Measurement of Levels of Heavy Metal Contamination in Vegetables Grown and Sold in Selected Areas in Lebanon

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Abstract

Vegetables sold in the Lebanese markets are likely to adsorb and/or absorb toxic heavy metals from implantation till they reach the consumer. The samples were purchased randomly from four major areas in Lebanon, namely Beirut, Jounieh, Tripoli, and Koura. The levels of four selected toxic heavy metals; lead, cadmium, chromium, and arsenic were determined in a total of 181 vegetable samples of which 66 are leafy vegetables, 84 are over ground vegetables, and 31 are underground grown vegetables. Overall, the levels ranged from non-detectable to 3.0904 µg/g for Pb, 0.0137 to 3.6170 µg/g for Cd, non-detectable to 19.55 µg/g for Cr, and non-detectable to 0.0636 µg/g for As, where µg/g refers to the weight of the metal per gram of dry sample weight. In all samples, the leafy vegetables contained considerably higher levels for all metals as compared to overground or underground vegetables. Washing the samples thoroughly with water before analysis has decreased the concentrations of toxic metals, more significantly so, for As.

Keywords: Heavy metals; Vegetables; Lebanon.

Introduction

Heavy metals are natural components of the Earth's crust and cannot be degraded nor destroyed. They enter the human body through food, water and air. Heavy metals are ubiquitous; therefore they tend to bioaccumulate thus causing an increase in their concentration in a biological system [1]. Chronic heavy metal toxicity has been the result of long term low level exposure to pollutants and is associated with many chronic diseases. Heavy metals are given significant interest throughout the globe due to their toxic, mutagenic and teratogenic effects even at very low concentrations[2]. Several cases of human diseases, disorders, malfunction and malformation of organs due to metal toxicity have been reported[3]. Reports indicate that lead, cadmium, chromium and arsenic may cause a wide variety of changes in biological systems, even at very low concentrations[4]. Some elements including

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arsenic, cadmium and chromium are carcinogenic. Others, such as lead and mercury have been associated with developmental abnormalities including autism in children [5].

The current work addresses one of the many health concerns which is the exposure of the Lebanese population to such toxic elements. For the past 30 years only about seven articles studied the presence of toxic elements in foodstuffs, particularly in fish [6-11]. Recently a study [12] has been carried out on the levels of certain heavy metals (lead, cadmium and mercury) in prepared and ready to eat foods. Unlike [12], the present study investigates heavy metal concentrations in the test samples of vegetables. This is very important because the Lebanese population relies heavily on vegetables in its diet and, knowing that different types of vegetables absorb and/or adsorb heavy metals differently, awareness of heavy metal contamination in each vegetable may possibly lead the Lebanese to minimize or hopefully avoid such exposure.

Heavy metals may be present either as a deposit on the surface of vegetables, or may be taken up by the crop roots and incorporated into the edible part of plant tissues. Heavy metals deposited on the surface can often be eliminated simply by washing prior to consumption, whereas bio-accumulated metals are difficult to remove and are of major concern.

Since in our region it is difficult to determine the exact source of contamination and the origin of the vegetables, the aim of this study is, therefore, restricted to evaluate the potential health hazard due to the consumption of various selected vegetables by analyzing the levels of toxic metals in them.

Materials and methods

Heavy metals determination was carried out using a Thermo-Electron (GFAAS) Graphite Furnace Atomic Absorption Spectrometer (M Series-AA Spectrometer) equipped with Deuterium and Zeeman background correction (Zeeman Furnace GF95Z) along with an FS95 auto-sampler (Thermo-electron Corporation, Germany). Polypropylene reagent bottles (10mL), sample cups (1mL) for the auto-sampler as well as graphite tubes were all supplied by the manufacturer. For analysis, coded hollow cathode lamps of Pb, Cr, Cd, and As were chosen. Argon gas of 99.999% purity (Chehab Industrial, Lebanon) was used at 300 ml/min flow as the internal inert gas during all stages of analysis except for atomization, when the flow was stopped automatically.

A grinder purchased locally, was used to homogenize all samples prior to acid digestion which is performed using Thermo Ethos1 (Advanced Digestion System, Milestone) microwave digestion oven. A programmable oven (Venticell) was also used at 60^{o}C overnight to completely dry the samples.

Distilled de-ionized (ddH_2O) water (Millipore Milli-Q system) was used throughout. Standard solutions of Pb, Cr, Cd, and As (1000 ppm reference solution, Romil-pure Chemistry) were used to prepare working standards. High quality ultra pure nitric acid (65%) was used to prepare wash solutions, diluents, and for the digestion of samples and their analysis in the GFAAS. All plastic and glassware were previously washed with tap water, soaked in 10% v/v nitric acid solution overnight, rinsed three times with ddH_2O followed by rinsing with 1% v/v nitric acid prior to use.

Samples

The study was carried out by random sampling from different locations in Lebanon namely Beirut, Jounieh, Tripoli, and Koura. Where applicable, sampling of each type of vegetable was carried out in three different places in each of the areas listed above and according to their seasonal availability.

The studied vegetable samples were grouped into three main categories; leafy, underground, and overground vegetables. The selected samples represent vegetables that are commonly used in the Lebanese diet. A total of 181 samples were collected, of which 66 are leafy, 31 are underground, and 84 are overground. The leafy vegetables are comprised of Lettuce, Zaatar (*Oregano*), Spinash, Parsley, Mint, Bakleh (*Purslane*), and Cabbage; The underground vegetables are comprised of Carrot, Potato, and Radish; While the overground vegetables are comprised of Cucumber, Tomato, Cauliflower, Zucchini, Eggplant, and Green pepper.

Sample Treatment

Once collected, samples are first washed as fresh vegetables using normal tap water to mimic the washing of fresh vegetables that would be normally carried out by any individual at home. Some of the vegetable samples namely cauliflower, cabbage, cucumber, green pepper, zucchini, tomato, potato and lettuce were each split in half. The first half were washed while the second half were left unwashed to observe whether adsorption is present and to what extent.

