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Nitrate accumulation in the winter and summer vegetables grown in the Punjab (Pakistan)

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Abstract

Vegetables occupy a pivotal place in the human diet, but unfortunately these contribute maximally to nitrate accumulation and pose serious health hazards. Nitrite and nitroso compounds which form when nitrite binds to other substances before or after ingestion e.g. the amines derived from proteins are toxic and can lead to severe pathologies in humans. Thus, the assessment of the health risk of nitrate to humans should encompass the toxicity of both nitrite and nitroso compounds. That's why a comprehensive study was carried out to determine the nitrate accumulation in winter and summer vegetables throughout the Punjab province of Pakistan. The winter vegetables accumulated more NO_3 content than summer vegetables. On the other hand leafy vegetables accumulated more nitrates than others. Among the winter vegetables radish and hybrid spinach remained at the top regarding NO_3 accumulation. Whereas among the summer vegetables i.e. brinjal and squish secured the highest position in this regard. The factors affecting the nitrate content viz season (winter and summer), growing intensity of agriculture fertilizer use and soil texture were also studied.

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Introduction

Nitrogen fertilization facilitates accumulation of nitrate in plant tissues as a result of an excess of nitrogen uptake over its reduction. When taken up in excess of immediate requirement, it is stored as free nitrate in the vacuole and can be remobilized subsequently when nitrogen supply is insufficient to meet the demand (Van der Leij *et al.,* 1998).

Application of nitrogenous fertilizers has been increased in fields to increase the crop yields. Since Nitrogen fertilizer plays imperative role both in crop yield and quality (Sisson *et al.,* 1991; Gastal and Lemaire, 2002; Wang *et al.,* 2002). Nitrate is often the major source of Nitrogen available to higher plants especially to vegetables. Nitrate uptake and distribution in crops is of major importance with respect to both environmental concerns and the quality of crop products.

Plants have shown a greater tendency for nitrate accumulation, especially in vegetables. Because edible parts contain very high concentrations of nitrate that has been implicated in the occurrence of methaemoglobinemia and possibly in gastric caner as well as other diseases (Bruning-Fann, 1993; Ishiwata *et al.,* 2002). Accumulation of nitrate in high amount in crops is becoming a major concern (Van der Boon *et al.,* 1990). It creates imbalance between translocation and intake of nitrates via xylem, and conversion of nitrates to ammonia that is rapidly assimilated into amino acids (Maynard *et al.,* 1976). Nitrates concentration in lettuce is affected depending upon source of N fertilizer and texture of soil (Gianquinot *et al.,* 1992; Gunes *et al.,* 1995). The NH_4-N-to-NO_3-N fertilizer-N ratio (Demsar and Osvald, 2003), the timing of fertilizer N release (Tesi and Lenzi, 1998), the light intensity and duration (Behr and Wiebe, 1992), crop season (Gianquinto *et al.,* 1992), and lettuce type and cultivar. In most types of lettuce, including the romaine type, the highest concentration of nitrates is normally observed in the external leaves (Abu Rayyan *et al.,* 2004). Plants that develop fruit or storage organs such as tomatoes or potatoes usually show a decline in nitrate concentration as the crop approaches harvest. Many workers have determined the nitrate and nitrite contents of the prepared infant cereal foods and common vegetables. The leafy vegetables contained large amount of nitrate and nitrite. The root vegetables like beets, turnips and radish were reported to have the highest nitrate contents (1000 to 2000 ppm). The potato tubers were found to contain less than 100 ppm nitrates. In tomatoes the concentration of nitrates ranged from 25 to 140 ppm. Heisler *et al.* (1974) reported a rapid increase of nitrite contents in storage abused spinach, beets and beet juice. Tamme *et al.* (2010) observed that the average levels of nitrate varied from zero to 9669 mg as KNO_3 Kg^{-1} in spinach, beetroots and radish. Highest level of nitrates found in leafy vegetables followed by root vegetables. Cooking had the effect of lowering the levels of nitrate in all types of fresh vegetables. No nitrite was formed during cooking.

Approximately 5% of all ingested nitrate is converted in saliva and the gastrointestinal tract to the more toxic nitrite (Pannala *et al.,* 2003). The toxic effects of nitrate are those resulting from the nitrite formed by its reduction by bacterial enzymes (Mensinga *et al.,* 2003). Nitrite and nitroso compounds which form when nitrite binds to other substances before or after ingestion e.g. the amines derived from proteins are toxic and can lead to severe pathologies in humans. Thus, the assessment of the health risk of nitrate to humans should encompass the toxicity of both nitrite and nitroso compounds. The best effect of nitrite is reaction with haemoglobin (oxyHb) which produce methaemoglobin (meth Hb) and nitrate.

$$NO2^- + oxyHb(Fe2+) \longrightarrow methHb\ (Fe3+) + NO3^-$$

As a consequence of the formation of methHb the oxygen delivery to tissue is impaired (Mensiga *et al.,* 2003). Generally, nitrate accumulating vegetables belong to the families of Brassicaceae (radish, mustard) Chenopodiaceae (Swiss chard, lettuce and Apiaceae (celery, parsley) (Santamaria, 2006). In this study, nitrate contents in winter and summer vegetables grown in the Punjab province of Pakistan have been measured.

