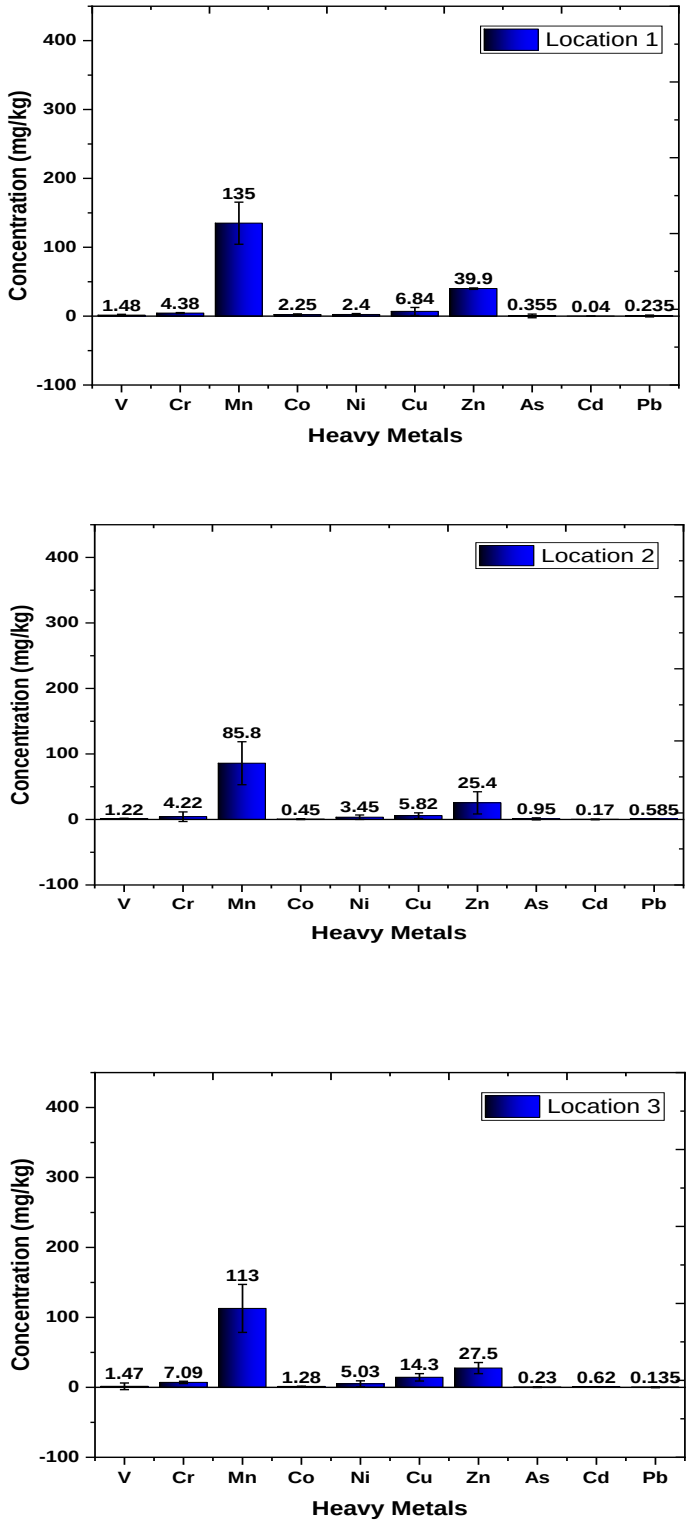

Figure 4. ICP-MS results for Hasawi mint taken from Location 1,2,3 and digested by **Closed- vessel microwave digestion**, mg/kg. Interval plots display 95% confidence intervals for the mean (two samples from each location, n=2).