All washed samples were carefully air dried, cut into small pieces and weighed immediately (fresh weight, W_f) before drying in the oven at 60^{o}C for 24 hours. When samples became fully dry, they were weighed again (dry weight W_d) so as to determine the original water content in each sample. This was followed by grinding and homogenization of the dried samples into fine powder using an electric grinder. The powdered samples are then stored in closed containers in absence of humidity.

Acid-Assisted Microwave Digestion of Samples

The method provides a fast, flexible, and reproducible procedure for the acid digestion of samples [13]**.** It utilizes closed reaction vessels in which high temperature and pressure combination is used to digest the samples causing the release of heavy metals of interest into solution. A 0.5 g of each powdered (dry) sample was introduced into a Teflon reaction vessel followed by the addition of 8.00 ml of concentrated nitric acid (65%). The vessel was swirled gently to distribute the powder evenly in the acid then sealed and placed in the rotor inside the microwave oven.

The unit was programmed to follow two basic steps. The first consisted of a 25 minutes period to raise the temperature from ambient to 180^oC at 1000 watts. During the second step, the same temperature was held for 10 minutes at 1000 watts. Once the program was finished, the vessels were air cooled to reach room temperature. The vessels were carefully opened under a fume hood where digests were transferred into 25.00 mL polypropylene volumetric flask followed by dilution up to the mark using ddH_2O.

Sample analysis by GFAAS

The Thermo-Electron M-series GFAAS used in this study is equipped with a software-incorporated cookbook which automatically sets up the optimized parameters for that type of metal under investigation. All samples, mother standard 100 ppb solution, and reagents are loaded into the auto-sampler according to the auto-sampler guide that is automatically generated by the software. The GFAAS's auto-sampler performed all necessary dilutions and preparations of the standard curves as well as automatic aspirations and injections of up to sixty samples for analysis in one run. Each sample was analyzed in triplicates where a mean value is obtained. The instrument was further programmed to re-measure the blank and/or a randomly selected standard calibration point every 10 samples to ensure accuracy, precision, and quality of the analytical data throughout the analysis. The auto-sampler's injection system was programmed to wash itself between samples using 1% v/v nitric acid so as to ensure the absence of carryover contamination between samples. The instrument was also programmed to clean the graphite tube after the analysis of each sample by raising its temperature to 2800^oC to ensure complete burning of residue, which were subsequently flushed out by passing argon gas through the graphite tube.

Results and discussion

In this study, the concentrations of Pb, Cd, Cr and As have been measured in the selected vegetables and the results are presented in table 1 and figures 1 to 8. The more elevated concentrations were found in zaatar (oregano), bakleh (purslane), parsley, spinach, lettuce and mint, all of which fall in the category of leafy vegetables. The results are presented in terms of µg/g of dry sample weight simply because the dry weight of samples remains constant unlike the fresh weight in which the water content might vary from one to another.

Lead

Elevated lead levels, due to plant uptake, is known to cause a series of metabolic changes in plants such as decreased growth, delayed flowering and reduction in quality [14]. In addition, this can bring Pb into the human food chain, thus becoming a major concern for health [15]. Like most heavy metals, Pb can bioaccumulate overtime and reside in the body for long periods, thus it is necessary to detect such metals even at very low concentrations.

In the analyzed samples of this study, lead levels ranged from non-detectable to 3.0904µg/g of dry sample (table 1). A similar study reported that lead concentrations ranged from non-detectable to 2.695 µg/g of dry sample which closely resembles data obtained in our study [16]. As shown in figure 1, the lead concentrations in leafy vegetables were much higher than other types of vegetables which are coherent with the literature [17-19]. The risk from lead may result from both lead contaminated particles adhered to the plant surface as well as direct uptake of lead into the plant tissue. Washing the leaves will only help remove the risk associated with the lead contaminated particles on the plant surface as demonstrated in figure 2.

Table 1: Summary of Lead, Cadmium, Chromium and Arsenic content determined in selected vegetable samples. (For each metal the range and its (mean value) are both expressed in µg of metal/g of dry sample weight)

Sample	n	Lead	Cadmium	Chromium	Arsenic
Cauliflower	10	n.d.-0.1575 (0.0112)	0.0559-0.6185 (0.2672)	0.0248-0.5671 (0.1826)	n.d.-0.0373 (0.0042)
Cabbage	8	0.0127-0.1528 (0.0645)	0.0401-0.4372 (0.1713)	0.0346-0.8746 (0.2188)	n.d.-0.0348 (0.0058)
Eggplant	10	n.d.-0.1025 (0.0403)	0.0476-0.8406 (0.3242)	0.0197-0.3903 (0.1536)	n.d.-0.0636 (0.0113)
Cucumber	10	0.0079-0.1678 (0.0558)	0.0587-0.7289 (0.2971)	0.0690-1.4954 (0.4587)	n.d.-0.0178 (0.004)
Green pepper	9	n.d.-0.1615 (0.0549)	0.1022-0.5975 (0.3189)	0.0249-0.3674 (0.1180)	n.d.-0.0165 (0.0002)
Zucchini	10	0.0373-0.1370 (0.0802)	0.0298-0.5112 (0.1470)	0.0344-0.5383 (0.1684)	n.d.-0.0502 (0.0064)
Tomato	10	0.0199-0.1638 (0.1070)	0.1731-0.8190 (0.4380)	0.0002-0.2890 (0.1301)	n.d.
Potato	9	0.0255-0.1607 (0.0764)	0.0411-0.8745 (0.3374)	n.d.-0.5643 (0.1618)	n.d.-0.0487 (0.0079)
Carrot	9	0.0579-0.3009 (0.1531)	0.0594-0.2798 (0.1357)	0.0255-0.1823 (0.0763)	n.d.-0.0227 (0.0026)
Radish	8	0.0726-0.5181 (0.1843)	0.1599-0.9031 (0.4809)	0.1466-0.4640 (0.2810)	n.d.-0.0255 (0.0084)
Zaatar	8	0.2119-0.9751 (0.4877)	0.1779-1.2261 (0.7210)	0.3762-3.0936 (1.4741)	n.d.-0.0579 (0.0149)
Bakleh	8	0.2362-1.5644 (0.5627)	0.2059-1.5572 (0.6362)	0.3151-1.331 (0.7164)	n.d.-0.0080 (0.0019)
Parsely	10	0.1932-1.0203 (0.5854)	0.0402-0.5536 (0.1793)	0.2384-7.5471 (1.2622)	n.d.
Spinash	8	0.3575-1.5862 (0.7389)	0.2950-3.617 (1.252)	0.6354-7.9818 (2.0982)	n.d.-0.0491 (0.0105)
Lettuce	10	0.0550-2.3427 (0.6245)	0.08985-1.1710 (0.6290)	0.0374-1.9008 (0.6104)	n.d.-0.0131 (0.0014)
Mint	10	0.2264-3.0904 (0.9057)	0.01366-0.3340 (0.0850)	0.5738-19.5550 (3.1609)	n.d.-0.0564 (0.0102)

