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叶类蔬菜的硝态氮累积及成因研究

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摘要：在菜园土壤上进行的田间试验，用禾谷类作物冬小麦作比较，研究了菠菜、小白菜、大青菜和油菜等叶类蔬菜累积硝态氮的特点。结果表明：硝态氮累积是一般旱作作物的共性，苗期更为明显，无论蔬菜还是冬小麦均有较高的硝态氮含量（367.8～1413.4μg/g）；但随生育期后延，蔬菜的硝态氮含量波动升高，冬小麦波动降低。盆栽试验表明，施入土壤的氮肥是蔬菜硝态氮累积的主要来源；过量施用氮肥所导致的蔬菜硝态氮吸收与还原转化不平衡是产生累积的根本原因；吸收与生长不协调更使累积过程加剧。

关键词：叶类蔬菜；硝态氮累积

The cause of nitrate accumulation in leafy vegetables

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Abstract: Field and pot experiments were carried out to study nitrate accumulation in leafy vegetables. N application rates at 0，90，180，270kg N/hm^2 were used in the field experiments with Chinese cabbage (*Brassica Chinensis* var. *Oleifera Makino et* Nenoto)，green cabbage (*Brassica Chinensis* L.)，spinach (*Spinacia oleracea* L.) and repe (*Brassica campestris* L.) as vegetables，and winter wheat (*Triticusp aestvum* L.) as a comparison crop. and they were planted on a vegetable field. The N rates of 0，0.20，0.40，0.60 and 0.80g N/kg soil were adopted for the pot experiments，using Chinese cabbage，spinach and rape as indicators，and the pots were placed in agreenhouse.

The field experiment results showed that the nitrate accumulation was a very common phenomenon for all crops in the experiments，especially at their seedling stages. The nitrate concentrations of the 4 leafy vegetable species ranged from 367.8 to 1413.4 μg/g，while those in winter wheat ranged from 730.1 to 807.4μg/g at the early stages. For comparison of species differences in nitrate accumulation，a mean value of different N rates were calculated for each crop. The data showed that the concentration in winter wheat was 16.4% lower than that in Chinese cabbage，14.6% lower than that in green cabbage and 39.9% lower than that in rape，while 59.2% higher than that in spinach. It is difficult to distinguish the crop more prone to accumulate nitrates between leafy vegetables and winter wheat at their early stage. However，with plant growth，the nitrate concentrations in the leafy vegetables increased，whereas those in winter wheat decreased in a fluctuating way. The average concentrations of the entire growing stage were significantly higher for the vegetables than those for wheat. It was 1069.3μg/g in Chinese cabbage，452.6μ/g in spinach，while only 338.7μg/g in winter wheat. Obviously，as a whole，the leafy vegetables had higher abilities to accumulate nitrate than winter wheat did.

Pot experiments showed that application of N fertilizer increased the vegetable growth at lower N rates，but inhabited it at higher N rates. Chinese cabbage reached its highest yield at 0.60g N/kg soil，and

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rape and spinach at 0.40g N/kg soil. When the N rates were increased to 0.80g N/kg soil, the yields of all vegetables were significantly decreased. Different from the yields, the nitrate-N concentrations in the leafy vegetables were in continuous increase as the N rates increased. Compared with that without N fertilization, nitrate-N concentrations were increased 5.4 to 79.7 times for Chinese cabbage, 3.4 to 104.4 times for rape, and 12.1 to 126.4 times for spinach. The nitrate-N concentrations were positively correlated with the N rates, the coefficients ranging from 0.933 to 0.957, significant at 0.01 level. Obviously, the N fertilizers added to soil were the major source for vegetables having high nitrate accumulation, being able to account for 87.0% to 91.6% of the nitrate accumulated in the 3 leafy vegetables.

