Liu et al., Int J Earth Environ Sci 2022, 7: 198
https://doi.org/10.15344/2456-351X/2022/198

International Journal of
Earth & Environmental Sciences

Short Communication Open Access

A Study on Nitrate Contents in Vegetables in the Watershed of Lake Tega in Japan

Huan Liu[1,2,*] and Guangwei Huang[1,2]

[1]*Institute for Studies of the Global Environment, Sophia University, 7-1 Kioicho, Chiyoda-ku, Tokyo, 102-8554, Japan*
[2]*Graduate School of Global Environmental Studies, Sophia University, 7-1, Kioicho, Chiyoda-ku, Tokyo, 102-8554, Japan*

Abstract

In this study, investigations on nitrate contents in vegetables are conducted in the watershed of Lake Tega in Japan. Four groups of vegetables, leafy, brassica, root and tuber, and fruiting, were sampled for testing. Among the four groups of sampled vegetables, nitrate contamination shows a decreasing order from the leafy vegetables, brassica, root and tuber, and fruiting vegetables. A nitrate concentration of 6,300 mg/kg, 4,000 mg/kg and 1,500 mg/kg were found in lettuce, spinach, and Chinese cabbage. The study focuses on the differences of the nitrate distributions in various crops. Findings from the detailed testing of nitrate distributions in various parts of crops suggests that some portions of vegetables, such as the inner part of Cabbage and turnip, can be considered as much suitable and healthier for young children. The nitrate content of vegetables before and after cooking is very different. After cooking, the nitrate content of vegetables is very much reduced.

Publication History:

Received: March 28, 2022
Accepted: April 11, 2022
Published: April 13, 2022

Keywords:

Nitrate contents, vegetables, Watershed of Lake Tega, Japan

Introduction

Vegetables are considered to be the most important dietary nitrate source [1]. High levels of nitrate contents in vegetables have been a significant health issue all over the world. Various studies have revealed that vegetables contribute to over 90 percent of our daily non-water dietary nitrate intake in the UK, Belgium, Finland, and Italy [2-5]. Furthermore, the intensive usage of chemical fertilizers in agricultural production also resulted in an increasing amount of nitrate concentration in the environment. Thus, there have been growing concerns about the nitrate concentration in human's daily dietary sources, especially in the vegetables [6].

With regards to the elevated nitrate level possibly leads to increased health risks, the former European Scientific Committee on Food (SCF) recommended an Acceptable Daily Intake (ADI) for nitrate ion of 3.7 mg/kg bodyweight per day, equivalent to 222 mg nitrate per day for a 60 kg adult [7]. This recommendation was also endorsed by the Joint Expert Committee on Food Additives (JFCFA) in 2002, which is an organization of the Food and Agriculture Organization (FAO) of the United Nations/World Health Organization (WHO) [8]. Meanwhile, the US Environmental Protection Agency (EPA) provides a References Dose for nitrate of 1.6mg nitrate nitrogen/kg bodyweight per day, equivalent to about 7.0 mg nitrate nitrogen/kg bodyweight per day [9].

Although various studies have been conducted regarding the daily nitrate intake, most of them were reported from Europe and the US. In Asian, the studies of Ishiwata et al. [10] and Guo et al. [11] also reported higher levels of nitrate dietary exposures compared to ADI. However, there are only limited case studies conducted in the watershed of Lake Tega. This research investigates the nitrate concentration in vegetables in the Watershed of Lake Tega in Japan. The objective is twofold: 1) to gain more insights on nitrate contamination in vegetables in this area; 2) to examine nitrate distribution in crops by measuring nitrate contents in various parts of crops; 3) to investigate the effects of fertilizers overuse and nitrate accumulation in vegetables on human health.

