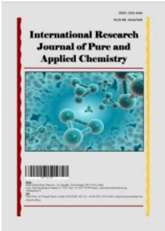

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Analysis and Determination of Lead and Chromium in Commercially Purchased Vegetables (Carrots, Potatoes and Tomatoes)

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Authors' contributions

This work was carried out in collaboration among all authors. The lab work was carried by author JIB and wrote the first draft while author CC performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Excess trace metal contamination in vegetables is a growing concern globally. Plants can be contaminated by trace metals, and it is important to understand the degree of contamination and the inherent risk. Vegetables are a staple in human diets, thus knowing the level of concentration of these metals within the diet is increasingly important. This project is designed to assess levels of trace metals in vegetables using Atomic Absorption Spectroscopy. Atomic absorption spectroscopy (AAS) is an analytical technique that determines unknown concentrations of elements using absorption of light from the desired elements with the aid of the working calibration curve obtained from the series of standards. Tests were done on three different commonly vegetable available in the UK to identify whether levels of trace metals fall within safe levels for human consumption. The vegetables selected were carrots, potatoes, and tomatoes. The concentration within organic vegetables did not significantly differ from the non-organic ones, though the results show, in some cases higher concentration of chromium in the potato and carrot peel. This is worthy of note from a health and nutrition perspective especially for those people that have a deficiency of these trace

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metals in their body or conversely wanted to manage their intake. The mean concentrations of these metals are in decreasing order of >Pb>Cr in most vegetables but in some Cr>Pb. The good thing is that the concentrations of these metals in vegetables purchased in the UK all fall below the maximum limits set by WHO/FAO and are thus considered safe for human consumption.

Keywords: Lead; chromium; vegetable; atomic absorption spectroscopy (AAS).

1. INTRODUCTION

Vegetables form an essential part of a healthy human diet because of their high nutritional value and antioxidants [1], which can be consumed either raw or cooked. Vegetables contain many of the nutrients necessary for the human diet such as carbohydrates, proteins, vitamins, minerals, and trace elements [2]. Trace metals are naturally occurring inorganic substance required in the human body and are a vital component of biological structures [1] but too much or too little of it can be harmful [3] and are associated with many adverse health effects [4]. When consuming vegetables, it is advisable to know the degree of contamination. The concentration levels of these heavy metals within the vegetables and whether it falls within the safe level for human consumption. Ingestion is one of the prime pathways for toxic elements into the human body whilst the vegetables absorb these metals through their roots from the growing soil [5]. The metal contamination in soil can come from various inputs [6] such as industrial waste, fertilisers [7], atmospheric pollution, sewage water, pesticides, and other human activities [3].

Heavy metals such as lead (Pb) and chromium (Cr) can pose a danger to humans through exposure or food intake if more than the maximum limit required is consumed.

Lead (Pb) is a highly toxic metal [8] and is commonly found in the environment and diet. There are no small or minute amounts [9] of lead (Pb) that are safe, and it is particularly harmful to children [10] as their growing bodies absorb lead more easily and their brains and nervous systems are more sensitive to its damaging effects. Clearly there are many ways in which lead can be introduced into a child's environment, from paint to exhaust fumes [11], but it is interesting to note that certain vegetables could also contribute to this. Heavy metals do not biodegrade [3], and some are naturally occurring in the earth crust. Many metals can be removed by washing [12] the vegetables before consumption and the importance of washing vegetables is often stressed in government literatures and health instruction as a result, but some can't be removed by water alone. The FAO and WHO, however, have come out with a provisional tolerable weekly intake of lead which is 25µg/kg though being over this amount does not necessarily mean that health is at risk as it is also a factor of the period of time [13] over which it is taken. Lead poisoning is a classic disease and it can be through chronic or acute, inhalation, skin contact or ingestion. Chronic exposure leads to mental retardation [12,10], disruption of biosynthesis of haemoglobin, birth defects, miscarriages [8], autism, allergies [11], and many more.

Chromium is an abundant element on earth and can be released into the environment through the burning of natural gas, oil, or coal, from where it will be deposited into the soil or water. Other sources of chromium in the environment can be fertilisers, chromium steel and plating as well as tanneries. It occurs in several oxidation states [12] but the most commonly occurring forms are trivalent Cr^{3+} and hexavalent Cr^{6+}.The former is generally harmless in the environment because of its weak membrane permeability but the latter is more active penetrating the cell membrane [11]. Chromium is an important part of human biology particularly regarding glucose absorption and by extension development of type 2 Diabetes. There are many positive health claims about the benefits of Chromium in the body in the correct amounts, however the negative effects of an excess can include gastrointestinal, respiratory, and immune problems as well as cancers [6].