With the increase of nitrate-N concentration, nitrate reductase activities were obviously increased. The activities of the nitrate reductase reached 1.19μg $NaNO_2$/g • h. for Chinese cabbage and rape, and 1.97μg $NaNO_1$/g • h. for spinach at 0.60g N/kg soil, while the corresponding values were only 0.16, 0.21, and 0.10 μg $NaNO_2$/g • h. at 0.20g N/kg soil. The nitrate reductase is a key enzyme for plants to reduce nitrate, and high activities of the enzyme usually mean high nitrate reduction abilities. However, in this experiment the high activities did not lead to reduction of the nitrate in the leafy vegetables to a low concentration. The reason for this phenomenon may be related with the physiological characteristics of the nitrate reductase. The nitrate reductase is a substrate-inducible enzyme and its activities closely depend on supply of the substrates: the more nitrate in the medium, the higher activity of the enzyme will be. With the increase of N rates, the vegetables took up more nitrate that stimulated the activities of the nitrate reductase in plant tissues. However, the increase of nitrate reduction ability might not be as much as the increase of the nitrate uptake amount caused by over-fertilization of N. The unbalance between nitrate absorption and reduction may be the principal cause for such occurrence of nitrate accumulation.

Further comparison was made between the vegetable growth and the nitrate accumulation. The results showed that with the increase of N rates, both the total amounts of nitrate in the leafy vegetables and their yields were increased. However, their increased magnitudes were different: the total nitrate always increased more than did the yields. For Chinese cabbage, as commpared with that without N fertilization, the total nitrate increased 33.7, 205.5, 367.6 and 327.4 times respectively at 0.20, 0.40, 0.60 adn 0.80g N/kg soil, while their yields only correspondingly increased4.2, 4.4.4, 4.9 and 3.1 times. This trend was also observed in rape and spinach. This phenomenon indicatied that faster enrichment of total nitrate over yields accelerated the increase of the nitrate-N concentration in the vegetables.

Nitrate accumulated in the vegetables originates from the nitrate existing in soil, and application of N fertilizers either in organic or inorganic forms further increased its amounts, making the dryland soil having a high nitrate contents and resulting in much more nitrate uptake by crops. In the field experiments, the nitrate-N concentrations in the leafy vegetables had reached Pollution Level 3 (NO_3^--N≥325μg/g) at no N fertilization, and continuously increased with the N rate from 90 to 180 kg/hm^2. Different from the field results, the pot experiments showed that the yields of the 3 vegetables at low N rates (0.20g N/kg soil) were of no significant difference with the highest ones, which were obtained at higher N rates, while their nitrate-N concentrations were significantly lowed, ranging from 43.0 to 72.1μg/g, much less than Pollution Level 1 (NO_3^--N<98μg/g). Therefore, the key measures for regulating nitrate accumulation in vegetables lie in reasonable N fertilizer management.

Key words: leafy vegetable; nitrate accumulation

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硝态氮(NO_3^--N)广泛存在于自然界。随饮食进入人体的硝态氮,会在肠胃中经细菌作用还原成亚硝态

氮（NO_2^--N）。后者能迅速进入血液，将血红蛋白中的低铁氧化为高铁，使其形成无法运载氧气的高铁血红蛋白，造成人体缺氧，患高铁血红蛋白症。这一疾病对幼儿威胁更大，严重者可引起死亡。亚硝态氮还可与各种胺类反应，生成强致癌物质亚硝胺，诱发消化系统癌变[1]。已确定的120多种亚硝胺化合物中，90多种有致癌作用[2]。

蔬菜是人类必需的植物性食物，又是人类摄入硝态氮的主要来源，占摄入总量的72%～94%[3]； 而且，其中的硝态氮在被食用之前就可部分还原成亚硝态氮。Aworth等发现，新鲜菠菜在20℃气温下存放2d，硝态氮减少30%～40%，亚硝态氮却升高到100mg/kg左右[1]。周根娣等发现，雪里蕻盐渍4d，亚硝态氮由3.1mg/kg上升到66.7mg/kg[4]。蔬菜被人类食用后至少还会有5%的硝态氮可在人体内转化为亚硝态氮[5]。由此可见，蔬菜中的硝态氮转化形成亚硝态氮的数量相当可观。联合国世界卫生组织和粮农组织（WHO/FAO）1973年规定，每公斤人体重的硝态氮日允许摄入量为0～1.06mg，亚硝态氮为0～0.04mg[6]。欧共体食物科学委员会（SCF）1992年又将亚硝态氮的允许量降低到0～0.02 mg[7]。按人均体重60kg，日食鲜菜0.5kg，加工处理过程使蔬菜硝态氮降低50%[4]计，则每kg蔬菜的硝态氮含量应不超过254.4mg，方能达到这一要求。各地的调查表明，多数蔬菜，特别是叶菜硝态氮含量已超过这一上限[4,8,9]。研究蔬菜累积硝态氮的特点和成因，寻求相应的调控措施已成为当务之急。

