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TRACE METAL ANALYSIS OF ORGANIC VEGETABLES SOLD IN SOME SUPERMARKETS IN MANILA, PHILIPPINES

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ABSTRACT

Recent years have seen the rapid growth of the *organic* food products industry, primarily driven by the consumers' desire for a healthier lifestyle. Similar to worldwide trend, explicitly-labeled "Organic" food products have become ubiquitous in the Philippines, with the consumers most of the time having no information on the quality of the products. In the Philippine setting, very few researches have focused on the analysis of *organic* vegetables, and in this study, the trace-metal (cadmium, copper, iron, nickel, and zinc) concentration of specific organic and conventional vegetables (cabbage, celery, leek, lettuce, and spinach) that are being sold in some shops in the cities of Makati and Manila, Philippines were determined using atomic absorption spectroscopy. The mean concentration for copper, iron, nickel and zinc in the samples were calculated to be between 0.0146-0.881 mg/kg, 0.648-13.1 mg/kg, 0.0409-2.04 mg/kg, and 0.266-2.87 mg/kg, respectively, while cadmium levels varied from 0.005-0.772 mg/kg (with some samples below the limit of detection). Nevertheless, statistical analysis ($p < 0.05$) showed more organic vegetables having no significant differences than conventional ones indicating that in terms of the content of these trace metals, being organic may not necessarily mean better.

1. Introduction

Vegetables are very important when it comes to the eating habits of humans in order to be healthy. These food products provide many health benefits which include being a good source of nutrients such as folate or folic acid, fibers, potassium, vitamins A, E, and C, in addition to having antioxidant effects (Slavin, 2012). These nutrients found in vegetables are crucial and important in the health and well-being of the body, but they may also pose negative effects to a person's health due to potential occurrence of high concentration of certain metals. Research has shown that vegetables grown in contaminated farmlands absorb chemicals which cause heavy metal contamination of crops such as cadmium, iron, nickel, etc. (Wuana, 2011). These heavy metals cannot be processed by the body and can undergo bioaccumulation inside an organism.

Heavy metals can infiltrate the food cycle, vegetables in particular, through environmental contaminations such as the application of certain agricultural aids like fertilizers and pesticides to the soil or to the crop (Wuana et al., 2011). Crops that are grown without the use of these agricultural aids and instead are grown with renewable resources so as to improve its environmental quality and health benefits are called "organic",vand are reported to have lower levels of heavy metals.

Plants have diverse capabilities when it comes to the removal and the accumulation of metals (Chibuike, 2014). Vegetables that are leafy tend to absorb and accumulate more metals from its environment (Ali et al., 2012). There is even the possibility that metal contamination in vegetables may increase due to an escalation in the heavy metal concentrations of certain soils due to the rapid increase in industrial and urban activities. Nevertheless, consuming certain metal-contaminated vegetables (e.g.. high heavy metal content) may pose serious health risks to humans since these are not easily metabolized and thus can be bioaccumulated in certain vital organs, causing various types of sickness depending on the chronic and acute exposure (Chibuike et al., 2014).

The organic industry of the Philippines was valued at US $10 million during the year 2003, with an estimated 20% annual growth rate (Pearl2, 2007). In 2014, the Philippines had the fourth largest number of organic producers and most organic agricultural land at 165,974 producers and 101,278 hectares respectively among Asian countries (Willer, 2016). The consumption of vegetables in the Philippines has been increasing through the years from 1990-2005; the 1.6% increase in annual consumption of vegetables surpassed the 1.5% increase in annual vegetable production (Porciuncula, 2014). Unfortunately, reports on trace metal content of organic and conventional vegetables being sold in the Philippine market are lacking, and since buying organic produce usually costs higher, it is important for consumers to know if they are indeed getting their money's worth. In this study, trace metal content of different vegetables and brands, both organic and conventional, being sold specifically in Makati and Manila, were determined and analyzed to help serve as a guide for consumers in their decision-making process.