"n" refers to number of samples
'n.d.' refers to non-detectable

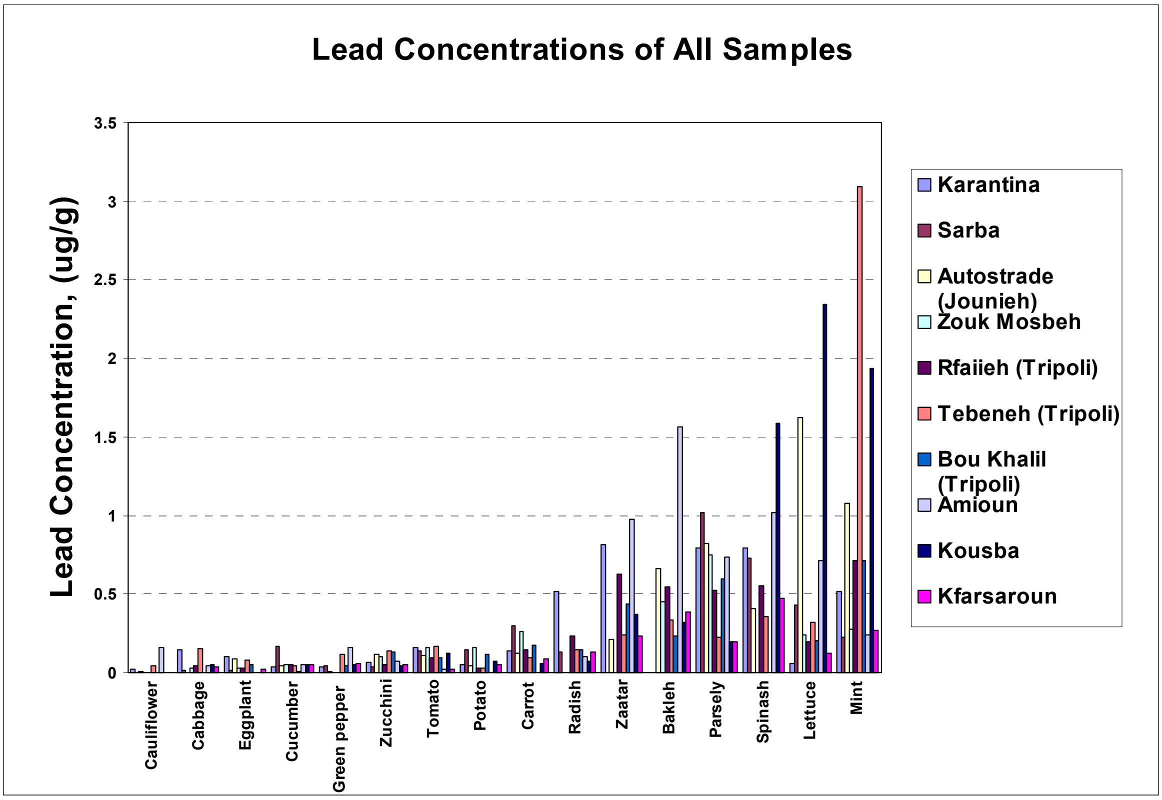

Figure 1: Lead concentrations found in samples from different areas. Concentrations are expressed in µg/g based on the dry weight analyzed. For each sample type, the order in which the areas appear (colored bars) corresponds to the same order found on the most right-hand legend from top to bottom. In case where no bars exist for a specific area of a certain type of sample, the concentration is considered to be non-detectable.

