A Comparative Study on Nitrate Contents in Vegetables between Northern Part of China and the Watershed of Lake Tega in Japan

Huan Liu¹,², * and Guangwei Huang¹,²

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

*h-liu-0t6@sophia.ac.jp

Abstract. Humankind is continuously exposed to nitrate through water and food. Excessive intake of nitrate could lead to health problems. Among the food products consumed by human beings, both fresh and processed vegetables are the major source of dietary nitrate intake. In this study, investigations on nitrate contents in vegetables are conducted in the northern part of China and the watershed of Lake Tega in Japan. Four groups of vegetables, leafy, brassica, root and tuber, and fruiting, were sampled for testing. The study focuses on the differences of the nitrate distributions in various crops. As a developed country, Japan has a much stricter regulation on the utilization of nitrogen in fertilizers, with a recommended yield goal N rate of 250 kg/ha/year. Whereas in China, the recommended N rate is 450 kg/ha/year. Meanwhile, the watershed of Lake Tega in Japan has an average annual precipitation of 1348 mm, which is much higher than the Northern part of China. High concentration of nitrate was found in vegetables grown in both Northern China and the watershed of Lake Tega, although the social and environmental conditions are drastically different in these two regions.

1. Introduction
Nitrate is one of the most common chemical compounds in nature, and it is widely found in soil, water, and food. Three of human’s main nitrate intake sources are vegetables, water, and cured meat [1] [2] [3]. Among them, vegetables are considered to be the most important dietary nitrate source [4]. 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 US, the UK, Belgium, Finland, and Italy [5] [6] [7] [8] [9]. 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 [3].

Although Epidemiological data proposed that nitrate may have some positive influences on human health, such as its function as an effective host defense against gastrointestinal pathogens, as well as its values for carotenoids, vitamins C, Selenium, dietary fiber, and so forth [10] [11], it is generally considered that the increasing dietary nitrate intake by human is also positively correlated with an increased risk of cancer, methemoglobinemia, hyperparathyroid, children polyuria, hypertension,
carcinogenic nitrosamines, and so on [12] [13]. Infants under 3 months are particularly susceptible to infantile methemoglobinaemia due to their normal intestinal flora contributing to the generation of methemoglobin. Reduced oxygenation of the tissues can generate significant adverse implications for infants, and result in coma and death under the most severe circumstances.

The nitrate circulation cycle starts with the oxidation of organic wastes by the nitrogen-fixing bacteria [14]. Plants then absorb the nitrogen from soil in the form of nitrate and ammonia. Nitrate is mainly found in cell vacuoles and is transported in the xylem [12] [15]. The main function of the xylem is to conduct water and dissolved nutrients like nitrogen upward from the root to the leaves, whereas the phloem is responsible for transporting the soluble organic materials made during photosynthesis from the leaves to the growth points of the plant [16]. These mechanisms redistribute the nitrate across different parts of the plants, such as leaves, stems, and storage organs. It implies that leafy vegetables, such as lettuce, cabbage, and spinach, may contain relatively high nitrate concentration whereas root vegetables, such as potato tubers, carrots, onions, and seeds, may show relatively low concentration. In addition, the transport system also results in younger leaves containing lower nitrate concentration than older leaves. A study on cabbage leaves has verified this characteristic, where the highest nitrate concentration was found in the outer leaves and the lowest nitrate concentration was found in the innermost leaves [17].

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 [12]. 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) [18]. 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 [19].

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. (2002) [20] and Guo et al. (2011) [21] also reported higher levels of nitrate dietary exposures compared to ADI. However, there are only limited case studies conducted in Asian countries. This research investigates the nitrate concentration in crops in two different Asian locations: the Northern Part of China and the Watershed of Lake Tega, Japan. The objective is twofold: 1) to gain more insights on nitrate contamination in vegetables in Asia; 2) to examine nitrate distribution in crops by measuring nitrate contents in various parts of crops.

2. Materials and methods

2.1. Study areas

Three study areas were chosen for this research: Zhangye City in Gansu, China; Luan County in Hebei, China, and the watershed of Lake of Tega in Japan.