2. Materials and methods

2.1 Vegetables Samples

Three brands of organic vegetables were identified and purchased in supermarkets in Makati and Manila, Philippines. For each organic brand, five vegetables were bought, namely cabbage, celery, leek, lettuce, and spinach. For conventional samples, the same five vegetables were bought from side-road vendors.

2.2. Preparation of vegetable samples

For the duration of the experiment, only the leaves of the vegetables were used. The collected leaves were rinsed in distilled water and wrapped individually, separating one vegetable from another, with properly labelled aluminum foil. Rinsing and drying of samples were done in batches, based on the brand of vegetables, over the course of 3 weeks. The samples were dried in an oven at 100^{o}C to 120^{o}C, each batch requiring 3 - 4 hours for the drying process to be completed. After drying, the samples were then subjected to acid digestion (Hunt, 1982).

Two grams of each vegetable was accurately weighed using an analytical balance and transferred to 250 mL Erlenmeyer flask. 25 mL of 12 M HCl was added to each flask, followed by heating using the hot plate at low setting for twenty minutes under a hood. Afterwards, 10 mL of distilled water was added. The solution was filtered to 50 mL volumetric flask three times using filter papers. The filtrate was diluted to the mark with distilled water passing through the filter paper. The entire process was done in triplicates for each vegetable.

2.3. Trace Metal Analysis

The concentrations of copper, iron, nickel, and zinc in each of the digested samples were determined using the AA-6300 SHIMADZU atomic absorption spectrophotometer. A stock solution containing 100 ppm of all four metals to be analyzed was purchased from Thermo Fischer Orion. Varying amounts of the stock solution were transferred to 50 mL volumetric flasks and diluted to the mark using distilled water to prepare standard solutions having concentrations ranging from 0 ppm to 15 ppm. The type of gas used was air-C_2H_2, with burner height of 7 mm and slid width of 0.7 nm. For all of the trials, the fuel gas flow rate was 1.8 L/min while the support gas flow rate was 15.0 L/min. A standard curve was also formulated for each of the metal analyzed. The wavelengths used for the analysis were 228.8 nm, 324.7 nm, 248.3 nm, 232.0 nm, and 213.9 nm for cadmium, copper, iron, nickel, and zinc respectively.

2.4. Statistical Analysis

The data gathered were compared and analyzed using one-way analysis of variance and Tukey multiple comparisons test ($p < 0.05$) using Graphpad Prism ver. 6.07.

3. Results and discussions

Despite their apparent necessity in human diet, studies have shown that vegetables can absorb and accumulate metals from the soil due to sewage effluents, waste water irrigation, conventional pesticides, and synthetic fertilizers (Alloway, 2013). Concerns on the health risks posed by excessive intake of trace metals, among others, have increased the consumers' demand for organic food products that are deemed to be the healthier option compared with conventionally grown produce. However, there is a lack of scientific evidence in support of this claim, especially in the Philippine market.

Using atomic absorption spectroscopy, samples of five organic and conventional vegetables (cabbage, celery, leek, lettuce, spinach) were analyzed for their trace metal (Cd, Cu, Fe, Ni, Zn) concentrations. In the experiment, metal content in the vegetables generally followed this trend: Fe > Zn > Ni > Cu > Cd (Figs. 1 and 2). These results were found to be in accordance with several studies reported earlier. In a study comparing Fe, Cu, Cd and other metal concentrations in 13 vegetable samples taken from agricultural sites in Punjab, India, Fe was accumulated the highest and Cd was accumulated the lowest for every vegetable (Sharma, 2016). Furthermore, a study done by Hoque et. al. concluded that among Zn, Cu, and Ni, the highest average concentration of trace metals in 8 samples (tomato, bottle gourd, green amaranth, red amaranth, chili, and banana) was found to be Zn, while Ni had the lowest concentration among the three metals (Hoque, 2014). Another study done produced a similar trend with regards to trace metal concentration in spinach, lettuce, cabbage, coriander, radish, and cauliflower (Anwar, 2008). Other studies concerning the estimation of heavy metals in other vegetables reported similar results (Arora, 2008; Singh, 2010; Uwah, 2011).