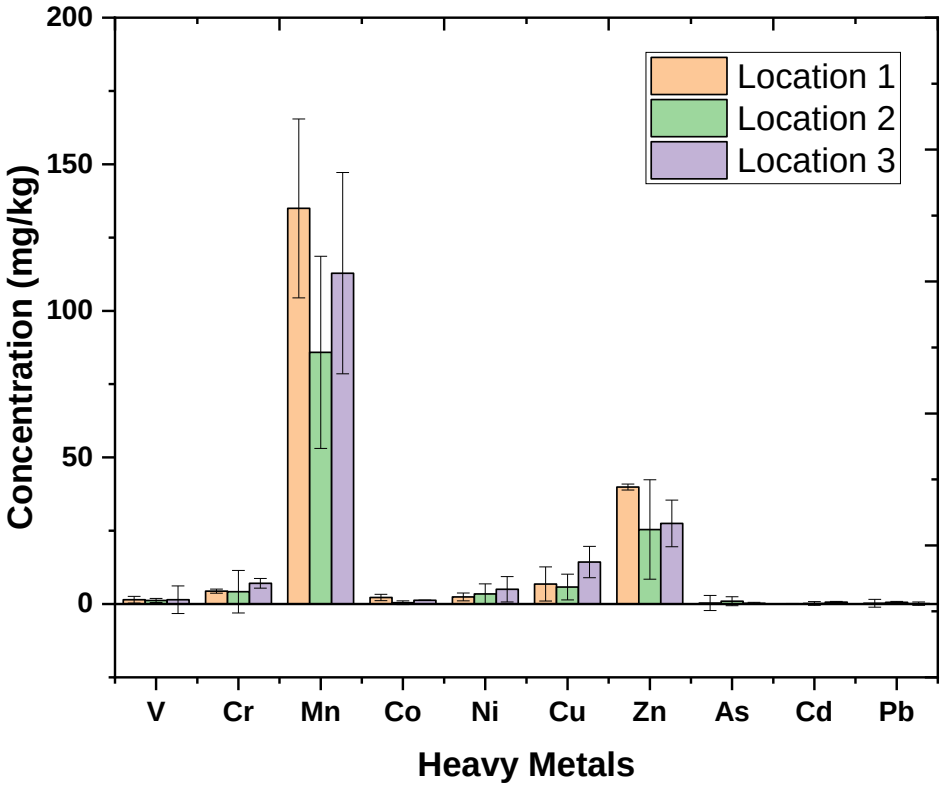

Figure 5. A clustered bar graph of ICP-MS results for Hasawi mint taken from Location 1,2,3 and digested by Closed-vessel microwave digestion method, mg/kg. Interval plots display 95% confidence intervals for the mean (two samples from each location, n=2).

Table 3. Summarizes the lowest and highest concentration range with 95% CI for each metal in all three locations using the microwave digestion method, there were three locations, and every location was repeated twice (n=6).

Heavy metals	**(Lowest± 95%CI) to (Highest± 95%CI) (mg/kg), n=6**	**Heavy metals**	**(Lowest± 95%CI) to (Highest± 95%CI) (mg/kg), n=6**
V	(1.22 ± 0.70) to (1.48 ± 1.14)	Cu	(5.82 ± 4.38) to (14.32 ± 5.34)
Cr	(4.22 ± 7.24) to (7.09 ± 1.65)	Zn	(25.41 ± 16.96) to (39.93 ± 1.02)
Mn	(85.85 ± 32.78) to (134.95 ± 30.49)	As	(0.23 ± 0.38) to (0.95 ± 1.52)
Co	(0.45 ± 0.64) to (2.26 ± 1.08)	Cd	(0.04 ± 0.13) to (0.62 ± 0.25)
Ni	(2.41 ± 1.33) to (5.03 ± 4.32)	Pd	(0.14 ± 0.57) to (0.59 ± 0.32)