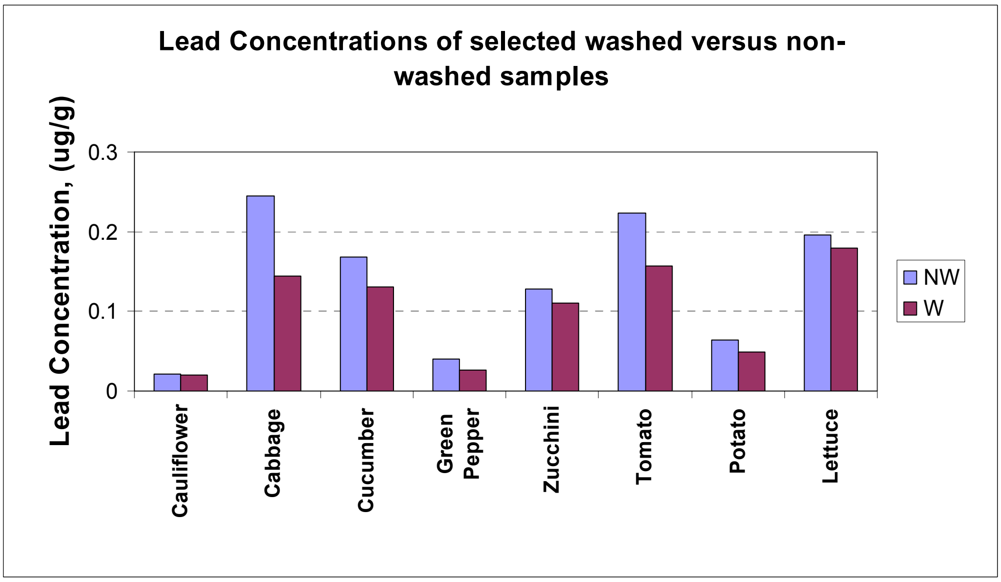

Figure 2: Lead concentrations found in washed (W) versus non-washed (NW) selected samples. Concentrations are expressed in µg/g based on the dry weight analyzed.

The maximum allowable levels of lead are reported in the literature as follows: 6 µg/day for individuals less than 6 years, 15 µg/day for greater than 7 years, 25 µg/day for pregnant women, and 75 µg/day for other adults set by the USFDA in 1993 [16, 20]. Another study reported a maximum allowable level of 0.01 mg of Pb/kg based on fresh weight [21]. This refers to metal intake from various sources namely food, water and air and takes into consideration the maximum tolerable level of metal that the body can handle and excrete before causing any signs of toxicity.

Our results show that the samples analyzed contain high concentrations of Pb which can easily add up to the maximum allowable values especially in the case of children. For example, the level of lead obtained upon the consumption of say 500 g of fresh cabbage (or its equivalent from the consumption of different vegetables) per day will add up to 7 µg Pb/day which is high as compared to the maximum allowable levels for children and even for adults. This becomes even more pronounced knowing that food is not the only source of Pb intake into the human body.

Cadmium

Cadmium is the most toxic heavy metal because it bio-accumulates, has a long half life of about 30 years [1] and may cause health disorders even at low doses. The use of contaminated water for irrigation, fertilizers, sewage and composts can remarkably increase the uptake of cadmium into plant tissues [22, 23]. Based on plant species, their physical and chemical properties, plants can readily absorb cadmium from soil where upon ingestion will enter into the human food chain. In 1998, Reeves and Vanderpool indicated that sunflowers tend to remove cadmium from the soil and deposit it in their seeds [24]. The same was found in peanuts by McLaughlin in 2000 [25].

The cadmium concentration in all samples studied varied from 0.0137 to 3.6170 µg/g (table 1). The higher concentrations were found in the leafy vegetables such as zaatar (Oregano), bakley (Purslane), lettuce and especially higher in spinach (figure 3). Similar results were obtained by Schuhmacher in 1991 [26]. Washing has decreased the cadmium concentration significantly as observed in figure 4. The cadmium tolerable levels have been reported by Kachenko and Singh [21] and Cabrera [4] to be 0.01 mg/kg of fresh weight and 57-71 µg/day respectively. In the samples studied, almost half of the vegetables show a high concentration of cadmium, whereby, consumption of 400 g of spinach per day, as an example, will yield an intake of 86.44 µg/day, which exceeds the intake limit of an adult.

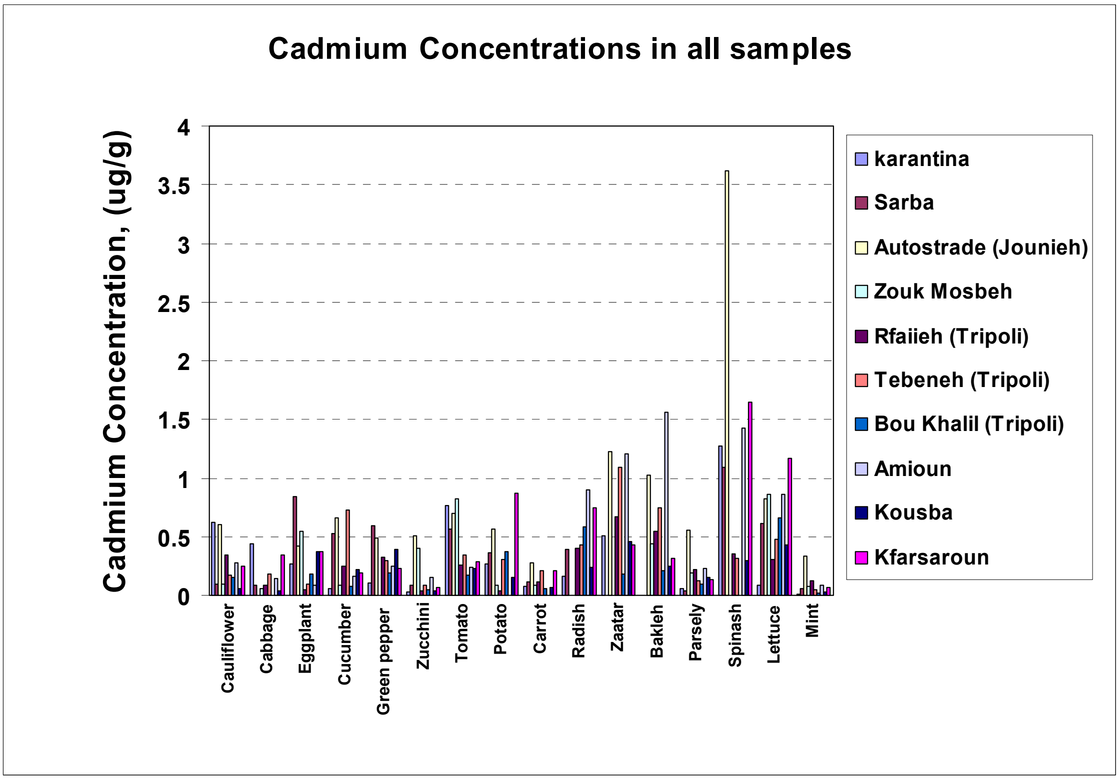

Figure 3: Cd concentrations found in samples from different areas. Concentrations are expressed in µg/g based on the dry weight analyzed. For each sample type, the order in which the areas appear (colored bars) corresponds to the same order found on the most right-hand legend from top to bottom. In case where no bars exist, for a specific area of a certain type of sample, the concentration is considered to be non detectable.

