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Nitrate Residues in Fruits, Vegetables and Bread Samples and Their Health Consequences

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Abstract

Application of mineral fertilizers such as Nitrate or urea derivatives to crops is an essential agricultural step for fruits and vegetable production. This step may lead to accumulation of Nitrate levels in fruit and vegetables creating health risks such as cancer. This study aimed to determine nitrate residues in beard, fruits, vegetable and water samples collected from different locations and to correlate them with potential cancer cases in Gaza. Results showed elevated levels of Nitrate in some breads samples and all fruits, vegetable and water samples. This suggests high potential risk of population to cancer cases. So far, the growing incidence of cancer cases in Gaza strip may be attributed to high level of nitrate contents in bread, fruits, vegetable and water samples. It is recommended to exclude nitrate fertilization from agricultural process or replace it with other N-fertilizer source.

Keywords

Nitrate Residues, Cucumber, Tomato, Fruits

1. Introduction

Contamination of food is a worldwide problem. Contamination of food may occur directly on the field throughout agricultural steps such as pesticide and fertilizers application, indirectly throughout postharvest applications of preservatives and/or throughout processing steps. So far food contamination can be subdivided into three main categories: 1) organic contamination of food, which includes pesticide residues, antibiotics, preservatives; 2) inorganic contamination such as heavy metals, nitrates, phosphates and; 3) organometallic contaminations.

So far organic contamination of food samples with pesticides has intensively

studied. This included determination of dioxins, dibenzofurans and poly chlorinated biphenyls (PCBs) in human human milk and food [1] [2] pesticide residues in cucumber, tomatoes, strawberries, green pepper, potatoes, *Vicia faba*, green bean and green peas [3]-[8], leachate of solid wastes [9] [10], herbicide residues in water [11]-[23].

Furthermore, inorganic contamination included heavy metals in water samples [24] [25], vegetables [26] [27] [28] [29]. These studies found considerable concentrations of heavy metals in water samples and found nitrate levels exceeded the maximum residue limits set by USEPY. Additionally, some authors investigated the health impacts associated with these contaminants. The investigators found oxidative stress among general populations [30]-[35], impaired human health effects [36] [37] [38] [39] [40], toxicity to non-target organism [41]-[48]. Gaza Strip has an intensive agricultural activity to secure vegetables, fruits and bread to the local population. This activity has been associated with intensive application of mineral fertilizers such as the use of large quantiles of nitrate along with phosphate as inorganic fertilizers. Application of fertilizers in Gaza Strip raised public concern on Nitrate concentration in fruits, vegetables and bread and their health consequences.

Cancer cases in Gaza Strip are progressively increased; the incidence rate reached high level of Ministry of Health [49]. Previous studies correlated the incidence of cancer cases with pesticide application [38] [50] and/or medical exposure treatments [51]. Furthermore, it can be suggested that a correlation between nitrate levels in food and cancer cases are existed. Previous authors [52] correlated cancer cases with nitrate levels in food. So far, few reports around the world and no reports published from Gaza on nitrate levels in bread, fruits and vegetables are available and their health consequences. Residues of nitrate in bread, fruits and vegetables are poorly investigated or remains on its primary stages. The author of this study focused his efforts on nitrate determination in beard, fruits and vegetables for the possibility to respond to the growing concern of public about nitrate residues in food samples.

2. Materials and Methods

Description of study site. Gaza Strip is an important part of Palestine. It contained five Governorates, the northern area, Gaza, the middle (Deir al-blah), Khan Yunis and Rafah Governorates. The Gaza Strip, as one of the most densely populated areas in the world (2638 people/km^2), has limited and declining resources and has already started to experience deterioration of environmental quality. More details on study site are shown in recently published work [8].

3. Experimental Design

3.1. Data Collection

Data on Nitrate fertilizers used in agricultural crops were collected from ministry of agriculture, Gaza Palestine.

3.2. Determination of Nitrate Levels

3.2.1. Bread Samples

Bread samples were collected from main five big bakeries from Gaza city where most inhabitants purchased their daily bread. About 5 bags (3 kg each and counted 50 bread pieces) were collected from each bakery house through two hours during the intensive period of work to insure randomization of bread selection.