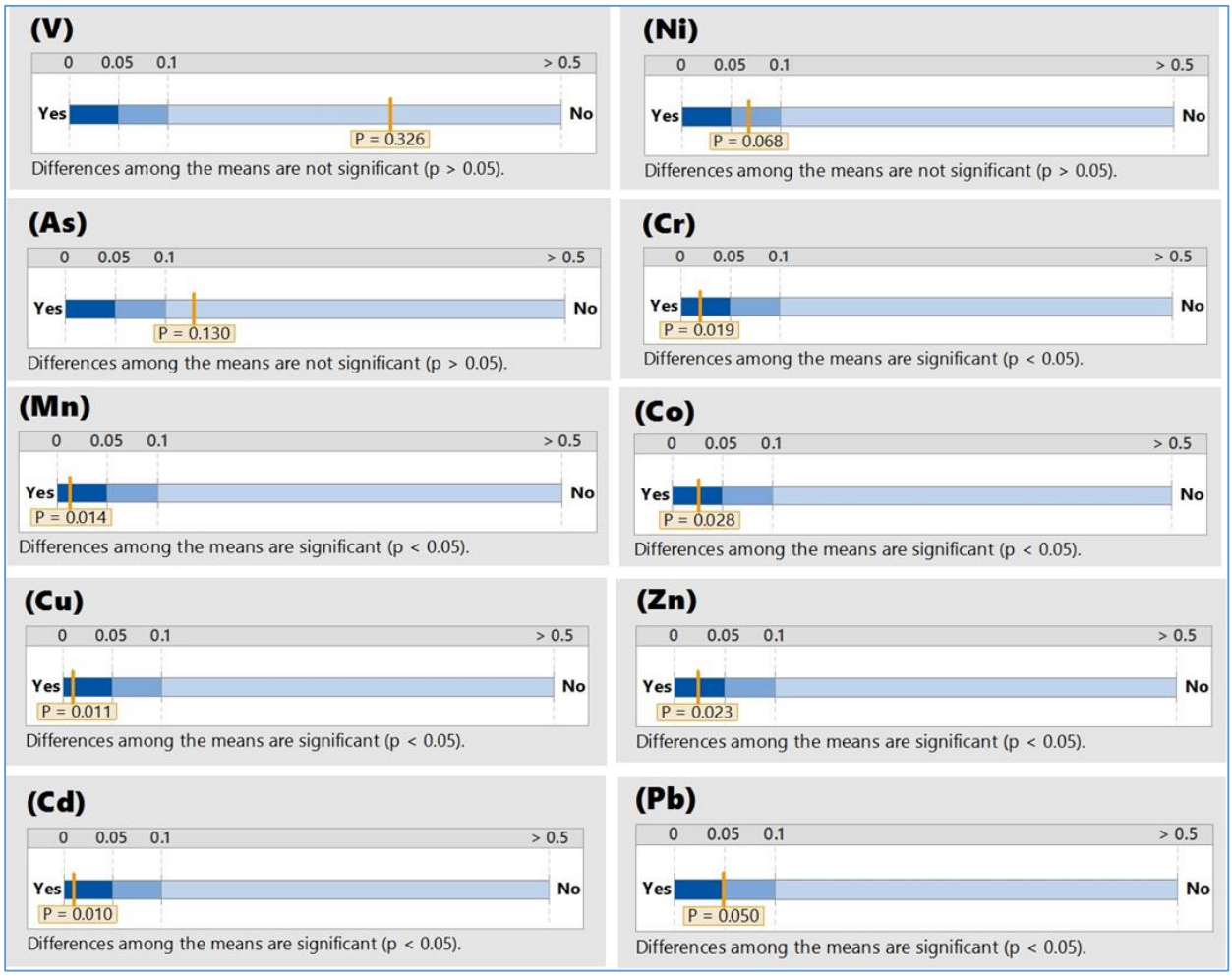

Figure 6. Comparison between the means of each heavy metal in the three locations using One-Way ANOVA.

4. Conclusions

In this research mint samples (Hasawi mints) were collected from three different locations and digested by two digestion methods: 1) acid digestion method and 2) a microwave digestion system. The results by ICP-MS from both digestion methods revealed that the following 10 metals were presented: V, Cr, Mn, Co, Ni, Cu, Zn, As, Cd and Pb.