Materials and Methods

Study areas

Lake Tega covers a total land area of 6.5 square kilometers with a perimeter of 38 kilometers. There are approximately 530,000 populations living in the nearby cities in its catchment area, including Matsudo, Kashiwa, Nagareyama, Abiko, Kamagaya, Inzai, Shiroi and a village, Motono. Lake Tega has been rated as the most polluted lake in Japan for 27 consecutive years since the year of 1974, when Japanese Environment Agency first launched its annual nationwide survey on lake and marsh water quality. It is worth noting that there are no large-scale factories or other industrial facilities in this catchment area, so household wastewater is the main source of pollutant leading to the degradation of the water quality in this area. The household wastewater, in this context, is defined as the water used and discharged due to daily household activities, such as cooking, washing, bathing, and toilet wastewater.

Methodology

Our testing methodology first extracts a small amount of plant juice (in a range of 0.3 ml to 2 ml) from crop samples by squeezing them into a container; and then measures its nitrate ion concentration using a Horiba compact nitrate ion meter (LAQUAtwin-B741). This meter is based on the Ion Selective Electrode method. It is one of the most frequently used potentiometric sensors for determining various ions concentrations dissolved in aqueous solutions in both laboratory analysis and industrial and environmental monitoring. Compared to other analytical techniques, for instance, the ion chromatography and the colorimetric method, Ion Selective Electrode method is simple to

[*]**Corresponding Author:** Huan Liu, Institute for Studies of the Global Environment, Sophia University, 7-1 Kioicho, Chiyoda-ku, Tokyo, 102-8554, Japan; E-mail: liulittlehuan@gmail.com

Citation: Liu H, Huang G (2022) A Study on Nitrate Contents in Vegetables in the Watershed of Lake Tega in Japan. Int J Earth Environ Sci 7: 198 doi: https://doi.org/10.15344/2456-351X/2022/198

Citation: Liu H, Huang G (2022) A Study on Nitrate Contents in Vegetables in the Watershed of Lake Tega in Japan. Int J Earth Environ Sci 7: 198 doi: https://doi.org/10.15344/2456-351X/2022/198

use, it has an extremely wide range of applications. More importantly, its measurement range is well suited for measuring nitrate ion distribution in crops [12]. Furthermore, it is an instrument that enables on-site measurements without sample dilutions, which significantly accelerates the analysis of nitrate ion concentration in vegetables and provides critical information on nitrate dynamics in remote regions.

Sampling

We randomly sampled four types of vegetables from the watershed of Lake Tega, including leafy, brassica, root and tuber, and fruiting vegetables. Then we measured their nitrate contents using the methodology described above. Specifically, 25 of the species of the vegetables we tested such as spinach, Chinese cabbage, lettuce, broccoli, turnip, green pepper, eggplant, onion, carrot, corn grain, tomato, cucumber, and apple. We chose them for this study because they are popular vegetable choices of the local population. Additionally, all measurements were done under fine weather during daytime.

Nitrate levels in different parts of the vegetables were analyzed separately. Firstly, nitrate contents in outer leaves; innermost leaves, and stems were separately analyzed for leafy vegetables (Chinese cabbage and lettuce). Secondly, nitrate concentration in stem, root, and flower head were measured for broccoli. Thirdly, for the fruit samples like corn and tomato, nitrate contents in stems and fruits were measured. Lastly, nitrate concentrations in turnip were tested before boiling and after boiling.

Results and Discussions

With regard to the experimental results of nitrate contents in the four types of vegetables, higher levels of nitrate concentration were found in leafy vegetables such as lettuce and Spinach, whereas lower levels occur in tubers or fruits such as kiwifruit, blueberry, and tomato. It indicates a decreasing sequence of nitrate concentration from leafy, brassica, root and tuber, to fruiting vegetables as depicted in Figure 1. A nitrate concentration of 6,300 mg/kg, 4,000 mg/kg and 1,500 mg/kg were found in lettuce, spinach, and Chinese cabbage, respectively. Moderate nitrate contents were found in root and tuber vegetables such as broccoli, turnip and ginger with values of 3,400 mg/kg, 3,000 mg/kg and 2,200 mg/kg, respectively. Nevertheless, lowest nitrate concentration was found in one of the fruiting vegetables - kiwifruit with a value of 75 mg/kg. This finding is consistent with the results of nitrate contents reported from European countries [1].