Materials and methods

Collection and processing of vegetable samples

A survey of the 36 districts (8 Divisions) of the Punjab province was carried out to procure the summer and winter vegetable samples. From each district nine vegetable samples were taken. From four samples from each side within the municipal limit (urban area) and four samples from the rural area (4-5 km) and one sample was collected from the centre of the city center (Fig. 1).

The samples of these matured crops were collected and wrapped in polyethylene bags. The samples of leafy and other vegetable were stored in refrigerator at 4°C temperature during transportation and prior to analysis. This procedure was carried out to avoid the reduction of nitrate into nitrite during storage. For leafy vegetables the samples were washed with distilled water and in case of fruits it was chopped into small portions, dried with blotting paper and then dried for 48 hours at 70°C and weighed to determine plant dry weight. Dried plants were ground in a mill (40 mesh). Then nitrate (NO_3) contents were determined by method reported by Ryan *et al.* (2002). All results are expressed as Means on dry weight basis.

Statistical analysis

Results were analyzed by analysis of variance using the SAS computer program (SAS Institute, 1988).

Data was subjected to multivariate statistics such as Mean and standard Error of Mean, Analysis of Variance, and Duncan Multiple Range Test (DMRT). A probability level of $P \leq 0.05$ was chosen to establish statistical significance.

Results and discussion

Season and Nitrate Accumulation

The data regarding NO_3 accumulation in winter vegetables in the eight Divisions of the Punjab province is presented in Table 1.

Table 1. Nitrate accumulation (mg kg^{-1}) in different winter vegetables in eight divisions of Punjab province of Pakistan.

Division	Carrot	Hybrid Spinach	Local Spinach	Radish	Turnip	Potato
Rawalpindi	1309c	-	1925bc	5273b	904c	787g
Gujranwala	1631a	5535b	1998b	5755ab	955abc	954a
Lahore	1608a	4667f	2106a	6065a	922bc	930c
Faisalabad	1570a	5532c	1991b	5671ab	886c	909d
Sargodha	1570ab	5940a	2001b	5667ab	973abc	885e
Multan	1426bc	-	1885c	5604ab	1080ab	-
Bahawalpur	1496ab	5050e	1841c	5586ab	807c	795f
Dera Ghazi Khan	1638a	5206d	2022ba	5810ab	1091a	-
Average	1531	3991	1971	5679	952	658

DMRT Means with same letter are not significantly different.

On average, the nitrate contents of winter vegetables ranged from 657 to 5679 mg kg^{-1}. Results indicated that significant variations among the Divisions in different winter vegetables were existent. The maximum NO_3 contents (5678 and 3991 mg kg^{-1}) were accumulated in radish and hybrid spinach respectively. The minimum NO_3 contents (952 and 657 mg kg^{-1}) were present in turnip and potato respectively.

The data relating to NO_3 content in summer vegetables of the eight divisions of the Punjab province is presented in Table 2. In general, brinjal and squash accumulated more nitrate contents than onion, okra and tomato. Population quantum exerted significant effect on the nitrate accumulation in different vegetables (Table 3 and 4). The vegetables collected from large cities accumulated high nitrate contents.

Table 2. Nitrate accumulations (mg kg^{-1}) in different summer vegetables in eight divisions of Punjab province of Pakistan.

Division	Okra	Brinjal	Squash	Onion	Tomato
Rawalpindi	998abc	1993ab	1354a	1524a	410a
Gujranwala	981bc	1765d	1448a	949g	352dc
Lahore	1075a	2075a	1954a	1019e	376bc
Faisalabad	992abc	1863dc	1456a	1014f	355dc
Sargodha	1044ab	2018a	1562a	1333b	400ab
Multan	972bc	1886bc	1338a	912h	345d
Bahawalpur	928c	1894bc	1802a	1030d	357dc
Dera Ghazi Khan	952c	1964abc	1618a	1043c	390ab
Average	993	1932	1567	1103	373

DMRT Means with same letter are not significantly different.

Table 3. Effect of population on the Nitrate accumulation (mg kg^{-1}) in different winter vegetables in Punjab province of Pakistan.