The microwave digestion system was the best for all studied heavy metals in term of sample preparation procedures, linearity (≥ 0.999), recovery (97%- 101%, n=2) and precision (R.S.D% ≈ 1-2 %, n=2). The microwave digestion system was the best for all studied heavy metals in term of sample preparation procedures, linearity (≥ 0.999), recovery (97%- 101%, n=2) and precision (R.S.D% ≈ 1-2 %, n=2). Mint samples from first location contained 7 metals within the permissible limit while the remaining three metals (Cr, Cu and Zn) had higher levels. The second location contained 9 metals within the permissible limit but only one metal (Cu) had a higher level. The third location contained 6 metals within the permissible limit while it had 4 metals (Cr, Ni, Cu, Cd) with higher level. All locations contained As, Cd and Pb within permissible limit except the third location which had a cadmium amount of (0.6 mg/kg) while the cadmium permissible level is (0.3 mg/kg).

Overall, the lowest and the highest concentration range for each metal in all three locations with 95% CI (n=6) using the microwave system are summarized in the following:

V, from (1.22 ± 0.70 mg/kg) to (1.48 ± 1.14 mg/kg), **Cr** (4.22 ± 7.24 mg/kg) to (7.09 ± 1.65 mg/kg), **Mn** (85.85 ± 32.78 mg/kg) to (134.95 ± 30.49 mg/kg), **Co** (0.45 ± 0.64 mg/kg) to (2.26 ± 1.08 mg/kg), **Ni** (2.41 ± 1.33 mg/kg) to (5.03 ± 4.32 mg/kg), **Cu** (5.82 ± 4.38 mg/kg) to (14.32 ± 5.34 mg/kg), **Zn** (25.41 ± 16.96 mg/kg) to (39.93 ± 1.02 mg/kg), **As** (0.23 ± 0.38 mg/kg) to (0.95 ± 1.52 mg/kg), **Cd** (0.04 ± 0.13 mg/kg) to (0.62 ± 0.25 mg/kg) and **Pd** (0.14 ± 0.57 mg/kg) to (0.59 ± 0.32 mg/kg). Statistically difference among the means of location 1, location 2 and location 3 were

significant ($p<0.05$) except for Vanadium, Nickle and Arsenic which were not significant ($p>0.05$).

Some farmers use composts (from inorganic sources), fertilizers and plant nutrients in relatively large amounts that are rich in some metals and this might explain some of the higher value of metals in the mint. Moreover, contamination from industrial activities nearby mint farms might also be expected. To tackle the environmental and health issue associated with high heavy metal amounts, a combination of a good agricultural practice and monitoring is required.

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References

[1] P. De Voogt, Reviews of Environmental Contamination and Toxicology, 2015. doi:10.1007/978-3-319-20013-2.

[2] I. Baranowska, K. Srogi, A. Wlochowicz, K. Szczepanik, Determination of Heavy Metal Contents in Samples of Medicinal Herbs, Polish Journal of Environmental Studies. 11 (2002) 467–471.

[3] A. Prkić, J. Giljanović, S. Petričević, M. Brkljača, M. Bralić, Determination of Cadmium, Chromium, Copper, Iron, Lead, Magnesium, Manganese, Potassium, and Zinc in Mint Tea Leaves by Electrothermal Atomizer Atomic Absorption Spectrometry in Samples Purchased at Local Supermarkets and Marketplaces, Analytical Letters. 46 (2012) 367–378. doi:10.1080/00032719.2012.718825.

[4] F. Burke, S. Hamza, S. Naseem, S. Nawaz-ul-Huda, M. Azam, I. Khan, Impact of Cadmium Polluted Groundwater on Human Health: Winder, Balochistan, SAGE Open. 6 (2016) 1–8. doi:10.1177/2158244016634409.

[5] S.J. Roney, Nickolette; Smith, Cassandra; Williams, Malcolm; Osier, Mark; Paikoff, Toxicological Profile for Uranium, in: ATSDR's Toxicological Profiles, Agency for Toxic Substances and Disease Registry (US), 2010. doi:10.1201/9781420061888_ch157.