Varying amounts of metals in vegetables can be attributed to a number of factors such as natural and anthropogenic sources, oxidation and reduction reactions, adsorption and dissolution reactions, and nonorganic and organic complexations of these trace metals in the soil. For Fe, its abundance is due to it being a biologically essential component of every living organism (Aisen, 2001; Lieu, 2001). In addition, sources of Fe in the soil are mineral compounds that can constitute about 30-40% of the earth's composition, deteriorating metals, and industrial wastes (Polanski, 1988). For these reasons, it is sensible that several studies recorded iron concentration to be among the highest in various vegetable samples. On the other hand, Cu accumulates mainly in the root system of the crops because under excessive concentration of Cu, root tissues of plants have shown a strong capability to hold Cu from being transported to the shoots (Bigdeli, 2008). Thus, only a small fraction of Cu will be found in the shoot system of the crop. Since this report only analyzed trace metal concentrations in the leaves, this may be a probable reason behind lower Cu concentration found in the samples compared to other trace metals. It was also remarked that Cu deficiency can be observed in soils that are rich in organic matter which reduce mobility and availability of Cu (Oorts, 2013). This is due to its tendency to be adsorbed by carbonates and clay minerals, both of which having a rather large binding capacity for Cu (Logan, 1997). Compared to other

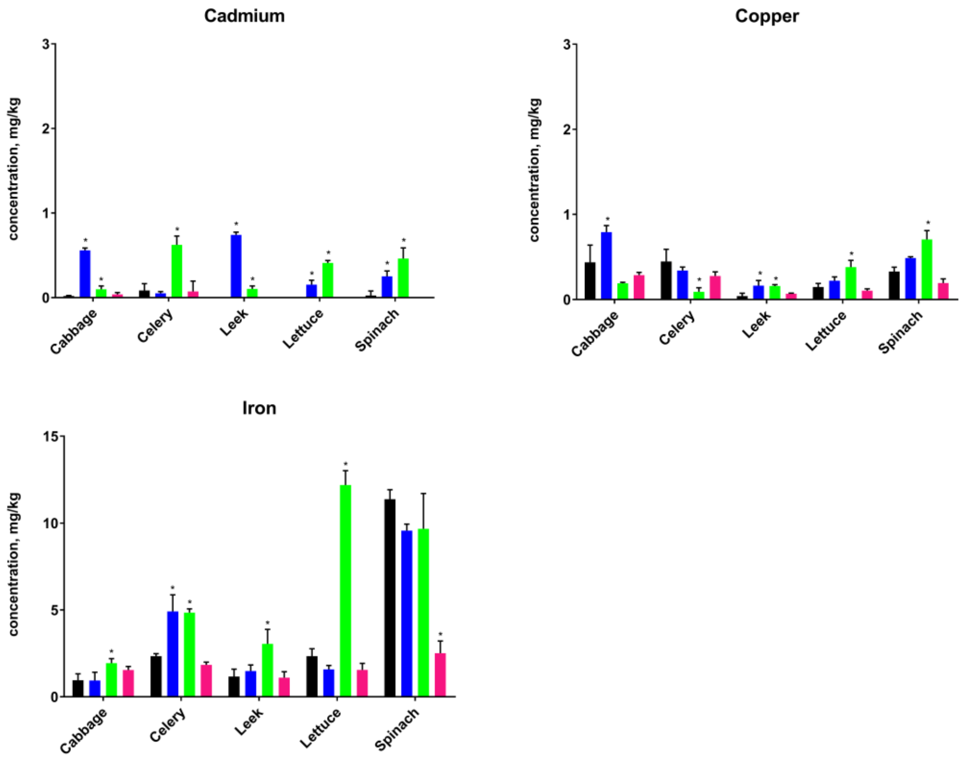

Figure 1. Average concentration of cadmium (Cd), copper (Cu), and iron (Fe) in various vegetables and brands. Black bars represent conventional vegetables, while blue, green, and pink bars represent Brand A, B, and C organic vegetables, respectively. Some samples have Cd concentrations that are below the limit of detection. Error bars indicate standard deviations. Asterisk indicate significant differences with the conventional vegetables ($p < 0.05$). The y-scale for Fe is different from the other metals.