Our finding is elucidated by nitrate translocation and physiological structure of plants. In a plant, nitrate is mainly stored in cell vacuoles and is transported in a tissue called xylem, whose function is basically to carry water along with dissolved nutrients from the roots to the leaves, whereas the phloem mainly transports the soluble organic substances that are made during the phase of photosynthesis from the leaves to the growth points of the plant. Thus, the least nitrate concentration was found in fruiting organs such as seeds and fruits. Since fruits are mainly composed of parenchyma cells whose primary function is to store the organic materials produced from the photosynthesis of leaves or roots. Higher nitrate content was found in leafy vegetables and tubers such as Chinese cabbage, lettuce, broccoli, and turnip in which the majority of inorganic nitrate ion is accumulated and cannot be assimilated through photosynthesis.

Furthermore, highest nitrate concentration was found in the leafy vegetables of lettuce, spinach, and Chinese cabbage with a value of 6,300 mg/kg, 4,000 mg/kg and 1,500 mg/kg, respectively. In the Commission Regulation No. 1881/2006, the European Commissions has laid down the maximum levels of nitrate in lettuce during harvest seasons [7]. For lettuce harvested from 1st Oct to 31st Mar, the recommended nitrate concentration is 4,000 mg/kg for those grown in the open air, and 4,500 mg/kg for those grown under cover. For lettuce harvested from 1st Apr to 30th Sep, the recommended nitrate concentration is 2,500 mg/kg for those grown in the open air, and 3,500 mg/kg for those grown under cover. On the contrary, the Japanese government has not established any legal standards for nitrate concentration as contaminant in food. Based on the European standards, nitrate contents in lettuce produced from Tega in Japan has significantly exceeded the standard with a value of 2,500 mg/kg

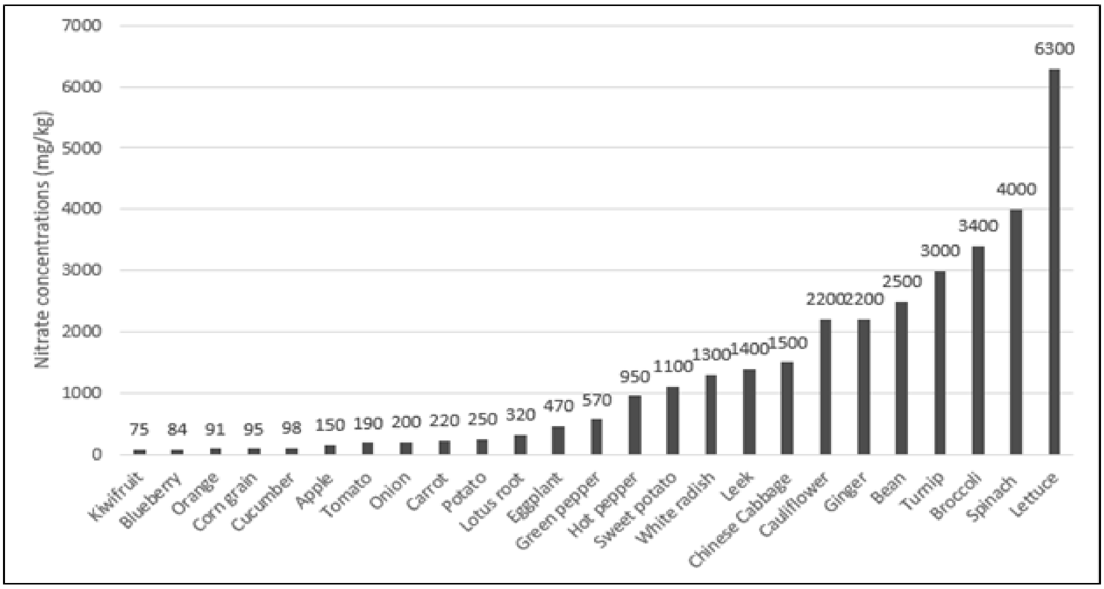

Figure 1: Nitrate contents in measured vegetables.