[6] M. Bost, S. Houdart, M. Oberli, E. Kalonji, J.F. Huneau, I. Margaritis, Dietary copper and human health: Current evidence and unresolved issues, Journal of Trace Elements in Medicine and Biology. 35 (2016) 107–115. doi:10.1016/j.jtemb.2016.02.006.

[7] T.P. Phan, T.T.T. Pham, K.L. Nguyen, T.K.O. Nguyen, T.T.T. Ha, K.B.T. Nguyen, T.T.H. Chu, The impacts of lead recycling activities to human health and environment in Dong Mai craft village, Hung Yen, Vietnam, Journal of Vietnamese Environment. 8 (2016) 266–270. doi:10.13141/jve.vol8.no5.pp266-270.

[8] E.J.M. Joy, A.J. Stein, S.D. Young, E.L. Ander, M.J. Watts, M.R. Broadley, Zinc-enriched fertilisers as a potential public health intervention in Africa, Plant and Soil. 389 (2015) 1–24. doi:10.1007/s11104-015-2430-8.

[9] A.J. McMichael, R.E. Woodruff, S. Hales, Climate change and human health: Present and future risks, Lancet. 367 (2006) 859–869. doi:10.1016/S0140-6736(06)68079-3.

[10] M.K. Wong, P. Tan, Y.C. Wee, Heavy metals in some Chinese herbal plants, Biological Trace Element Research. 36 (1993) 135–142. doi:10.1007/BF02783172.

[11] M. Tüzen, A COMPARISON OF SAMPLE PREPARATION PROCEDURES FOR THE DETERMINATION OF HEAVY METALS IN LICHEN SAMPLES BY GFAAS, Analytical Letters. 35 (2002) 1667–1676. doi:10.1081/AL-120013046.

[12] R. Mohamed, B.H. Zainudin, A.S. Yaakob, Method validation and determination of heavy metals in cocoa beans and cocoa products by microwave assisted digestion technique with inductively coupled plasma mass spectrometry, Food Chemistry. 303 (2020) 125392. doi:https://doi.org/10.1016/j.foodchem.2019.125392.

[13] S. Kilic, M. Soylak, Determination of trace element contaminants in herbal teas using ICP-MS by different sample preparation method, Journal of Food Science and Technology. 57 (2020) 927–933. doi:10.1007/s13197-019-04125-6.

[14] S.R.N. Endah, A. Nofriyaldi, Validation Methods of Analysis Heavy Metals Level Lead (Pb) and Cadmium (Cd) with Oxidator Variations by Atomic Absorption

Spectrofotometry in Herbal Medicine, in: Journal of Physics: Conference Series, Institute of Physics Publishing, 2020: p. 62010. doi:10.1088/1742-6596/1477/6/062010.

[15] L. Xia, Y. Li, Y. Liu, G. Li, X. Xiao, Recent advances in sample preparation techniques in China, Journal of Separation Science. 43 (2020) 189–201. doi:10.1002/jssc.201900768.

[16] B. Hu, J. Xue, Y. Zhou, S. Shao, Z. Fu, Y. Li, S. Chen, L. Qi, Z. Shi, Modelling bioaccumulation of heavy metals in soil-crop ecosystems and identifying its controlling factors using machine learning, Environmental Pollution. 262 (2020) 114308. doi:https://doi.org/10.1016/j.envpol.2020.114308.

[17] E. Boros-Lajszner, J. Wyszkowska, J. Kucharski, Phytoremediation of soil contaminated with nickel, cadmium and cobalt, International Journal of Phytoremediation. (2020) 1–11. doi:10.1080/15226514.2020.1807907.