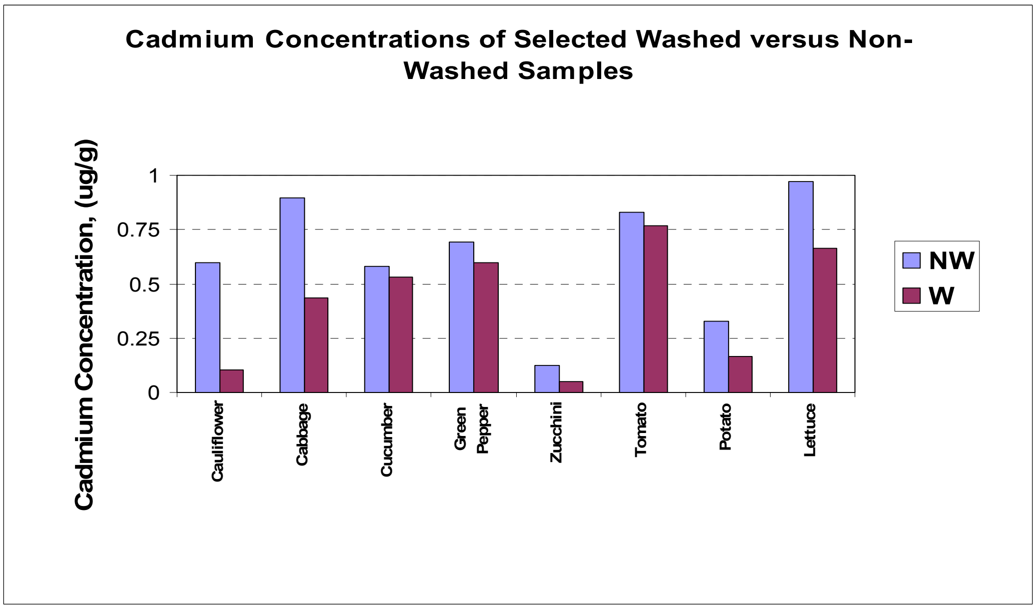

Figure 4: Cadmium concentrations found in washed (W) versus non-washed (NW) selected samples. Concentrations are expressed in µg/g based on the dry weight analyzed.

Chromium

Exposure to chromium may occur through breathing air, drinking water, or eating food containing chromium or even through skin contact. In human beings and animals, it is considered to be an essential metal for carbohydrate and lipid metabolism within a certain range of concentrations (up to 200 µg/day). However, exceeding normal concentrations leads to accumulation and toxicity that can result in hepatitis [36], gastritis, ulcers and lung cancer [3, 27-30].

The chromium content of the samples studied ranged from non-detectable to 19.55µg/g of dry sample (table 1). Elevated Cr concentrations were observed for all leafy vegetables as shown in figure 5. Non leafy samples had Cr levels ranging between non-detectable to 1.4954 µg/g of dry sample which are comparable to data from various published studies [4, 29-31]. Washing the vegetables has proven once again, to be efficient in reducing the amount of Cr adhered as shown in figure 6.

The normal dietary intake of Cr in adults ranged from 13 to 61µg/day of total intake by ingestion, depending on age, country and gender as has been reported by Mann and Truswell [32]. This range clearly excludes the intake from water and air where the sum of dietary, air and water intake should not exceed 200µg/day as set by the national research council [27-29]. A fresh weight of about 1kg/day of cucumber yields a total Cr intake of 12.62 µg which falls on the lower end of the normal intake range as it is the case for the rest of the non-leafy samples. However, the study shows that the risk increases in consuming leafy vegetables such as parsley, mint and spinach where high concentrations of Cr have been detected (figure 5). Since, the three leafy samples are normally consumed in a much lower levels (<<1 kg) then there will be no risk from consuming such vegetables.