The samples of each bakery were brought to the laboratory, mixed thoroughly then, three samples 1 kg each were randomly collected from the 5 bags of each bakery house. Each 1 kg of bread sample was transferred to a 3 litter glass conical flask. Two litter of distilled water was added to each flask and left for four hours then the total volume of water and bread was brought to 3 L by adding distilled water up to the mark. The flasks were left under magnetic stirring for an overnight period. Then bread water solution was separated by centrifugation at 3000 g for 15 min at 10˚C. Nitrate concentrations in the superintendents were determined using salicylic acid method [28] [29]. The analysis included 15 replicates.

3.2.2. Fruits Samples

Representative samples (15 samples, 3 kg each) tomato, *Solanum lycopersicum*, cucumber *Cucumis sativus,* samples, sugar cane *Saccharum spontaneum* L. and pomegranate *Punica granatum* samples were randomly selected and purchased from the central market in Gaza and stored in the laboratory at our university. The samples were coded according to the laboratory procedure. One kg tomato and/or cucumber was added to one litter distilled water and crushed using a blinder for 15 min. Then the mixture was homogenized using a special homogenizer. Then the supernatants were collected using centrifugation method at 5000 g for 20 min at 10˚C. The analysis included 15 replicates.

Sugar cane and pomegranate fruits were squeezed using a special device for each of them. The supernatant was cleaned up by filtration using normal filter paper.

Nitrate levels in the supernatants were determined by salicylic acid method as mentioned above [28]. The analysis included 13 replicates.

In this method Nitrate concentration were allowed to react with salicylic acid to form nitro-salicylic acid in the presence of sulfuric acid. The nitro salicylic acid is colorless in the acidic media where the reaction took place. Then a sodium hydroxide was added to the solution to neutralize the sulfuric acid. At neutralization, a yellow color would appear and its intensity represents the concentration of nitrate in the solution. More details are shown in the following equations

$$\begin{aligned} &NO_3 + \text{Salicylic acid} + \text{Sulfuric acid} \rightarrow \text{P} \\ &-\text{Nitro} - \text{Salicylic acid} + \text{Sulfuric acid} \end{aligned} \quad (1)$$

$$\begin{aligned} &(\text{Equation 1}) + \text{NaOH} \rightarrow \text{P} - \text{Nitro} - \text{Salicylic} \\ &\text{acid } (\text{yellow color}) + H_2O + Na_2SO_4 \end{aligned} \quad (2)$$

The yellow color is being determined by spectrophotometer at 420 nm.

3.2.3. Water Collection

Water samples were collected from the bakery themselves and analyzed for Nitrate levels. Additionally, water samples were collected from local wells supplying human consumption. Nitrate levels were analyzed as mentioned above.

3.3. Statistical Analysis

Mean and standard deviation were calculated for each type. T-test was used to detect significant differences among means. P-value = 0.05 or less indicates significant differences.

4. Results

4.1. Nitrate Fertilizers

Nitrate fertilizers used in agricultural crops are shown in Table 1. It can be seen that large fraction of nitrogen fertilizers (urea and organic fertilizers) were applied in the tested agricultural produces. The amounts of fertilizers can slowly be converted to nitrate in soil and become available for plant uptake.

4.2. Nitrate Levels in Bread

Nitrate levels in bread samples are shown in Figure 1. It is obvious that nitrate levels in all bread samples are below 20 mg/kg except bread from Gaza-Y has Nitrate level above 60 mg/kg.

Furthermore, statistical analysis detected significant differences only with Gaza-Y bread. So far we marked the data with different letter where statistical analysis was detected.

4.3. Nitrate Level in Fruits and Vegetables

Nitrate levels in cucumber, tomato (vegetables) and sugar cane, pomegranate (fruits) some fruits and vegetables are shown in Figure 2.

It is obvious that Nitrate concentrations exceeded the maximum residue limit set by USEPA and WHO for nitrate concentration in drinking water quality. Regardless to the fact that these products are not drinking water but water consist more that 90 of its contents. Consequently, we can apply drinking water standards to judge the quality for human consumption. It can be seen that

Table 1. Nitrate fertilizers in agricultural crops.