Citation: Liu H, Huang G (2022) A Study on Nitrate Contents in Vegetables in the Watershed of Lake Tega in Japan. Int J Earth Environ Sci 7: 198 doi: https://doi.org/10.15344/2456-351X/2022/198

for those grown in the open air. This implies that there is a potential health risk for the local people in the Tega region.

We also measured the nitrate distributions between the inner and outer parts of the leaves for Chinese cabbage, lettuce, and cabbage. High nitrate content was found in the outer parts of those three vegetables with a value of 1,900 mg/kg, 3,200 mg/kg and 1300 mg/kg, respectively, whereas low nitrate levels was found among the inner parts with a value of 1,200 mg/kg, 1,100 mg/kg and 650 mg/kg, respectively. Thus, nitrate concentration in leafy vegetables illustrated a remarkably decreasing trend from outer (older) leaves to inner (young) leaves as depicted in Figure 2.

The distribution of nitrate concentration in leafy vegetables is mainly driven by the vacuoles and nutrients transport systems. Vegetables store most of their nitrate in the central vacuoles [13]. Large and water-filled central vacuoles in mature living vegetable cells can occupy 80 to 90 percent of the overall volume of the cell, vegetable cells increase their sizes basically by taking up water into those large central vacuoles, thus the osmotic pressure of the vacuoles are maintained at a sufficiently high level so that water molecules can enter from the cytoplasm. Furthermore, similar to the plasma membrane, which regulates uptake into the cell, tonoplasts (membranes of vacuoles) are primarily responsible for regulating the transport of inorganic ions and metabolites between the vacuole and the cytosol. While there are many different nitrate transporters, nitrate can be selectively chosen and carried into the tonoplasts and then stored in the vacuoles. There are less nitrate ions absorbed and accumulated in those inner (young) leave cells because they have smaller and less central vacuoles than the outer (older) leave cells. In addition to that, the amount of nitrate reductase activities occurring in the young leaf cells is significantly higher than the older leaves. The combination of these two mechanisms results in the observed nitrate concentration decreasing in the order of leave ages. Furthermore, as nitrate ions get transported into leaves via their xylems, they are stored in the vacuoles. This is consistent with our observation where we measured higher nitrate concentration in the outer leaves than the inner leaves, as the older leaves tend to have more mature and powerful xylems than younger leaves.

Detailed nitrate contents of a brassica vegetable (broccoli) were tested within its stem, root and flower head as shows in Table 1. Nitrate concentration from the root of broccoli indicates a significant decreasing order from the bottom to the top, and the stem contains the highest nitrate concentration. However, nitrate contents in the flower heads, the edible part of broccolis, are found to be much lower than their roots and their stems. In addition, nitrate concentration in different parts of flower heads also shows an organized distribution that the nitrate concentration in the central part of the flower heads is much higher than the ambient part of the flower heads.

Organ	Nitrate (Japan)
Root 1(bottom)	8400
Root 2(middle)	3400
Root 3(middle)	1800
Root 4(top)	1000
Stem (closed to root 1)	9800
Flower head 1(branch)	1700
Flower head 2(branch)	2200
Flower head 3(central)	3400

Table 1: Nitrate contents (mg/kg) in various parts of Broccoli.