Zhangye city, formerly known as Ganzhou, is located in the Zhangye Oasis, the center of Hexi Corridor, Gansu Province, Northwest China. It is a part of the ancient Silk Road, and it is located in the middle reaches of the Heihe River basin. It has a population of approximately 1.28 million with a land area of 39.4 thousand square kilometers. This city serves as an important agricultural center for seed corn and vegetable cultivation in China due to its fertile soil and suitable climate for growing crops. Nearly 70% of the land is used for agriculture. Zhangye Oasis has a typical dry continental climate with a mean annual precipitation of 117mm, an average temperature of 7.6 Celsius, and a mean evaporation rate of 2,390 mm per year. The seed corn grown in the Zhangye Oasis is heavily cultivated through applying chemical nitrogen fertilizers [22]. Just in the year of 2005 alone, there were more than 300 kg/ha of nitrogen fertilizers used on growing seed corn. In more recent years, the number has come to exceed 450 kg/ha/year [23].
Luan County is located at the southern foot of Yanshan Mountain in the province of Hebei and in the middle reaches of the Luan River. This county has a total population of 540,000 and a total area of 999 square kilometers. At the end of 2009, 71.5 percent of its land was used for agricultural purposes. The second-class agricultural land usages in Luan County include cultivated land, garden plot, woodland, and other agricultural use land [24]. Luan County has a warm-temperate monsoon climate with a mean annual precipitation of 760 mm and an average temperature of 11 Celsius.

Lake Tega covers a total land area of 7 square kilometers with a perimeter of 38 kilometers. There are approximately 480,000 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. This area has a warm-temperate climate with a mean annual precipitation of 1328mm and a mean temperature of 14.6 Celsius. It is also worth mentioning that in the year of 2008; the total amount of fertilizers used in Japan was 259 kg/ha/year (FAO), which is significantly less than China.

2.2. 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 use, it has an extremely wide range of applications. More importantly, its measurement range is well suited for measuring nitrate ion distribution in crops [25]. 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.

2.3. Sampling
We randomly sampled four types of vegetables from the local farmlands of the Northern part of China and from the local farmlands near Lake Tega in Japan, including leafy, brassica, root and tuber, and fruiting vegetables. Then we measured their nitrate contents using the methodology described above. Specifically, the species of the vegetables we tested were Chinese cabbage, lettuce, broccoli, turnip, beetroot, bell pepper, eggplant, long pepper, corn, tomato, cucumber, pear, and apple. We chose them for this study because they are popular vegetable choices of the local population. All the nitrate content measurements were conducted on-site to obtain better accuracies. 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 brassica and broccoli. Lastly, for the fruit samples like corn and tomato, nitrate contents in stems and fruits were measured.

3. Results and discussions
With regard to the experimental results of nitrate contents in the four types of vegetables, higher levels of nitrate concentration was found in leafy vegetables such as Chinese cabbage and lettuce, whereas lower levels occur in tubers or fruits such as beetroot 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 8,100 mg/kg and 6,300 mg/kg were found in Chinese cabbage and lettuce, respectively. Moderate nitrate contents were found in root and tuber vegetables such as broccoli, turnip and beetroot with values of 3,400 mg/kg, 3,000 mg/kg and 1,400 mg/kg, respectively. Nevertheless, lowest nitrate concentration was found in one of the fruiting vegetables - cucumber with a value of 73 mg/kg. This finding is consistent with the results of nitrate contents reported from European countries [4].

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 beetroot in which the majority of inorganic nitrate ion is accumulated and cannot be assimilated through photosynthesis.

![Figure 1](image_url)  
**Figure 1.** Nitrate contents in measured vegetables.

Furthermore, highest nitrate concentration was found in the leafy vegetables of Chinese cabbage and lettuce with a value of 8100 mg/kg and 6300 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 [12]. 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 Chinese and 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 for those grown in the open air. This implies that there is a potential health risk for the local people in the Tega region.

There are many factors that can significantly accelerate nitrate accumulation in vegetables, such as temperature, light, vacuole, carbohydrates, xylem transportation, the age of vegetables, nitrate reductase activity, fertilizer usage, nitrogen fertilization, and light intensity, etc. Interestingly, despite that the social and environmental conditions are very different between the northern China region and the watershed of Lake Tega in Japan, the nitrate contents found in the vegetables collected from these two regions are very consistent. In light of the result from Figure 2, high nitrate concentration was
found in Chinese cabbage from both the northern part of China and the Lake Tega in Japan with a value of 8,100 mg/kg and 7,300 mg/kg, respectively. Meanwhile, relatively low levels of nitrate were measured in tomato juice with a value of 170 mg/kg from both China and Japan. And broccoli was found to have a higher nitrate level in Japan than that in China.