Crop	Urea 46% (kg/ha)	Organic N
Cucumber	130	500
Tomato	220	500
Sugar cane	200	500
Pomegranate	200	500

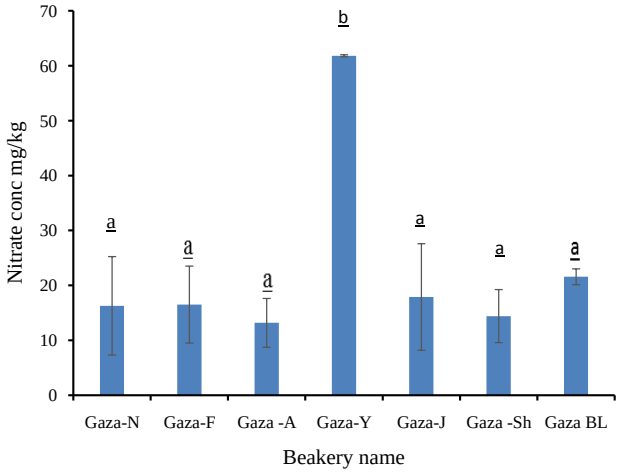

Figure 1. Nitrate levels in bread samples collected from different bakeries in Gaza strip. Error bars represent standard deviation. Columns have the same letter are not significantly different at P-vale = 0.05.

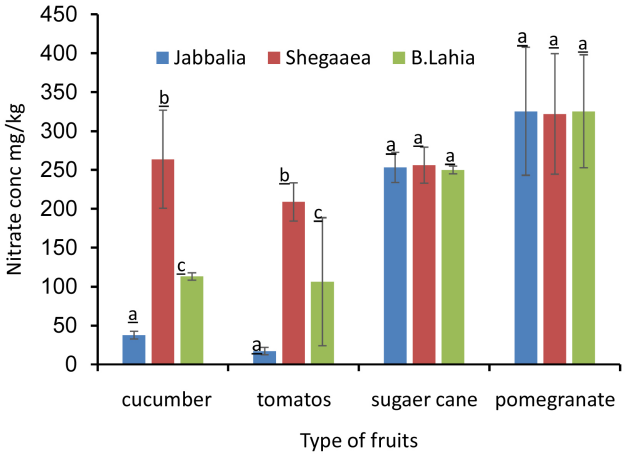

Figure 2. Nitrate levels in different fruits and vegetables from different locations. Error bars represent standard deviation. Columns have the same letter are not significantly different at P-value = 0.05.

significant differences in nitrate levels were detected in cucumber and tomato obtained from different locations. This suggests different nitrate application and uptake by plants. So far fruit samples did now show significant difference among locations. This suggests that similar nitrate program was applied for fruits in different location.

4.4. Nitrate Levels in Water

Nitrate levels in drinking water are shown in **Figure 3**. It is obvious that Nitrate level exceed WHO standards [53]. These data suggest high potential risk to local inhabitant who used large fraction of water.

4.5. Cancer Cases in Gaza Strip

Cancer cases registered in Gaza strip during 2009-2014 are listed in **Table 2**.

Table 2. Cancer case registered in Gaza 2009-2014 [49].

	Incidence rate case/100,000		
Type of cancer	Children (n = 476)	Male (n = 3219)	Female (n = 3850)
Breast cancer	-	-	49.1 (n = 1283)
Colo-rectal	-	45.3 (n = 378)	42.7 (n = 709)
Lung	-	44.9 (n = 370)	18.8
Prostate	-	32.4	-
Thyroid	-	-	32.4
Leukemia	24.5	30.1	29
Lymphoma	11.6	28.6	21
Brain	16.3	25.5	18.3
Neuroblastoma	6.1	10.1	-
Nephroblastoma	5	-	-
Rhabdomyosarcoma	2	-	-
Retinoblastoma	1.4	-	-
Urinary bladder	-	21.4	-
Stomach	-	17.4	13.8 (n = 227)
Bone	6.3	11.3	10.5
Uterus	-	-	21.9
Ovary	-	-	16.3

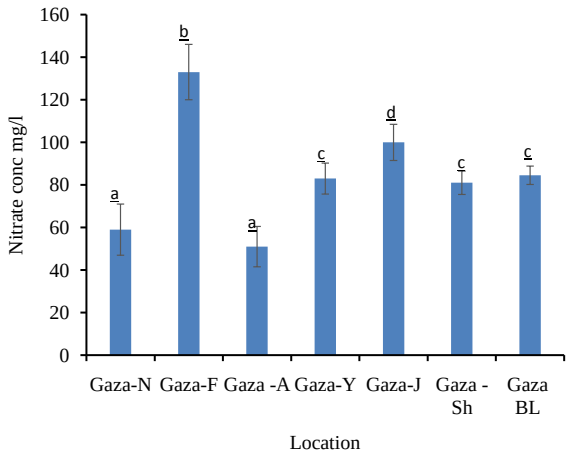

Figure 3. Nitrate levels in water samples from different locations. Error bars represent standard deviation. Columns have the same letter are not significantly different at P-value = 0.05.