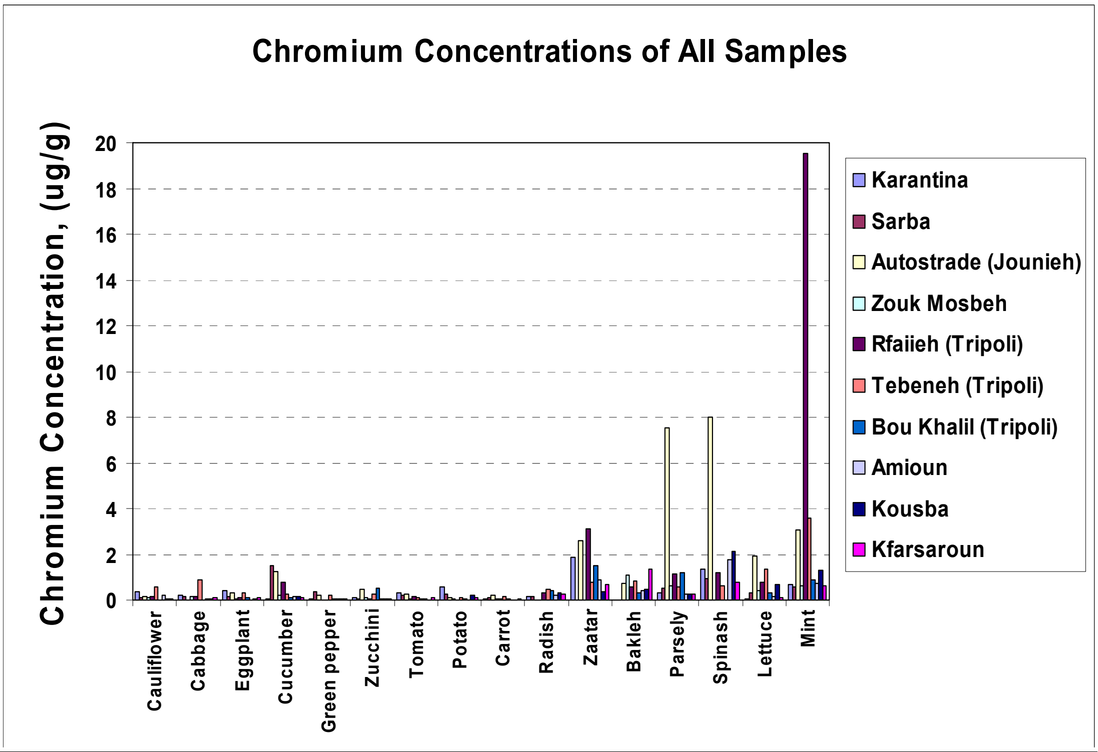

Figure 5: Cr concentrations found in samples from different areas. Concentrations are expressed in µg/g based on the dry weight analyzed. For each sample type, the order in which the areas appear (colored bars) corresponds to the same order found the most right-hand legend from top to bottom. In case where no bars exist, for a specific area of a certain type of sample, the concentration is considered to be non detectable.

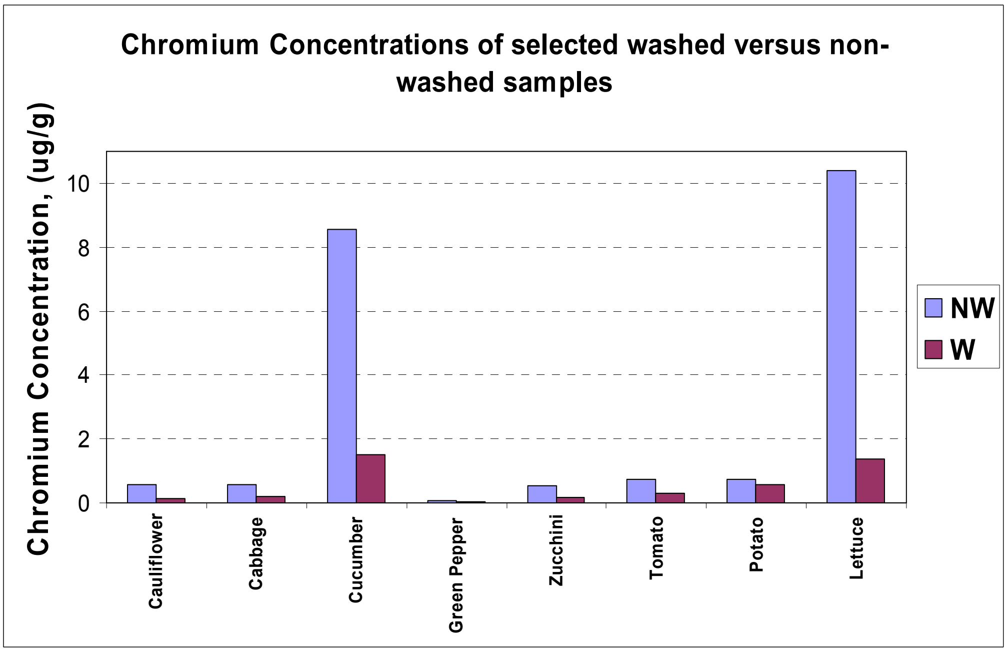

Figure 6: Chromium concentrations found in washed (W) versus non-washed (NW) selected samples. Concentrations are expressed in µg/g based on the dry weight analyzed.

Arsenic

Arsenic has a short half-life of several weeks in the body, but its effects can be seen years after exposure has ceased and is considered to be a human carcinogen [33]. Fortunately, plants do not absorb arsenic as reported by researchers who have studied vegetable samples grown in agricultural soils high in As content [34] whereby the levels in edible crop samples were below the analytical detection limit of 0.075µg/g of As and, therefore, could not be detected.

In this study, Arsenic concentrations ranged from non-detectable to 0.0636µg/g of dry sample (table 1 and figure 7). With a maximum allowable value of 150 µg/day[35], the results suggest that arsenic levels do not pose any risk from any of the studied edibles and, as well, reflect the fact that plants do not absorb Arsenic. figure 8 shows that washed samples display a non-detectable level of Arsenic which agrees with the results reported in [34]