As a developed country, Japan has a much stricter environmental conservational practice than China. For instance, the annual mean utilization of nitrogen fertilizer in Japan is 250 kg/ha/year, whereas in China the regulation is 450 kg/ha/year [23]. Meanwhile, Lake Tega in Japan has an average annual precipitation of 1348 mm, which is much higher than the Northern part of China with values of 117 mm and 760 mm in Zhangye and Luan County, respectively. Nevertheless, nitrate levels from both the northern part of China and the watershed of Lake Tega in Japan indicate high nitrate contaminants in vegetables despite their drastically different social and environmental conditions.

![Figure 2. A comparison of nitrate contents between the northern part of China and the watershed of Lake Tega in Japan.](image)

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 7,300 mg/kg, 6,300 mg/kg and 1300 mg/kg, respectively, whereas low nitrate levels was found among the inner parts with a value of 2,100 mg/kg, 2,200 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 3.

![Figure 3. Distribution of Nitrate contents in three different vegetables.](image)
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 leaf 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, respectively from both North China and Japan 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.

Comparing the nitrate concentrations measured in the broccolis harvested in the two sampling locations, we found that the nitrate levels in all parts of the broccoli grown in Lake Tega, Japan are consistently higher than the broccolis grown in northern China. This indicates Japan has a higher nitrate contamination than North China.

| Organ                     | Nitrate (Japan) | Nitrate (China) |
|---------------------------|-----------------|-----------------|
| Root 1 (bottom)           | 8400            | 3000            |
| Root 2 (middle)           | 3400            | 2200            |
| Root 3 (middle)           | 1800            |                 |
| Root 4 (top)              | 1000            |                 |
| Stem (closed to root 1)   | 9800            | 5300            |
| Flower head 1 (branch)    | 1700            | 860             |
| Flower head 2 (branch)    | 2200            |                 |
| Flower head 3 (central)   | 3400            |                 |

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 [16]. This results in a decreasing order of nitrate concentration from the bottom to the top along the taproot.

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 levels in the fruit of tomato were measured with a value of 160 mg/kg, whereas it was as high as 2,300 mg/kg in the stem. In addition to that, nitrate concentration in the stem illustrated a slight decrease from the bottom to the top with values of 2,300 mg/kg and 1,900 mg/kg, respectively as shows in Figure 4.

In light of aforementioned translocation mechanisms of nutrients in vegetables, nitrate ions are mainly transported in xylem and stored in central vacuoles of vegetables. Passive diffusion of nitrate ions in the xylem results in the decreasing trend of nitrate concentration from the bottom to the top in the stems. Furthermore, fruit cells of tomato are primarily parenchyma cells with central vacuoles storing organic materials that are assimilated and reduced through photosynthesis. These result in a relatively low value of nitrate concentration in the fruit of the tomato compared to that in stems. This also suggests that for those vegetables with stems above the ground such as corn, tomato, apple and so forth, nitrate levels in their fruits are significantly lower than other parts.

![Figure 4. Nitrate contents (mg/kg) in tomatoes.](image)

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        |

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 [27]. 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.

4. Conclusions
This study investigates the nitrate contamination in four types of vegetables, including leafy, brassica, root and tuber, and fruiting vegetables. For vegetables sampled in the Northern part of China, a nitrate level up to 8,100 mg/kg was found in Chinese cabbage. For vegetables sampled in Japan, a nitrate level up to 7,300 mg/kg was found in Chinese cabbage and the nitrate concentration in broccoli was found to be higher than broccoli sampled in China. These revealed that nitrate contamination of vegetables has occurred in both the arid regions of northern China and the water-abundant areas of Japan despite the agricultural practices, the fertilizer applications, and the average annual precipitation in the two regions are very different. 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. In addition, 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 present study is mainly designed for problem identification; further in-depth study regarding the relationship of nitrate contamination and fertilizer usage in both countries will be conducted.

Acknowledgments
The authors acknowledge the generous support and cooperation provided by Prof. Che Tao, Prof. Wang Jian, Prof. Wang Weizhen, Prof. Huang Chunlin and Prof. Li Xin, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences.

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