The finding of the decreasing nitrate concentration from bottom to top in root cells of broccoli can be explained by the nitrate absorption mechanism through the root system. A root system selectively absorbs water and solutes to support plant growth and development of tissues. Particularly, depending on nitrate concentration in the soil solution, nitrate is actively carried into root cells by numerous membrane proteins called nitrate transporters (NRT), which ensure the capacity of root cells to uptake nitrate ions through high affinity and low affinity systems. Once nitrates have been absorbed into the symplast of the root at the epidermis or cortex, they must be first loaded into the xylems, and then through the xylems, they are transported from the root to the shoot for further assimilation. Nevertheless, the final movement of loading nitrate into the xylem is driven by the mechanism of passive diffusion [14]. This results in a decreasing order of nitrate concentration from the bottom to the top along the taproot.

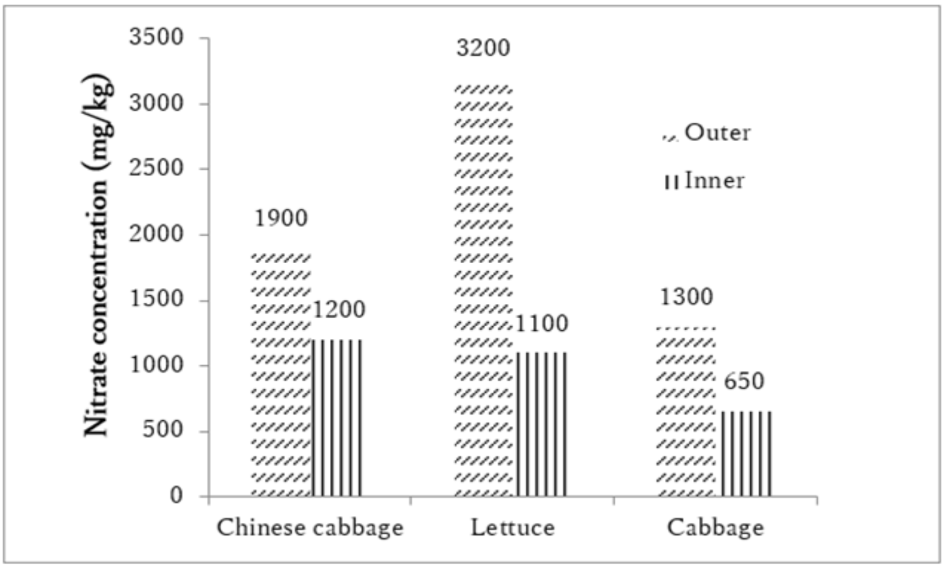

Figure 2: Distribution of Nitrate contents in three different vegetables.

Citation: Liu H, Huang G (2022) A Study on Nitrate Contents in Vegetables in the Watershed of Lake Tega in Japan. Int J Earth Environ Sci 7: 198 doi: https://doi.org/10.15344/2456-351X/2022/198

The nitrate that got transported into leaf cells is being stored in vacuoles and consumed during photosynthesis. The stem of the plant does not have a direct nitrate consumption mechanism like the leaves do, therefore nitrate ions easily accumulate in the stem cells and leads to the highest nitrate concentration measured among all parts of the plant. Moreover, the passive diffusion transport system in xylem also results in distinctive nitrate levels in branches and central parts of broccoli's flower heads, and the nitrate concentration in the central part of flower head is higher than that in the branches.

Nitrate contents in various parts of stems and tubers of turnip were measured, respectively. Nitrate concentration in the inner stem of turnip was found as high as 8600 mg/kg. Our results also illustrate the nitrate contents increase significantly from inner part to outer part inside of a tuber. On the contrary, the nitrate concentration decreases remarkably from inner stem to outer stem as shows in Table 2.

Samples	Inner stem	Outer stem	Inner tuber	Outer tuber
1	8600	Null	1000	2700
2	3100	230	310	910
3	4300	200	480	1700

Table 2: Nitrate contents (mg/kg) in turnip.