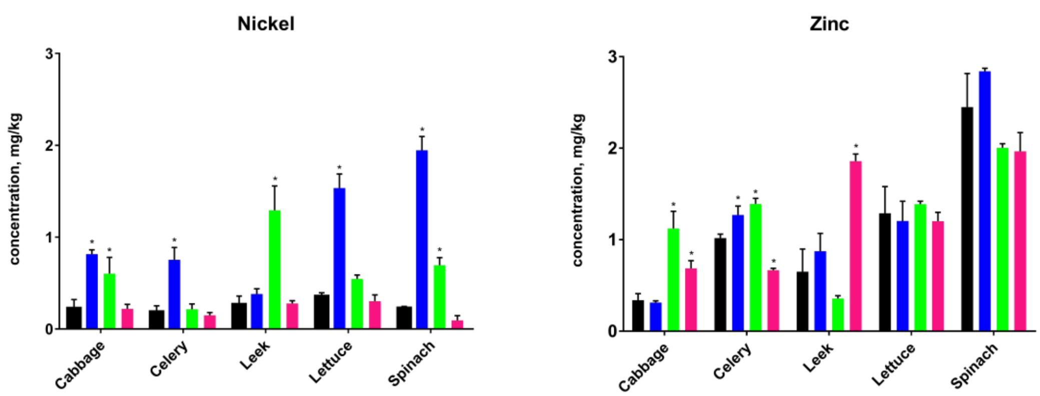

Figure 2. Average concentration of nickel (Ni) and zinc (Zn) in various vegetables and brands. Black bars represent conventional vegetables, while blue, green, and pink bars represent Brand A, B, and C organic vegetables, respectively. Error bars indicate standard deviations. Asterisk indicate significant differences with the conventional vegetables ($p < 0.05$).

metals, concentration of Cd in earth's crust is relatively low at 0.1 to 0.5 ppm (Jensen, 1992) and this may be linked to the low Cd concentration detected in the vegetable samples.

Despite the similar trend on metal concentration found in a variety of vegetables, it is important to note that the range of metal concentrations differ from each study. This difference can be attributed to several environmental factors surrounding vegetable growth and development. Several sources of contamination of the environment where vegetables are planted include emissions from the rapidly expanding industrial areas, disposal of wastes, animal manures, leaded gasoline and paints, land application of fertilizers, mine tailings, pesticides, wastewater irrigation, coal combustion residues, spillage of petrochemicals, and atmospheric deposition (Khan, 2014; Zhnag, 2014)

Furthermore, different vegetables have different characteristics that may enable them to accumulate more metals than others. In this report, spinach contained higher metal concentrations compared to other vegetables in all but a few cases (Figs. 1 and 2). Spinach has leaves with a large surface area that makes it more likely to accumulate metals (Ali, 2012). Spinach also has a higher transpiration, in which moisture is carried from the roots to the leaves of the plants to be released. Due to this and spinach's faster growth rate, translocation of metal in spinach is faster and has higher rate of metal absorption (Muchuweti, 2006; Alia, 2015). These findings are also consistent with previous literature on spinach and its capability to absorb and retain trace metal (Osaili, 2016; Hoefkens, 2010; Bvenura, 2012).