蔬菜为什么会累积硝态氮？迄今为止，虽进行了不少研究，但未得到一致结论。不少学者认为，硝态氮在植物体内富集是一种奢侈消耗，在土壤氮素供应充足的情况下，作物会以超过本身代谢需要的速率吸收，以便在氮素供应不足时维持正常生长[10]，但这一论点并未确证。揭示蔬菜硝态氮累积的内在原因，会为蔬菜生产中合理施用氮肥、降低硝态氮累积提供重要理论依据。本研究以硝态氮累积量高、广泛食用的菠菜、小白菜、大青菜和油菜等4种叶类蔬菜和禾谷类作物冬小麦为供试材料，通过大田和盆栽试验，进一步探讨这一问题。

1 材料与方法

1.1 田间试验

用于比较蔬菜与禾谷类作物累积硝态氮的异同。试验于1994年8月～1995年7月，在西北农林科技大学蔬菜站菜园土上进行。菠菜（宁夏圆叶），小白菜（黑油菜），大青菜（矮抗青），油菜（秦油1号），冬小麦（小偃六号新选系）。在每公顷施112.5kg P_2O_5的基础上，分别设0.0，90.0，180.0，270.0 kg N 4个水平。磷肥用过磷酸钙，做基肥。氮肥用尿素，1/2做基肥，播种时和磷肥一起施入；1/2做追肥，于1995年3月13日随灌水施入。

小区面积为2.0 m^2，间距0.3 m。区间以深0.4 m的塑料薄膜隔开，以防肥料侧渗。试验采用成条分组法排列：不同施肥处理按条带设置，同一作物集中成组，重复2次。蔬菜生长期间，根据土壤湿度人工洒水，每次不超过20mm，以防肥料淋失。

1.2 盆栽试验

用于研究氮肥用量、蔬菜生长、硝态氮吸收及还原转化对蔬菜硝态氮累积的影响。试验于1995年8月～12月在西北农业大学农作一站温室内进行。供试土壤采自该站大田耕层。试验用小白菜（黑油菜）、菠菜（宁夏圆叶）和油菜（秦油1号）为指示作物，在每kg土施0.3g P_2O_5的基础上，分别施0.0，0.2，0.4，0.6，0.8g N。氮肥用尿素，磷肥用过磷酸钙，均在播种时作基肥施入。

试验用深棕红色、硬质、不透光塑料盆，每盆装土4.5kg。装盆过程中轻轻振动，使土壤松紧合适。装土4.15kg以后，灌水700 ml，使土壤含水量达25%左右，确保出苗前不再灌水。水分完全渗入土壤后，播种并将剩余土壤覆于表面，厚度约1cm。重复4次。蔬菜生长期间采用重量法灌水。

1.3 采样及测定

根据试验要求在蔬菜不同生长阶段分期采样。采下的植物样立即装入塑料袋、标记密封，放入致冷箱；带回实验室后，用自来水冲去根系表面粘附的泥土，并迅速用无氮吸水纸吸干，地上部分不冲洗。然后把蔬菜按器官部位分开分别迅速称重。用于测定硝态氮的样品分别切碎混匀，装入塑料袋，标记密封，放于冰箱，在0～4℃保存。浸提硝态氮在采样后当日或次日进行，采用研磨浸提法[11]，制成待测液。待测液中的硝

态氮用连续流动分析仪测定。用于分析硝酸还原酶活性的蔬菜样品均在在采样当天，采用体内法[12]，利用内源基质测定。

2 结果与分析

2.1 硝态氮累积是一般旱作作物的共性

小白菜、大青菜和油菜属十字花科，菠菜属藜科，冬小麦属禾本科。这几种作物均可在在秋季播种，营养和生殖生长基本上遵循同一进程。生育进程的吻合为在同一田块上种植这些作物，并在同一时间采样、比较其硝态氮含量差异及其随生育期的变化提供了方便。