Since the inner stems and leaves grow earlier than the outer stems and leaves, they are essential in transporting inorganic nutrients such as nitrate ions and water molecules absorbed from the roots to the older leaves to synthesize them into organic materials for growth and further development of tissues and organs. As turnip matures, unreduced nitrate will accumulate in the vacuoles and xylems of stems and leaves, which results in the fairly high concentration of nitrates in stems, especially in inner stems.

Anatomically, the swollen structure of turnip predominantly consists of secondary xylem, which is made up of non-lignified xylem parenchyma cells [15]. These are the only living components in the xylem tissue and they are specialized for storing organic materials to be used by photosynthesis. These results in an accumulation of organic matters and therefore the relatively low nitrate levels of the tuber compared to values from the stems. Meanwhile, more parenchyma cells are located at the inner tubers of turnip, whereas relatively less parenchyma cells are located in the outer tuber in which nitrates are transported, leading to the lower nitrate concentration in the inner tubers compared to the outer tubers.

Nitrate concentration differs in the various parts of a plant. The vegetable organs can be listed by decreasing nitrate content from leaf, stem, root, tuber, fruit, and seed. However, the present study found that the average nitrate level in the outer stem was 215 mg/kg. It is lower than that in tubers with a mean value of 1,183 mg/kg. Only in the inner stems, the nitrate content shows a decreasing trend from stems to roots with an average value of 5,333 mg/kg.

In this study, nitrate content in lettuce and turnip before and after boiling was compared and analyzed (Figure 3). The nitrate concentrations of No.1 lettuce, No.2 lettuce and No.3 lettuce samples before treatment were 5000 mg/kg, 3000 mg/kg and 3400 mg/kg, respectively. Then, after boiling 1 minute, the corresponding nitrate concentrations of the three lettuce samples after cooking were 2900 mg/kg, 2300 mg/kg and 1800 mg/kg. The results showed that the nitrate content of three lettuce samples decreased by 42%, 23.33%, and 47.06%, respectively.

The nitrate concentration in turnip was 2025 mg/kg before boiling. After boiling for 1 minute, the nitrate content in the turnip was 260 mg/kg, which was 87.16% less than that before boiling. Therefore, the nitrate content of vegetables before and after boiling is greatly different.

Results and Discussion

This study investigates the nitrate contamination in four types of vegetables, including leafy, brassica, root and tuber, and fruiting vegetables. It is found that the concentration of nitrate in different types of vegetables is different, among the four groups of sampled vegetables, nitrate contamination shows a decreasing order from the leafy vegetables, brassica, root and tuber, and fruiting vegetables. The order of nitrate accumulation was lettuce > spinach > broccoli > Chinese cabbage > cauliflower > radish > eggplant and fruit. A nitrate concentration of 6,300 mg/kg, 4,000 mg/kg and 1,500 mg/kg were found in lettuce, spinach, and Chinese cabbage. Findings from the detailed testing of nitrate distributions in various parts of crops suggests that some portions of vegetables, such as the inner part of Cabbage and turnip, can be considered as much suitable and healthier for young children. The nitrate content of vegetables before and after cooking is very different. After cooking, the nitrate content of vegetables is very much reduced. The present study is mainly designed