Population	Carrot	Hybrid Spinach	Local Spinach	Radish	Turnip	Potato
Large	1524a	6088a	2109a	5767a	1008a	891a
Medium	1579a	5323b	1960b	5770a	977a	885c
Small	1508a	5321c	1938b	5587a	933a	886b
Average	1537	5577	2002	5708	973	887

DMRT Means with same letter are not significantly different.

The concentration was highest in radish (5708 mg kg^{-1}) followed by hybrid spinach (5577 mg kg^{-1}). The minimum concentration was found in potato (887 mg kg^{-1}) and turnip (927 mg kg^{-1}). While in summer vegetables (Table 4), on average, brinjal and squash accumulated nitrates as 1943 mg kg^{-1} and 1566 mg kg^{-1} respectively while tomato accumulated nitrate contents (372 mg kg^{-1}) followed by Okra (1014 mg kg^{-1}) and onion (1056 mg kg^{-1}).

Table 4. Effect of population on the Nitrate accumulation (mg kg^{-1}) in different summer vegetables in Punjab province of Pakistan.

Population	Okra	Brinjal	Squash	Onion	Tomato
Large	1083a	2023a	1554a	1074b	390a
Medium	992b	1900b	1652a	972c	360b
Small	968b	1907b	1492a	1107a	367b
Average	1014	1943	1566	1051	373

DMRT Means with same letter are not significantly different.

Table 5. Effect of sewage water on the Nitrate accumulation (mg kg^{-1}) in different winter vegetables in Punjab province of Pakistan.

Sewage water use	Carrot	Hybrid Spinach	Local Spinach	Radish	Turnip	Potato
High	1613.0a	5493a	2046.0a	5896.4a	1038.3a	923.8a
Moderate	1475.2b	5297b	1887.7b	5382.0b	899.7b	851.7c
Low	1368.0c	5226c	1870.4b	5543.6b	817.1c	865.0b
Average	1485.4	5338.7	1934.7	5607.3	918.4	880.2

DMRT Means with same letter are not significantly different.

Use of sewage water and nitrate accumulation

The data concerning the impact of sewage water on nitrate accumulation in vegetables is shown in Tables 5 and 6. The data depicted where the frequency of sewage water uses was more the nitrate accumulation was also high. On average radish (5607 mg kg^{-1}) and hybrid spinach accumulated nitrate contents, while the minimum concentration (880.2 mg kg^{-1} and turnip 918 mg kg^{-1}) in winter vegetables.

The similar trend was observed in Summer vegetables such as brinjal (1887 mg kg^{-1} and squish 14.95 mg kg^{-1}) accumulated more nitrate then tomato (358 mg kg^{-1} and Okra 959.5 mg kg^{-1}).

Table 6. Effect of sewage water on the nitrate accumulation (mg kg^{-1}) in different summer vegetables in Punjab province of Pakistan.

Sewage water use	Okra	Brinjal	Squash	Onion	Tomato
High	1046.3a	2001.0a	1628.6a	1071a	382.2a
Moderate	946.4b	1817.4b	1529.3a	1038c	363.9b
Low	885.9c	1844.4b	1329.9a	1044b	330.5c
Average	959.5	1887.6	1495.9	1051	358.9

DMRT Means with same letter are not significantly different.

Growing industry and nitrate accumulation

The data showing the impact of growing intensity on nitrate accumulation is presented in Tables 7 and 8. Medium growing industry affected nitrate concentration in both the season. Radish and spinach accumulated low nitrate compared to potato and turnip growing under the same conditions in winter. While in Summer Brinjal and Squash accumulate more nitrate contents than Tomato and Okra.

Table 7. Effect of growing intensity on the nitrate accumulation (mg kg^{-1}) in different winter vegetables in Punjab province of Pakistan.

Growing Intensity	Carrot	Hybrid Spinach	Local Spinach	Radish	Turnip	Potato
High	1530b	5386b	1964ab	5738a	948b	895b
Medium	1657a	5421a	2014a	5683a	1036a	897a
Low	1315c	5100c	1933b	5356b	892b	832c
Average	1501	5302	1970	5592	959	874

DMRT Means with same letter are not significantly different.

Table 8. Effect of growing intensity on the Nitrate accumulation (mg kg^{-1}) in different summer vegetables in Punjab province of Pakistan.

Growing Intensity	Okra	Brinjal	Squash	Onion	Tomato
High	1000ab	1921a	1578a	982c	363b
Medium	955b	1905a	1594a	1157b	379ab
Low	1030a	1948a	1310a	1331a	384a
Average	995	1925	1494	1157	375

DMRT Means with same letter are not significantly different.