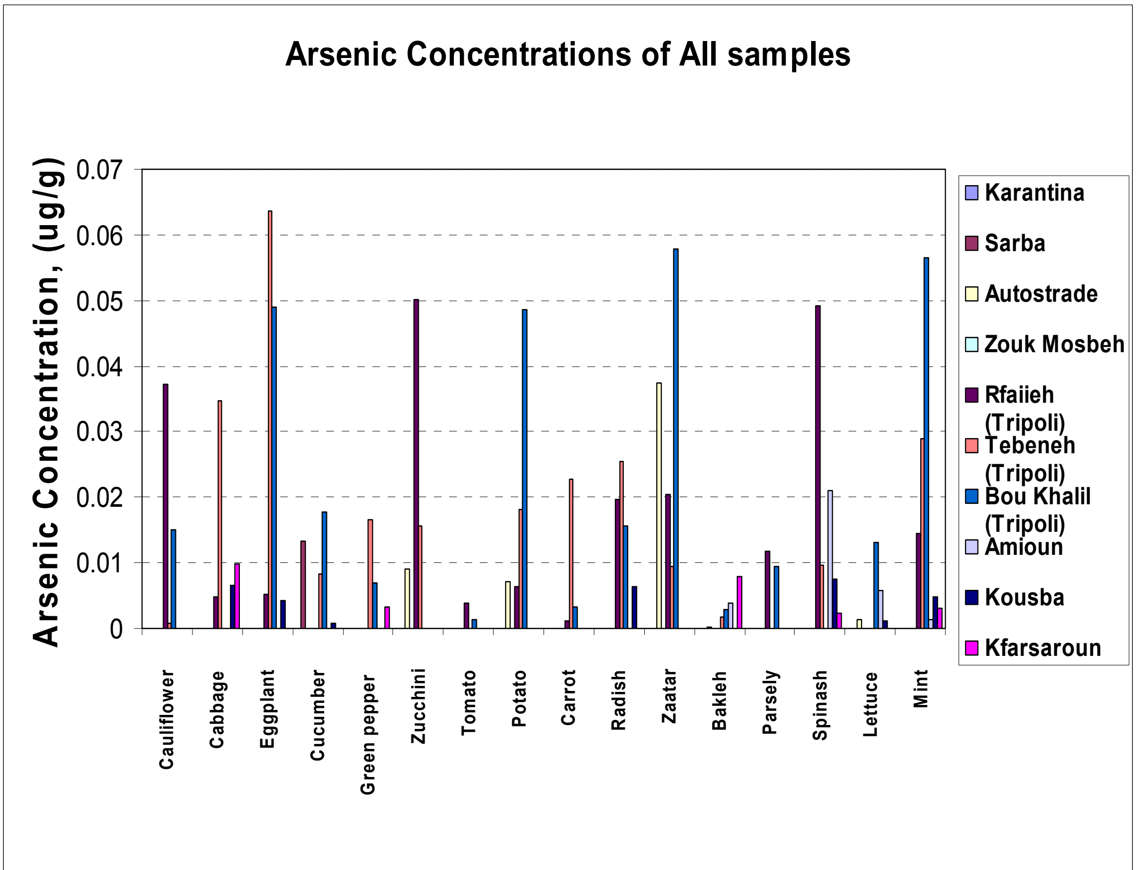

Figure 7: As concentrations found in all samples from different areas. Concentrations are expressed in µg/g based on the dry weight analyzed. For each sample type, the order in which the areas appear (colored bars) corresponds to the same order found on the most right-hand legend from top to bottom. In case where no bars exist, for a specific area of a certain type of sample, the concentration is considered to be non detectable.

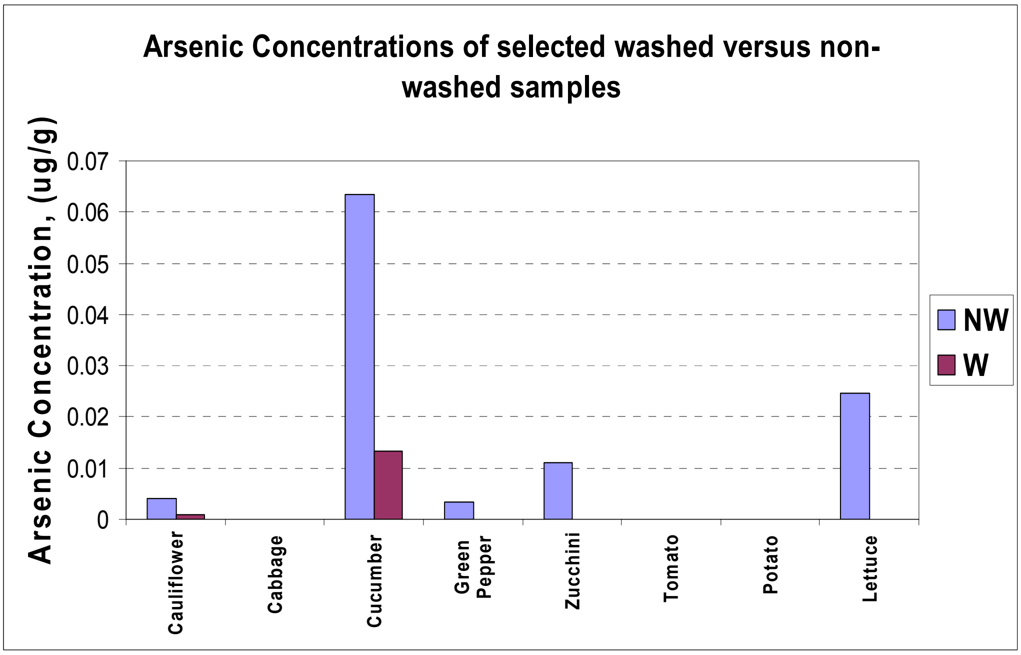

Figure 8: Arsenic concentrations found in washed (W) versus non-washed (NW) selected samples. Concentrations are expressed in µg/g based on the dry weight analyzed.

Conclusions

The concentrations of Pb, Cd, Cr and As were determined and the results show that the leafy vegetables in particular, contain higher levels of all metals studied. Since the sources of metal contamination of samples were difficult to determine, the study therefore, was centered on assessing the level of metals in vegetable samples only, while excluding other sources such as water and air.

In general, it was found that all metals with the exception of arsenic, can be absorbed into the plant tissues where they accumulate and in turn are passed on to humans through ingestion. The results obtained show that children are at higher risk from heavy metals particularly Pb, Cr, and Cd than adults. Thorough washings of edibles was found to have decreased the levels of the adsorbed metal.

Since this is the first report to discuss levels of selected metals in individual samples of vegetables from various locations in Lebanon, the results obtained encourage for further and future detailed research that should consider important factors such as soil types and their metal contents. In addition, it would also be beneficial to carry out a detailed study where edible plants can be classified according to which metal they tend to absorb more, so as to give the consumer the full awareness regarding such toxic elements thus helping them to decide on what products or vegetables to consume safely and what should be minimized.