[18] G. Quan, Q. Fan, L. Cui, A.R. Zimmerman, H. Wang, Z. Zhu, B. Gao, L. Wu, J. Yan, Simulated photocatalytic aging of biochar in soil ecosystem: Insight into organic carbon release, surface physicochemical properties and cadmium sorption, Environmental Research. 183 (2020) 109241. doi:https://doi.org/10.1016/j.envres.2020.109241.

[19] H.R. Gebeyehu, L.D. Bayissa, Levels of heavy metals in soil and vegetables and associated health risks in Mojo area, Ethiopia, PLoS ONE. 15 (2020) e0227883. doi:10.1371/journal.pone.0227883.

[20] B. Wang, C. Chu, H. Wei, L. Zhang, Z. Ahmad, S. Wu, B. Xie, Ameliorative effects of silicon fertilizer on soil bacterial community and pakchoi (Brassica chinensis L.) grown on soil contaminated with multiple heavy metals, Environmental Pollution. 267 (2020) 115411. doi:https://doi.org/10.1016/j.envpol.2020.115411.

[21] M. Mokarram, H. Amin, A. Setoodeh, Assessment of heavy metals contamination and the risk of non-cancerous diseases in vegetable using electromagnetic-chemical, Environmental Science and Pollution Research. 27 (2020) 36362–36376. doi:10.1007/s11356-020-09690-4.

[22] H.S. Mekky, E. Abou El-Anwar, S.A. Salman, A.A. Elnazer, W. Abdel Wahab, A.S. Asmoay, Evaluation of Heavy Metals Pollution by Using Pollution Indices in the Soil of Assiut District, Egypt, Egyptian Journal of Chemistry. 62 (2019) 1673–1683. doi:10.21608/ejchem.2019.9720.1654.

[23] S. Zheng, Q. Wang, Y. Yuan, W. Sun, Human health risk assessment of heavy metals in soil and food crops in the Pearl River Delta urban agglomeration of China, Food Chemistry. 316 (2020) 126213. doi:https://doi.org/10.1016/j.foodchem.2020.126213.

[24] S. Qayyum, I. Khan, K. Meng, Y. Zhao, C. Peng, A review on remediation technologies for dense metals polluted soil, Central Asian Journal of Environmental Science and Technology Innovation. 1 (2020) 21–29. doi:10.22034/CAJESTI.2020.01.03.

[25] H.I. Abdel-Shafy, A.H. Kamel, Groundwater in Egypt issue: Resources, location, amount, contamination, protection, renewal, future overview, Egyptian Journal of Chemistry. 59 (2016) 321–362. doi:10.21608/ejchem.2016.1085.

[26] N.K. Soliman, A.F. Moustafa, Industrial solid waste for heavy metals adsorption features and challenges; a review, Journal of Materials Research and Technology. 9 (2020) 10235–10253. doi:https://doi.org/10.1016/j.jmrt.2020.07.045.

[27] A. Rohanifar, N. Alipourasiabi, G.S. Shyam Sunder, J.G. Lawrence, J.R. Kirchhoff, Reversible chelating polymer for determination of heavy metals by dispersive micro solid-phase extraction with ICP-MS, Microchimica Acta. 187 (2020) 1–10. doi:10.1007/s00604-020-04308-5.

[28] N. Al-Masoud, M. Habila, Z.A. ALOthman, T.S. S. Alomara, N. Alraqibah, M. Sheikh, A.A. Ghfar, M. Soylak, Nano-Clay as Solid Phase Microextractor of Copper, Cadmium and Lead for Ultra-trace Quantification by

ICP-MS, Analytical Methods. (2020). doi:10.1039/d0ay01343a.

[29] H. El Hadri, J. Gigault, S. Mounicou, B. Grassl, S. Reynaud, Trace element distribution in marine microplastics using laser ablation-ICP-MS, Marine Pollution Bulletin. 160 (2020) 111716. doi:https://doi.org/10.1016/j.marpolbul.2020.111716.