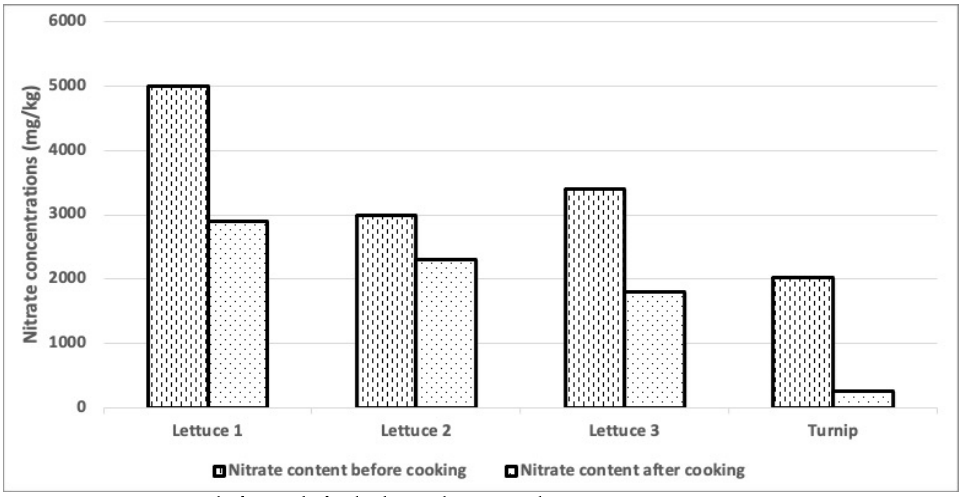

Figure 3: Nitrate content before and after boiling in lettuces and turnips.

for problem identification; further in-depth study regarding the relationship of nitrate contamination and fertilizer usage in both countries will be conducted.

Competing Interests

The authors declare that they have no competing interests.

Refrences

1. Santamaria P, Elia A, Parente A and Serio F (1998) Fertilization strategies for lowering nitrate content in leafy vegetables: chicory and rocket salad cases. J Plant Nutrition 21: 1791-1803.
2. National Research Council (1981) The Health Effects of Nitrate, Nitrite, and N-Nitroso Compounds: Part 1 of a 2-Part Study. Washington, DC: The National.
3. Knight T M, Forman D, Al-Dabbagh S A and Doll R (1987) Estimation of dietary intake of nitrate and nitrate in Great Britain. J Food Chem Toxicol 25: 277-285.
4. Dejonckheere W, Steurbaut W, Drieghe S, Verstraete R and Braeckman H (1994) Nitrate in food commodities of vegetable origin and the total diet in Belgium (1992-1993). J Microbyol Aliment Nutr 12: 359-370.
5. Dich J, Jarvinen R, Knekt P and Penttila P L (1996) Dietary intakes of nitrate, nitrite and NDMA in the Finnish Mobile Clinic Health Examination Survey. J Food Addit Contam 13: 541-552.
6. Du S T, Zhang Y S, Lin X Y (2007) Accumulation of nitrate in vegetables and its possible implications to human health. J Agricultural Sciences in China 6: 1246-1255.
7. EFSA (2009) J EFSA 69: 1-79.
8. Joint FAO/WHO Expert Committee on Food Additives. Meeting (44th : 1995: Rome, Italy) & International Programme on Chemical Safety. (1996). Toxicological evaluation of certain food additives and contaminants in food / prepared by the forty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). World Health Organization.
9. Mensinga T T, Speijers G J A and Meulenbelt J (2003) Health implications of exposure to environmental nitrogenous compounds. J Toxicol Rev 22: 41-51.
10. Ishiwata H, Yamada T, yoshiike N, Nishijima M, Kawarnoto A and Uyama Y (2002) Daily intake of food additives in Japan in five age groups estimated by the market basket method. J. European Food Research and Technology. 215: 367-374.
11. Guo KX, Yao CX, Chen Y, Yang YF and Lu LM (2011) Nitrate contents in autumn vegetables and assessment of nitrate intake in Shanghai. J Environmental Science 32: 1177-1181.
12. Ott-Borrelli K A, Koenig R T and Miles C A (2009) A comparison of rapid potentiometric and colorimetric methods for measuring tissue nitrate concentrations in leafy green vegetables. J Hort Technology. 19: 439-444.
13. Martinoia E, Heck U and Wiemken A (1981) Vacuoles as storage compartments for nitrate in barley leaves. Nature 289: 292-294.
14. Taiz L and Zeiger E (2010) Plant Physiology ed Fifth, Sinauer Associates, Inc
15. Langer R H M and Hill G D (1981) Agricultural Plants Cambridge University Press