2. MATERIALS AND METHODS

2.1 Instrumentation

Atomic absorption spectroscopy is an analytical technique that measures the concentrations of elements. It can analyse metals and metalloid in a sample. It makes effectively measures the light that is being absorbed by these elements. A detector measures the wavelengths of light transmitted by the sample and compares them to the wavelengths which originally passed through the sample to measure their concentration [14]. Elements to be analysed need to be in atomic state to quantify the absorption of ground state atoms in the gaseous state. It utilises the principle that elements in the gas phase absorbs light at very specific wavelengths which gives the technique excellent specificity and detection limits [15]. The atoms absorb frequency of lights and make a transition to higher energy levels. The amount of light absorbed will determine the concentration of analyte. AAS includes 4 basic components; a light source which is a hollow cathode lamp, an atomizer, a monochromator, a detector and a read-out data processing. Through working calibration curve and after calibrating the instruments with liquids of known concentrations the concentration of unknowns can then be measured.

The characteristics and operating of the instrument are: (Agilent's 28 FS AA, single-element lamps and the instrument parameters for chromium are fixed at lamp current (7 mA), fuel (acetylene), support (air), wavelength (357.9 nm), slit width (0.2 nm) and optimum working range of 0.06 – 15.0 µg/mL). Lead fixed working parameters are at lamp current (5 mA), fuel (acetylene), support (air), wavelength (217.0 nm), slit width (1.0 nm) and optimum working range of 0.1 – 30.0 µg/mL).

2.2 Sample Preparation and Digestion

All samples were purchased from a supermarket (ASDA) Bangor Farrar Road in north Wales United Kingdom. Most ASDA vegetables are supplied by local farmers. Carrots, parsnips and pumpkins are being supplied from Kellington, North Yorkshire UK.

Digestion is required in every analytical experiment to extract samples from a solid material and turn into solution for analysis. It can either be done through an open-air procedure or closed method.

The principle of nitric acid-digestion is that HNO_3 acts as both an acid and oxidising agent. As an acid it dissolves in inorganic oxides e.g.

$$CaO + 2H_3O \rightarrow Ca^{2+} + 3H_2O$$

and as an oxidising agent HNO_3 can oxidise zero valence inorganic metals [16] and non-metals into ions e.g.

$$Fe + 3H_3O^{+} + 2HNO_3 \rightarrow 2NO_2 + 3Cu^{2+} + 10H_2O.$$

Consequently, HNO_3 does not form any insoluble compounds with metals and non-metals whereas other acids do such as H_2SO_4 and HCl, which makes it ideal for use in this process. HNO_3 changes to gaseous NO_2 which suitable in the sense that it will evaporate in the medium reaction i.e. $H_3O^{+} + HNO_3 + e^{-1} \rightarrow NO_2 \uparrow$(brown) + $2H_2O$. In the hot plate open-air procedure an aliquot of samples (0.5 g) in digestion tubes were digested in an acid HNO_3 (5 mL) and heated for 4 hours in the fume hood – first at 80˚C for an hour, 100˚C for another hour, and finally120˚C for 2 hours. Multiple digestion was done at different times for each vegetable to obtain the number of repeats required. At the start of the digestion, a foamy, cloudy and dark-orange vapour from the mixture was observed but after the 4 hours of digestion the solution in the tube turned clear suggesting digestion is complete. Nitric-acid digestion was the only method used in this experiment due to water-solubility of the by-products. Nitric-acid salts are soluble in water and that is safer and can be disposed of accordingly. All samples were digested in triplicates. Gravity filtration was applied to remove any remaining solids that may cause blockage for AAS analysis. The experimental materials are five different samples of plant products (duplicated for organic ones); new potatoes, carrots, and cherry tomatoes. Potato and carrot peels were analysed separately.

Each sample was cut individually into bite-size or strips and dried overnight in the laboratory oven at 105°C. The dried samples were then finely powdered using the cyclone miller. Each sample was weighed (0.5 g) and digested with HNO_3 (5ml) in digestion tubes for four hours at increasing temperature (1 hour at 80°C, another hour at 100°C and finally 2 hours at 120°C). The clear solution was cooled down and filtered to remove any form of solid residue left after the process.

Table 1. Permissible max limit of heavy metals in vegetables from different published paper [18]

	Pb	Cr
Permissible Max limit	0.30 (µg/g) 10 mg/kg 25 µg/week	30 mg/kg

Table 2. Concentrations in mg/kg of investigated vegetables in Pb and Cr of mean ± standard error

Elements	Samples	potato		carrot		tomato
		meat	peel	meat	peel	
Cr	Non-org	0.5 ±0.06	0.8 ± 0.06	0.03 ± 0.06	0.3 ± 0.03	0.08 ± 0.005
	Organic	0.3 ± 0.02	0.1 ± 0.04	0.3 ± 0.04	0.3 ± 0.06	0.1 ± 0.03
Pb	Non-org	0.15 ± 0.1	0.15 ± 0.1	1.8 ± 0.14	2.3 ± 0.1	0.15 ± 0.12
	Organic	1.1± 0.2	1.3 ±0.6	1.4 ± 0.1	1.0 ±0.1	1.3 ± 0.2

Table 3. Limit of detection and limit of quantification of some heavy metals

µg/L	Pb	Cr
Limit of detection (LoD)	0.2	0.02
Limit of quantification (LoQ)	0.8	0.07

The aim of this work was to investigate the concentration of lead and chromium in commercially purchased vegetables (carrots, potatoes, and tomatoes) and to compare these values to the available literature and the of WHO/FAO to see if the are within safe limits.