[30] X. Zhang, X. Wei, X. Men, Z. Jiang, W.-Q. Ye, M.-L. Chen, T. Yang, Z.-R. Xu, J.-H. Wang, Inertial-Force-Assisted, High-Throughput, Droplet-Free, Single-Cell Sampling Coupled with ICP-MS for Real-Time Cell Analysis, Analytical Chemistry. 92 (2020) 6604–6612. doi:10.1021/acs.analchem.0c00376.

[31] R. Komendova, The HR-CS-GF-AAS determination and preconcentration of palladium in contaminated urban areas, especially in lichens, Environmental Pollution. 256 (2020) 113468. doi:https://doi.org/10.1016/j.envpol.2019.113468.

[32] L.F. Rodrigues, R.F. Santos, R.C. Bolzan, F.A. Duarte, J.C.P. Mattos, E.M.M. Flores, Feasibility of DS-GF AAS for the determination of metallic impurities in raw material for polymers production, Talanta. 218 (2020) 121129. doi:https://doi.org/10.1016/j.talanta.2020.121129.

[33] S. S, A. SS, S.B. S, V. HK, M. PV, Determination of the bioavailability of zinc oxide nanoparticles using ICP-AES and associated toxicity, Colloids and Surfaces B: Biointerfaces. 188 (2020) 110767. doi:https://doi.org/10.1016/j.colsurfb.2019.110767.

[34] S. Singh, M. Oswal, S.W. Raja, S. Santra, R. Acharya, B.R. Behera, A. Kumar, K.P. Singh, Chemical characterization of Indian coal and coal residues by PIGE and PIXE spectroscopies using proton beams from tandem particle accelerators, Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms. 478 (2020) 205–217. doi:https://doi.org/10.1016/j.nimb.2020.06.045.

[35] P.A. Pérez, M.A. Bravo, W. Quiroz, Total mercury bias in soil analysis by CV-AFS: Causes, consequences and a simple solution based on sulfhydryl cotton fiber as a clean-up step, Analytical Methods. 12 (2020) 3756–3762. doi:10.1039/d0ay01035a.

[36] K.J. Lamble, S.J. Hill, Microwave digestion procedures for environmental matrices, Analyst. 123 (1998) 103–133. doi:10.1039/a800776d.

[37] M. Bettinelli, G.M. Beone, S. Spezia, C. Baffi, Determination of heavy metals in soils and sediments by microwave-assisted digestion and inductively coupled plasma optical emission spectrometry analysis, Analytica Chimica Acta. 424 (2000) 289–296. doi:10.1016/S0003-2670(00)01123-5.

[38] World Health Organisation. WHO., Quality control methods for medicinal plant materials World Health Organization Geneva, Who. (1998). doi:10.1016/j.pbi.2015.01.003.

[39] A. Varga, R.M.G. Martinez, G. Záray, F. Fodor, Investigation of effects of cadmium, lead, nickel and vanadium contamination on the uptake and transport processes in cucumber plants by TXRF spectrometry, Spectrochimica Acta Part B: Atomic Spectroscopy. 54 (1999) 1455–1462. doi:10.1016/S0584-8547(99)00105-6.

[40] D.R. Lessard, Currency changes and management control: a note on the integration of the international controller's and treasurer's functions, Science Advisor, Risk Assessment Forum, EPA 120/R-07/. 001 (1974). www.epa.gov/osa (accessed May 18, 2019).

[41] A. Ho, W. Morishita, D. Atasoy, X. Liu, K. Tabuchi, R.E. Hammer, R.C. Malenka, T.C. Südhof, Genetic Analysis of Mint/X11 Proteins: Essential Presynaptic Functions of a Neuronal Adaptor Protein Family, The Journal of Neuroscience. 26 (2006) 13089 LP – 13101. doi:10.1523/JNEUROSCI.2855-06.2006.

[42] FAO/WHO, Joint FAO/WHO food standards programme codex committee on contaminants in foods. Working document for information and use in discussions related to contaminants and toxins in the GSCTFF, 2011. http://www.fao.org/tempref/codex/Meetings/CCCF/CCCF5/cf05_INF.pdf (accessed May 18, 2019).