References

[1] Lenntech, "Water Treatment and Air Purification", 2006. In: http://www.lenntech.com/heavy-metals.htm
[2] Das, A.K., "Metal Ion Induced Toxicity and Detoxification by Chelation Therapy", 1st ed., Delhi: CBS, 1990.
[3] Avena, J.M., "Metallic Poisons". In Poisoning, fourth edition. Charles C Thomas Springfield, Illinois, 1979, pp. 252-258.
[4] Cabrera, C.; Lloris, F.; Gimenez, R.; Olalla, M., Lopez, M.C., *Sci Total Environ*, 2003, 308(1-3),1-14.
[5] Marshall, F.; Agrawal, R., "Wash your Spinach Twice". 2003. In: http://www.indiatogether.org/2003/apr/hlt-vegcontam.htm
[6] Acra, A.; Namaan, S.; Raffoul, Z., *Bull Environ Contam Toxicol*, 1981, 27(2), 209-212.
[7] Kouyoumjian, H.H.; Movsessian, M.; Najjar, E., *Lebanese Science Bulletin*, 1986, 2, 57-63.
[8] Kouyoumjian, H.H.; Tilbian, M.; Najjar, E., *Lebanese Science Journal*, 2001, 2, 37-44.
[9] Esseily, F.; Daher, G., *Annales de Recherche Scientifique (Liban)*, 2000, 2, 69-75.
[10] Hamdan, M.M., Assessment of Lead Pollution in an Urban Community, MSc. Thesis, American University of Beirut (AUB) Medical Library, W4H211a, 2003.
[11] Assaf, H.; Betbeder, A.M.; Creppy, E.E.; Pallardy, M.; Azouri, H., *Hum. Exp. Toxicol.*, 2004, 23(10), 495-501.
[12] Nasreddine, L.; Hwalla, N.; El Samad, O.; LeBlanc, J.C.; Hamze, M.; Sibril, Y.; Parent-Massin, D., *Food Addit. and Contam.*, 2006, 23(6), 579-590.
[13] Sahuquillo, A.; Rubio, R.; Rauret, G., *Analyst*, 1999, 124, 1–4
[14] Concon, J.M., "Food Toxicology: Contaminants and Additives", New York, Marcel Dekker, 1988.
[15] Shils, M.E.; Olson, J.A.; Shike, M., "Modern Nutrition in Health and Disease", Lea and Febiger, Malvern, 1994.
[16] Cabrera, C.; Gallego, C.; Lopez, M.C.; Lorenzo, M.L.; Lillo, E., *J. AOAC Int.*, 1994, 77, 1249-1252.
[17] Finster, M.E.; Gray, K.A.; Binns, H.J., *Sci. Total Environ.*, 2004, 320(2-3), 245-257.
[18] Rahlenbeck, S.I.; Burberg, A.; Zimmermann, R.D., *Bull. Environ. Contam. Toxicol.*, 1999, 62(1), 30-33.
[19] Sterrett, S.B.; Chaney, R.L.; Gifford, C.H.; Mielke, H.W., *Environ. Geochem. Health*, 1996,18,135-142.
[20] USFDA (United States Food and Drug Administration). Guidance Document for Lead in Shellfish. Washington, DC: Center for Food Safety and Applied Nutrition, 1993.
[21] Kachenko, A.; Singh, B., "Heavy Metals Contamination of Home Grown Vegetables Near Smelters in NSW", Proceedings of the Third Australian New Zealand Soils Conference, 2004, 5-9.
[22] Jackson, A.P.; Alloway, B.J., *Plant Soil*, 1991, 132,179-186.
[23] Jing, J.; Logan, T., *J. Environ. Qual.*, 1992, 21,1-8.
[24] Reeves, P.G.; Vanderpool, R.A., *J. Nutr. Biochem.*, 1998, 9(11), 639-644.
[25] McLaughlin, M.J.; Bell, M.J.; Wright, G.C.; Cozens, G.D., *Plant Soil*, 2000, 222(1-2), 51-58.
[26] Schuhmacher, M.; Bosque, M.A.; Domingo, J.L.; Corbella, J., *Bull. Environ. Contam. Toxicol.*, 1991, 46, 320-328.
[27] NRC, National Research Council. Recommended dietary allowances. Subcommittee on the Tenth Edition of the RDAs, Food and Nutrition Board, Commission on Life Sciences, National Research Council. Washington, DC: National Academy Press, 1989.
[28] Garcia, E.; Cabrera, C.; Lorenzo, M.L.; Sanchez, J.; Lopez, M.C., *Br. J. Nutr.*, 2001, 86, 391-396.
[29] Bratakos, M.S.; Lazos, E.S.; Bratakos, S.M., *Sci. Total Environ.*, 2002, 290(1-3), 47-58.
[30] Parveen, Z.; Khuhro, M.I.; Rafiq, N., *Bull. Environ. Contam. Toxicol.*, 2003, 71(6), 1260-1264.
[31] Schuhmacher, M.; Domingo, J.L.; Llobet, J.M.; Corbella, J., *Bull. Environ. Contam. Toxicol.*, 1993, 50, 514-521.
[32] Mann, J.; Truswell, A.S., "Essentials of Human Nutrition", Oxford, Oxford University Press,1998.
[33] ATSDR, Agency for Toxic Substances and Disease Registry, Toxicological profile for Arsenic. 2007. In: http://www.atsdr.cdc.gov/ toxprofiles/phs132.html.
[34] Kim, J.Y.; Kim, K.W.; Lee, J.U.; Lee, J.S.; Cook, J., *Environ. Geochem. Health*, 2002, 24, 215-227.
[35] Preedy, V.R.; Watson, R.R., Reviews in Food and Nutrition Toxicity. Volume 4; chapter 3. Taylor & Francis, 2005, pp. 57-84.
[36] Lança, S.; Alves, A.; Vieira, A.I.; Barata, J.; de Freitas, J.; de Carvalho, A., *Eur. J. Intern. Med.*, 2002,13(8), 518-520.