测定（表 1）表明，苗期是硝态氮容易累积的时期，不仅蔬菜累积硝态氮，冬小麦也累积硝态氮。但无论施氮与否，冬小麦的硝态氮含量总是低于小白菜、大青菜和油菜，而高于菠菜。从不同施氮水平的平均值来看，冬小麦的硝态氮含量分别比小白菜、大青菜和油菜低 16.4%，14.6%，39.9%，而比菠菜高 59.2%。从累积的硝态氮数量来看，苗期叶类蔬菜和禾谷类作物之间难分高低。

表 1　蔬菜与冬小麦苗期地上部分的硝态氮含量

Table 1　Nitrate N contents in above-ground parts of young seedlings of vegetables and winter wheat

施氮量 N rate (kg/hm²)	**硝态氮含量**(μg/g FW)Nitrate N content				
	小白菜 Chinese cabbage	**大青菜** Green cabbage	**油　菜** Rape	**菠　菜** Spinach	**冬小麦** Winter wheat
0.0	779.1	804.7	1146.0	367.8	730.1
90.0	937.8	855.4	1335.1	493.6	766.4
180.0	1010.7	888.9	1413.4	549.3	807.4
270.0	982.8	1081.5	1265.4	537.2	796.3
平均(Mean)	927.6	907.6	1290.0	487.0	775.1

注：**采样日期为** 1994 **年** 11 **月** 24～28 **日** Sampled from Nov. 24 to 28, 1994.

选择每公顷施氮 180kg 的小区，在不同生长阶段测定小白菜、菠菜和冬小麦的硝态氮含量，图 1 结果表明，随生育期后延，菠菜和小白菜的硝态氮含量波动升高，在开花期至结荚早期（4 月中旬～5 月上旬）含量最低，到种子成熟期又达高峰；冬小麦则不同，呈波动下降，在成熟期（6 月上旬）达最低值。就全生育期的平均值来看，两种蔬菜的硝态氮含量远高于冬小麦，小白菜的含量为每克鲜重 1069.3μg，菠菜 452.6μg，而冬小麦只有 338.7μg；小白菜累积的硝态氮是冬小麦的 3.16 倍，菠菜是冬小麦的 1.34 倍。可见，硝态氮累积是 2 种蔬菜和冬小麦的共性，但它们的累积能力随生育期而变化，就整个生长期而言，蔬菜高于冬小麦。

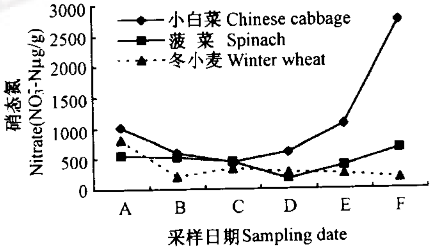

图 1　小白菜、菠菜和冬小麦地上部分的硝态氮含量随生长期的变化

Fig. 1　The changes of nitrate N content in the above ground-part of Chinese cabbage, spinach and winter wheat during their growing periods. (Sampling date, A: 1994-11-24～28; B: 1995-03-10～14; C: 1995-04-17～19; D: 1995-05-06～07; E: 1995-05-24; F: 1995-05-26(wheat: 1995\|06-03)

2.2 过量施用的氮肥是蔬菜硝态氮累积的主要来源

蔬菜累积的硝态氮归根结底来源于土壤。田间试验（表 1）表明：蔬菜的硝态氮含量在不施肥时就已达 3、4 级污染水平（硝态氮≥325μg/g）[8]，但随氮肥用量增加，仍显著升高。盆栽试验（表 2）也表明，蔬菜的硝态氮含量均随施氮量增加而成倍提高，小白菜比不施氮肥时提高 5.4～79.7 倍，油菜提高 3.4～104.4 倍，菠菜提高 12.1～126.4 倍。以每种蔬菜 2 次采样的 20 对数据进行相关分析表明，蔬菜的硝态氮含量和氮肥用量呈显著正相关。小白菜的相关系数为 0.957**，油菜为 0.955**，菠菜为 0.933**。由此可见，氮肥用量是决定蔬菜硝态氮累积多少的主要因素，施入土壤的氮肥是蔬菜累积硝态氮的主要来源。