Data gathered in this report were subjected to statistical analysis and the presence or absence of significant differences among the different samples were determined using the Tukey multiple comparisons test at $p < 0.05$ (Figs. 1 and 2). Results show that trace metal content of majority of organic vegetable samples did not differ significantly from their organic counterparts, and among those that are significantly different, organic samples were found to have higher metal concentrations than their conventional counterpart except in a few cases. Other literatures comparing conventional and organic vegetables have likewise reported that conventional vegetables have less metal concentration than the organic ones. As an example, a report has concluded that contaminants in organic vegetable samples were slightly higher than the conventionally grown vegetables (Hoefkens, 2010). Furthermore, studies comparing nutrients in conventional and organic vegetables showed lack of evidence that the two populations have significantly different nutrients contents (Bourn, 2002; Dangour, 2009; Williams, 2002). While a trend in the metal concentration of vegetable samples is observable, most of the trace metals analyzed are essential nutrients for various biochemical and physiological functions. Their functions in biological systems are well-known; for example, trace metals can participate in redox reactions, and they can also bind to proteins in enzymatic systems, and deficiency, diseases or syndromes may be evident when trace metals are supplied inadequately. However, upon reaching a certain level, accumulation of these same trace metals may result to serious health concerns. Excess Fe was determined to cause organ dysfunction due to the production of reactive oxygen species (ROS) (Kohgo, 2008). Similarly, accumulation of Cu produces ROS that lead to aging and the development of cancer and diseases of the nervous system (Halliwell, 1990). At higher concentrations, Zn and Ni are also associated with reduced immune function, lower levels of high-density lipoproteins, decreased body weight, and infections in the intestine (Hamilton, 2000; Henderson, 2012). Unlike the rest, Cd is not involved in human nutrition and higher biological systems. It is classified as a carcinogenic and exposure to higher levels of Cd is associated with prostate, renal, and lung cancers (Jarup, 2002; Waalkes, 2003). The mean concentrations of heavy metals in various vegetable species were compared with the standards set for vegetables by FAO & WHO (2014). These values are summarized in Table 1.

Table 1. Range of trace metal content (mg/kg) of various vegetables analyzed compared to safe limits established by WHO/FAO.

	Copper	Iron	Nickel	Zinc	Cadmium
Cabbage	0.182 – 0.881	0.648 – 2.24	0.106 – 0.867	0.266 – 1.33	0.0186 – 0.587
Celery	0.0391 – 0.605	1.74 – 5.98	0.106 – 0.908	0.649 – 1.46	0.00500 – 0.711
Leek	0.0146 – 0.231	0.716 – 3.91	0.156 – 1.60	0.330 – 1.92	0.0740[a] – 0.772
Lettuce	0.0852 – 0.464	1.17 – 13.1	0.235 – 1.71	0.954 – 1.49	0.119[a] – 0.442
Spinach	0.165 – 0.790	1.97 – 12.0	0.0409 – 2.04	1.77 – 2.87	0.0282[a] – 0.596
Tolerable Intake WHO/FAO*	0.5 mg/kg bw per day	0.8 mg/kg bw per day	1.2 mg/kg bw per day	0.3-1 mg/kg bw per day	25 µg/kg bw per month

* bw: body weight
[a] some samples had Cd levels below the detection limit

4. Conclusions

This study reports the trace metals (Cd, Cu, Fe, Ni, and Zn) content of cabbage, celery, leek, lettuce, and spinach sold in some supermarkters in Manila, Philippines, both organic and conventionally-produced. The order of the metal content in the vegetables generally followed this trend: Fe > Zn > Ni > Cu > Cd. The mean concentration for Cu, Fe, Ni and Zn in the samples were calculated to be between 0.0146-0.881 ppm, 0.648-13.1 ppm, 0.0409-2.04 ppm, and 0.266-2.87 ppm, respectively, while cadmium levels varied from 0.005-0.772 mg/kg with some samples containing amounts less than the instrument detection limit. Discrepancies between each sample and its metal content can be attributed to several factors. One of which is the type of vegetable used. Spinach was shown to absorb the highest metal concentration. This may be attributed to physical characteristics of spinach, its higher metal translocation, and faster metal absorption rate. Statistical analysis ($p < 0.05$) showed more organic vegetables having no significant differences than conventional ones indicating that in terms of the content of these trace metals, being organic may not necessarily mean better. The results in this study may serve as a guide for consumers in their choice between organic and conventionally produced vegetables, although more analysis is needed to give a complete picture of the chemical contents of these two types of products.

5. References

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