Table 2 shows the incidence of cancer among population. It is clear that there is a growing incidence of cancer among population.

5. Discussion

Nitrates and nitrogen containing fertilizers are essential plant nutrient. These

elements may occur naturally in soil due to geochemical cycle of Nitrogen gas or manmade fertilization. This may result in accumulation of Nitrate levels in fruit and vegetables. The presented data in **Table 1** clearly showed the large fraction of both types of nitrogen fertilizers that can be converted to Nitrate in soil and be absorbed by plant root systems. However, the conversions of these fertilizers to nitrate are a slowly process and depend on soil type. So far, plants can absorb all nitrate concentrations from soil and accumulate them in plant tissues as seen in the data in **Figure 2** and **Figure 3**. Furthermore, presented results in **Figure 1**, clearly demonstrates elevated levels of Nitrate in bread samples from different bakeries in Gaza. Regardless to the fact that the apparent level is nearly low, it can be at high risk to the local inhabitant due to the fact that bread is the main food that everybody consumes at least 0.75 - 1 kg/day/person. This tends to be high level. However, the nitrate residues in bread samples may be originated from water irrigation of wheat during agricultural processes or from water used for processing bread in different bakeries. This is supported by the data in **Figure 2** and **Figure 3**. This is also obvious from the data in **Figure 2** which shows high residue levels of nitrate in agricultural produces and high level in drinking water (**Figure 3**). Due to high daily intake of nitrate throughout bread consumption the local population may be exposed to potential hazardous reactions in human body that lead to cancer cell generation.

However, bread consumption with a high level of Nitrate may expose the stomach to form massive cancer cells. It is well known in the literature that bread stays at least 6 hr retention time in the stomach for acid digestion. This process may result in releasing Nitrate contents of food to the stomach and be absorbed and translocated to the liver, then be exposed to oxidase enzymes and be converted to Nitrite radical species or reactive oxygen species (ROS) that may react with the genetic memory of cell (RNA/DNA) forming nitrification product according to the Equation (3) and Equation (4):

$$NO_3 + \text{Oxidase Enzyme in liver} \rightarrow NO\bullet 2 \quad (3)$$

$$NO\bullet 2 + \text{RNA / DNA} \rightarrow \text{RNA-NO2} + \text{DNA-NO2} \quad (4)$$

It is obvious from Equation (3) that the formation ROS (NO_2) depends on the initial concentration of NO_3, as much bread being consumed as much of NO_3 excreted to the stomach, then the reaction proceed as in Equation (4). Our equations are in accordance with Sun *et al.* [54] who found different RNA sequencing due to exposure to nitrite concentrations in the range of 0.1 - 30 mg/l. additional supports to the equations can be obtained from Sun *et al.* [55] who revealed the influence of ROS generated in response to nitrite on oxidative stress defenses and the antioxidant system in *Megalobrama amblycephala*, the full length cDNA sequences.

It has been found that generation of ROS may lead to potential oxidative damage to cellular macromolecules [56]. So far, elimination of excessive ROS and protect the human body is depending on the activity of the defense systems which included antioxidant molecules like glutathione or antioxidant enzymes

such as superoxide dismutase, catalase and glutathione peroxidase [57] [58] [59] [60]. At normal activity of activity those molecules no damage may occur, but at partial or failure elimination of ROS may lead to oxidative damage [61] resulting in cancer cell formation.

So far, nitrite was shown to react with nitrostable compounds in human stomach to form nitroso compounds, many of these N-nitroso compounds have been found to be carcinogenic in all tested animals [53]. Thus a link between cancer risk and endogenous nitrosation as a result of high consumption of foods (bread, vegetables, fruits and drinking water) containing nitrate and/or nitrite and nitrostable compounds is possible [62] [63] [64].