2.3 蔬菜的硝态氮吸收与还原转化不平衡

进入植物体的硝态氮必须经硝酸还原酶作用还原成氨，才能形成氨基酸和蛋白质，参与植物体的氮代谢过程。硝酸还原酶活性是植物还原转化硝态氮能力高低的标志，活性高，还原转化能力就高，反之则低。

表2　不同氮肥用量对蔬菜硝态氮含量的影响

Table 2　Effects of N rates on nitrate N contents in the vegetables

施氮量 N rate (g/kg,soil)	硝态氮含量 (NO_3^--N μg/g.FW) Nitrate N contents		
	小白菜 Chinese cabbage	油　菜 Rape	菠　菜 Spinach
0.0	11.2	9.8	4.9
0.2	72.1	43.0	64.0
0.4	425.5	448.1	400.7
0.6	692.9	772.5	514.7
0.8	903.4	1032.6	624.3

* 采样日期为1995年11月28日～12月3日，12月4～9日．Sampled from Nov. 28 to Dec. 3 and from Dec. 4 to 9, 1995

表3　不同施氮水平对蔬菜叶片硝酸还原酶活性的影响

Table 3　Effects of N rates on the activity of nitrate reductase in leaf blades of the vegetables

施氮量 N rate (g /kg soil)	硝酸还原酶活性（μg $NaNO_2$/g FW·h） Nitrate reductase activity		
	小白菜 Chinese cabbage	油　菜 Rape	菠　菜 Spinach
0.2	0.16	0.21	0.10
0.6	1.19	1.19	1.97

采样日期同表2 Sampling date as indicated in Table 2

表4　不同氮肥用量对蔬菜生长量的影响

Table 4　Effects of N rates on fresh weights of vegetables

施氮量 N rate (g /kg soil)	生长量（g FW/plant） Fresh weights		
	小白菜 Chinese cabbage	油　菜 Rape	菠　菜 Spinach
0.0	13.7d	11.1c	7.0c
0.2	71.8b	65.9b	17.9ab
0.4	74.1ab	78.7a	21.4a
0.6	81.4a	75.8a	21.0a
0.8	55.6c	69.5ab	14.4b

生长量数据后面的英文字母表示方差分析结果，采样日期同表2．Letters after fresh weights are the results of variance analysis. Sampling date as indicated in Table 2

盆栽试验（表3）表明：施氮量增加，蔬菜还原硝态氮的主要器官叶片的硝酸还原酶活性均明显升高。但高的硝酸还原酶活性并未能使蔬菜的硝态氮含量降低。原因在于硝酸还原酶是基质诱导酶，介质中的硝态氮浓度愈大，酶活性就会因诱导而愈高。试验采用的尿素虽非硝态氮，但在通气良好的旱作土壤中会很快经水解、硝化形成硝态氮[13]，因此，土壤供氮水平高形成的硝态氮就多；蔬菜吸收的硝态氮增加，硝酸还原酶活性也随之升高。但由于蔬菜对硝态氮的吸收超过了还原能力，进入体内的硝态氮不能及时被还原转化，所以会出现大量累积。可见，过量施用氮肥时，蔬菜硝态氮吸收与还原转化不平衡是产生累积的根本原因。

2.4　蔬菜的硝态氮吸收与生长不协调

氮肥用量对蔬菜生长和硝态氮累积有明显的影响。盆栽试验两次测定结果的平均值（表4）表明，缺氮会抑制蔬菜生长，充足的氮肥供应是蔬菜获得高产的重要保证；但氮肥用量过高反而会使增产效应降低，甚至引起减产。氮肥用量较低时，3种蔬菜的生长量随氮肥用量增加而逐渐提高，小白菜在施氮量为0.6 gN/kg土时，生长量最高；油菜和菠菜在施氮量为0.4gN/kg土时，生长量最高。之后，随氮肥用量增加，生长量反而下降。