3. RESULTS AND DISCUSSION

Multiple analysis was carried out on each vegetable and as a comparison a similar set of tests were undertaken on vegetables sold as organic. The concentrations of the trace elements Lead and Chromium (Table 3) do vary from each other as expected but results are consistent through the numerous repeats done during the project. The results are not exactly in agreement with most of the published works as expected due to being carried out in different countries with different agricultural conditions, soil contamination, industrial regulations, environment and therefore different pollution levels and corresponding contamination. A recent study into lead in vegetables and fruit in the UK did return very similar results to this project confirming the variation to other published results is most probably environmental. The content of Cr in organic tomato is greater than that found in the non-organic tomato and is also higher than the levels of concentration reported in the literature on a study carried out in different countries [11,14]. The same was found with the organic tomatoes Cr concentration and the carrot peel both being higher than the non-organic. However, worryingly content of Pb in organic potato peel, carrot peel and tomato are also higher than the non-organic (Table 2). All results recorded are within the permissible amount (Table 1) for the human body. The results which a mean of 3 triplicates (Table 1) for organic vegetables were interesting if one considers the positive messages about the 'purity' of buying organic as in many cases they were not significantly different from their non-organic equivalent. In some cases, the results were slightly lower than for non-organic, and in others higher depending on the trace metal but not, it should be said, significantly except Cr and Pb concentrations in potato peel which came out higher than the literature [3]. It can be inferred that there are benefits to organic vegetables, but the contamination route is more complex than simple use of chemical fertilisers. The conditions in the environment [3] are an important factor in achieving the levels of `purity' suggested within the advertising and particularly regarding lead contamination which is of increasing global concern. Limit of detection and limit of quantification were calculated from running a blank (H_2O) with each analysis (Table 3).

4. CONCLUSION

The healthy human balanced diet involves having heavy portions of vegetables every day, the so called `5-a-day'. This study suggests that the general belief that vegetables are automatically a healthy option may not always be the case, particularly with regards to Pb. The results between organic and non-organic samples could suggest that this is not simply a matter of poor farming standards or methods. The regulations within the UK on farming and food standards are high and if anything, one could conclude that there are many environmental factors involved in the contamination of foodstuffs rather than simply farming practise, confirming for example the decision to move to lead free petrol [11]. There is increasing awareness of the dangers of some elements in food and the environment, lead is certainly one of the more worrying. There is a growing appreciation that this is becoming a problem due to imprudent agricultural practices [7] and industrial pollution with lead presenting a serious threat to human health [1]. Heavy metals in soil can affect quality of food [19] and plant growth.

The analysis results on these three chosen vegetables shows that they are not heavily contaminated with heavy metals and that the selection of organic vegetables will not significantly change the amount a consumer would ingest. Consumers in the UK can eat vegetables with a certain comfort that they are for the most part the healthy option. However, it is still worth remarking that carrot and potato peel show higher concentration than the flesh confirming the importance of peeling these vegetables should you wish to reduce the number of heavy metals ingested. Furthermore, growing vegetables organically is still worth doing despite the minimal difference in concentration compared to the non-organically grown ones, but it could be improved over the years by using more naturally made fertilisers such as manures etc and perhaps the true organic value may come to include the location of the farm, its soil quality and proximity to urban or industrial centres.

For future works, a suggestion would be to analyse washed vegetables in comparison to the unwashed to confirm the likely benefits of washing vegetables during preparation. Various digestion methods [19] could also be applied to improve the correlation of results as well as matrix recovery [16]. In this set one leafy vegetable was tested and some results explained by the larger surface area, testing more leafy vegetables would be useful to be able to confirm this causal link and see a wide range of results. It is important to weigh out samples meticulously, clean glassware thoroughly and filter solution properly. Use of a miller specifically dedicated to the study for the entire analysis would potentially improve the results as the machine could contaminate samples while milling due to powder residue from previous users being present. Cleaning the miller does not totally guarantee no-leftover especially the brown floppy air outlet dust filter which does not seem to clean-off that well and is where much of the remaining dust from samples collects via observation. Always check capillary tubes to ensure it is working well and is constantly sucking the solution in every analysis as sometimes solution can solidify or coagulate inside the tube causing blockage which hinders the solution passing through the flame and alter its absorption resulting in negative errors or higher concentration than expected. Other analytical techniques can also be considered such as X-ray fluorescence diffraction, ICP-MS, GFAAS and ICP-OS depending on the metal being analysed as there are always pros and cons to each technique. EG the ICP-MS, can analyse a lower detection limit than AAS but works on multiple elements. It is also suggested that estimation of Pb and Cr should be done in the water supplied for irrigation and in the soil in which these are grown. It is further suggested that root hair zone (absorptive zone of the respective roots), if done, will provide better information on the accumulation of these metals in vegetables investigated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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