So far, the nitro ribonucleic acid or nitro deoxyribonucleic acid began to grow independently far away from body control forming the 1st cancer cell that starts its growth resulting in a carcinoma growth that may lead to a massive cancer growth.

However, this reaction may take place in the liver forming liver cancer, or in lung forming a lung cancer and so on. This is in accordance with El-Nahhal and Lubbad [34] who found potential metabolic reactions of low concentrations of pesticide in chicken lever.

However, in less acid stomach, minority of population, bread content of nitrate may move to the intestine where the coliform bacteria are commonly residing, in this case nitrate may be reduced to molecular nitrogen and being execrated outside the body according to Equation (5) and Equation (6).

$$NO_3 + \text{COLIFORM BACTERIA} \rightarrow NH_4OH \quad (5)$$

$$NH_4OH \rightarrow NO_2 \rightarrow N_2O \rightarrow N_2 \quad (6)$$

It can be concluded that majority nitrate concentration may be harmful to the population due to the fact that the majority of stomachs are acidic leading to the chemical reactions detailed in Equation (3) and Equation (4). Additionally, the minority of nitrate levels be execrated from human body according to Equation (5) and Equation (6).

Equation (5) and Equation (6) are in accordance with previous reports [65] [66] that demonstrated the efficacy of bacterial to convert NO_3 to N_2.

The data in **Table 2** clearly demonstrate the distribution of cancer cases among population including children. It is obvious that incidence of cancer cases in the present study (**Table 2**) is nearly higher than the previous incidence rate [38] [50] indicating a growing rate. The explanation of these results is that the population consumed large quantities of bread that contained high concentration of nitrate (**Figure 1**). Nitrate concentration may be converted in the human body to potential carcinogenic agent such as Nitrite compound according to Equation (3) and Equation (4).

On the other hands, the population consumed large quantities of fresh fruits and vegetable due to their high content of anti-oxidants that would be of beneficial health effects against cancer [52] [67] but for our cases, consumption of fruits and vegetables with high nitrate levels (**Figure 2** and **Figure 3**) may be at

high cancer risk due to high level of nitrate contents. Our discussion agrees with Song *et al.* [68] who revealed the potential associations between dietary consumption of nitrates, nitrites, and nitrosamines and gastric cancer. Additionally, they verified that increased consumption of nitrites and N-nitrosodimethylamine seemed to be risk factors for cancer. Furthermore Dellavalle *et al.* [69] found that high dietary nitrate intake among population expected to have higher exposure to endogenously formed N-nitroso compounds increases risk of colorectal cancer. In the same context Inoue-Choi *et al.* [70] indicated that high nitrate levels in public drinking water and private well use may increase ovarian cancer risk among postmenopausal women. On the other hand, previous studies on cancer cases have been correlated with pesticides and residues accumulation in agricultural produces [38] [50]. In the present study, it appeared that nitrate levels in food dietary may have a strong correlation with increased cancer cases. Furthermore recent publication [51] correlated the cancer cases many causes.

6. Environmental Relevance

Cancer is not a communicable disease but it could refer to as an environmental disease that can be emerged due to exposure to certain environmental pollutants. Accordingly, improving the environmental quality may result in reducing the cancer cases among the population. Improving the environmental quality could be achieved by purifying drinking water by the use of organo-clays [71] [72] [73] bioremediation of soil pollution [74] [75] [76] [77] and the use of cyanobacteria [78] [79] [80]. Implementation of these options may lead to an improvement of the environmental quality and reduction of cancer case.

7. Conclusions

The rational of this work emerged from the fact that nitrate levels in food are a leading cause toward a massive carcinoma development in human body. The results revealed high levels of Nitrate residues in bread, vegetables, fruits and water cases in Gaza Strip. A progressive increase of cancer cases in Gaza Strip may be correlated with nitrate levels in bread fruits and vegetables.

It can be concluded that the population in Gaza Strip may become at risk due to high nitrate levels in bread, the main food in Gaza. It is highly recommended to exclude nitrate levels from bread, fruits vegetables and water samples. This can be achieved through reducing the amount of nitrate fertilizers in agricultural system.

Author Contribution

YE designed, performed the experimental work and wrote the manuscript.

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