比较施和不施氮肥时蔬菜的硝态氮累积总量（生长量与硝态氮含量的乘积）可以看出，随施氮量增加，硝态氮累积总量明显增加。在不同氮肥用量时，硝态氮总量的增加均大于生长量的增加（表5）。如小白菜在施氮量为0.2，0.4，0.6和0.8g N/kg土时，硝态氮累积总量分别比不施氮肥时增加33.7，205.5，367.6和327.4倍，而生长量仅相应增加4.2，4.4，4.9，3.1倍，说明硝态氮的增加速度远大于生长的增加速度。这种因生长增加慢而硝态氮吸收增加快引起的养分富集效应使蔬菜的硝态氮含量成倍升高。

据此可以推断，如果创造合适的外界条件，使蔬菜的生长增加量超过硝态氮累积增加量，硝态氮含量将会降低。本文进行的另外试验提供了这一方面的证据[14]，在采收前10d对同一施氮量的小白菜、菠菜进行不同的水分处理，结果土壤水分为20%和25%时，小白菜和菠菜的硝态氮含量比15%的土壤水分时降低19.4%～29.7%。增加土壤水分促进了蔬菜生长，也促进了硝态氮吸收，但生长和硝态氮吸收的增加并不同速，生长量增加快而硝态氮吸收量增加慢。这种由于增加土壤水分使生长增加较快引起的植物体内养分稀释效应，是导致蔬菜硝态氮含量降低的重要原因。

表 5　施氮时蔬菜累积的硝态氮和生长量比不施氮时增加的倍数

Table 5　Folds of NO$_3^-$-N and fresh weight increased by addition of different N rates to the vegetables as compared with those without N

施氮量 N rate (g /kg soil)	硝态氮累积量增加 Increased folds of accumulated NO$_3^-$-N			生长量增加 Increased folds of fresh weights		
	小白菜 Chinese cabbage	油　菜 Rape	菠　菜 Spinach	小白菜 Chinese cabbage	油　菜 Rape	菠　菜 Spinach
0.2	33.7	26.0	33.4	4.2	4.9	1.6
0.4	205.5	324.2	250.0	4.4	6.1	2.1
0.6	367.6	538.3	315.1	4.9	5.8	2.0
0.8	327.4	659.7	262.1	3.1	5.3	1.1

3　讨论

上述研究表明，硝态氮累积现象并非叶类蔬菜所特有，而是一般旱作作物的共性。这一特性在苗期表现尤为突出，叶类蔬菜与禾谷类作物冬小麦均能大量累积硝态氮，彼此难分高低。蔬菜的硝态氮累积之所以会危害人类，原因在于叶类蔬菜的收获物是硝态氮含量高的营养器官，且在土壤氮素供应充分、作物生长旺盛、吸收和累积硝态氮能力均较强的营养生长早期收获。蔬菜累积的硝态氮主要来源于施入土壤的氮肥，同时由于过量施用氮肥，所导致的蔬菜硝态氮吸收与还原转化不平衡是产生累积的根本原因，吸收与生长不协调加剧了这一累积过程。

调查表明，当前叶类蔬菜的施氮（N）量普遍在 1380～1800kg/hm^2。在肥力中等的菜园土壤上进行的田间试验（表 1）表明，在不施氮肥时，小白菜、大青菜、油菜和菠菜的硝态氮含量就已超过 3 级污染水平（硝态氮≥325μg/g 鲜重）[8]，每公顷施氮（N）90～180kg，硝态氮含量还能不断升高。可见，不合理的过量施用氮肥是蔬菜生产中的严重问题，是影响蔬菜品质的主要因素。盆栽试验（表 2，表 4）表明，在氮肥用量最低为 0.2g N/kg 土时，蔬菜仍显著高于不施氮肥时的生长量，且和最高产量相近；而其硝态氮含量却仅为 43.0～72.1μg/g 鲜重，尚达不到 1 级污染水平（硝态氮＜98μg/g 鲜重）[8]，可以生食、盐渍和熟食，属优质蔬菜。因此，合理控制和减少氮肥用量是蔬菜高产优质的关键所在。

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