Fertilizer use and nitrate accumulation

The intensity of fertilizer use (nitrogenous fertilizer) use exerted pronounced effect on nitrate accumulation. The higher the fertilizer use intensity the more nitrate accumulation was observed in vegetables in both the seasons. On average, radish

(5549 mg kg^{-1}) and hybrid Spanish (5314 mg kg^{-1}) accumulated nitrate contents. While the minimum nitrate concentration was observed in the order of potato (877 mg kg^{-1}) > turnip > (947 mg kg^{-1}) and carrot (1511 mg kg^{-1}) while in summer vegetables Brinjal was at the top (1919 mg kg^{-1}) while tomato remains at the lowest (372 mg kg^{-1}) (Tables 9 and 10).

Table 9. Effect of Fertilizer Use on Nitrate accumulation (mg kg^{-1}) of different Winter vegetables in Punjab Province of Pakistan.

Fertilizer Use	Carrot	Hybrid Spinach	Local Spinach	Radish	Turnip	Potato
High	1547a	5368b	1978a	5775a	971a	896b
Medium	1547a	5473a	1940a	5512ab	929a	909a
Low	1442a	5100c	1984a	5359b	942a	824c
Average	1512	5314	1967	5549	947	876

DMRT Means with same letter are not significantly different.

Table 10. Effect of Fertilizer Use on nitrate accumulation (mg kg^{-1}) of different winter vegetables in Punjab province of Pakistan.

Fertilizer Use	Okra	Brinjal	Squash	Onion	Tomato
High	1002a	1926ab	1600a	1026c	366b
Medium	957a	1858b	1489a	1093b	364b
Low	977a	1972a	1345a	1280a	386a
Average	979	1919	1478	1133	372

DMRT Means with same letter are not significantly different.

Soil texture and nitrate accumulation

Data regarding the effect of soil texture on nitrate accumulation in vegetables has been presented in Tables 11 and 12. It was observed that lighter the texture more would be the accumulation of nitrate compared to the heavy texture. Radish (5745 mg kg^{-1}) and hybrid spinach (5607 mg kg^{-1}) accumulated the nitrate at the par. While the lowest concentration was found in potato (898 mg kg^{-1}) and turnip (926 mg kg^{-1}).

During the summer the similar behavior was noticed. Brinjal (1926 mg kg^{-1}) and squish (1539 mg kg^{-1}) remained the higher accumulator of nitrate while tomato (372 mg kg^{-1}), Okra (1011 mg kg^{-1}) and onion (1075 mg kg^{-1}) remained the lower accumulator.

Table 11. Effect of Soil Texture on Nitrate accumulation (mg kg^{-1}) in different winter vegetables of Punjab province, Pakistan.

Soil Texture	Carrot	Hybrid Spinach	Local Spinach	Radish	Turnip	Potato
Loam	1474b	4976d	1903b	5567a	945ab	871c
Sandy Loam	1689a	5559c	2044a	5844a	1037a	867d
Clay Loam	1487b	5850b	2042a	5775a	930b	915b
Sandy Clay Loam	1589ab	6046a	2054a	5798a	903b	939a
Average	1560	5608	2011	5746	954	898

DMRT Means with same letter are not significantly different.

Table 12. Effect of Soil Texture on Nitrate accumulation (mg kg^{-1}) in different winter vegetables of Punjab Province, Pakistan.

Soil Texture	Okra	Brinjal	Squash	Onion	Tomato
Loam	970a	1906bc	1535a	954d	358b
Sandy Loam	1004a	1964ab	1639a	1259a	389a
Clay Loam	1038a	1827c	1513a	1048b	375ab
Sandy Clay Loam	1034a	2008a	1472a	1040c	370ab
Average	1012	1926	1540	1075	373

DMRT Means with same letter are not significantly different.

The limits set by European Commission (2002) regarding nitrate accumulation vary according to season indicating higher nitrate levels for winter grown vegetables compared to those grown in the summer. In fact, higher nitrate reductase activity was observed at increased light levels (Gaurdeau *et al.,* 1995) and a close negative correlation between photosynthetic activity and nitrate content has been reported for various lettuce cultivars (Behr and Wiebe, 1992).

Conclusion

The vegetables grown in winter season contained higher content of NO^-_3 –N than summer season. Vegetables namely, carrot, hybrid spinach, indigenous (Local) spinach, radish, turnip and potato grown in Gujranwala Division contained higher quantities of nitrates compared to other Divisions.

Furthermore, vegetables frequently irrigated by sewage water contained higher content of nitrates compared to moderate irrigated frequency areas. The accumulation of nitrates by various vegetables was greatly affected by various cropping growing intensity.

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