EC MICROBIOLOGY
Research Article

Effects of Nitrate in Domestic Water: Enugu Metropolis, Nigeria

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Abstract

The main aim of this study is to determine the quantity of nitrate in water samples collected from Enugu metropolis and also to discuss the health effects of high consumption of nitrate in water. Thirty seven water samples were collected from seventeen different locations with high population of inhabitants. Sterile universal sample containers were used to collect appropriate quantity of water. The water samples were properly sent to Safety Molecular and Pathology Laboratories, Enugu for quantification of nitrate present in them using the a spectrophotometer set at wavelength 520 nm. The nitrate levels present in most of the consumable water sources values (> 3 mg/L) were significantly within the range and lower than acceptable level of nitrate (50 mg/L) in water for consumption, ($P < 0.0001$). There is relatively low nitrate level (< 50 mg/L) in all water samples collected for analysis. This indicates that water found within Enugu metropolis is safe for human and aquatic consumption and pose no harm to human health.

Keywords: *Nitrate; Domestic Water; Nigeria*

Introduction

Nitrate is a polyatomic ion with molecular formula NO_3^- and a molecular mass of 62.0049 g/mol. They are naturally occurring ions formed by the oxidation of nitrogen and are an integral part of the nitrogen cycle in the environment. Nitrates are mainly produced for use as fertilizers in agriculture because of their high solubility and biodegradability. A rich source of inorganic nitrate in the human body comes from diets rich in leafy green foods such as spinach. Dietary nitrates may be found in cured meats, various leafy vegetables and drinking water. Apart from natural occurring nitrates found in vegetables and fruits, they are also used as preservatives and flavorings to meat and meat products [1]. Notwithstanding, nitrates improves texture, shelf-life, flavour and colour of meat and poultry products but its presence in food have been implicated in human toxicity and carcinogenicity due to their enzymatic conversion to nitrite (NO_2^-) in the body [1]. Also, nitrites and water can be converted in the body to nitric oxide which could reduce hypertension.

Nitrate poisoning can occur through enterohepatic metabolism of nitrate due to nitrite being an intermediate. Nitrites oxidize the iron atoms in hemoglobin from ferrous iron (II) to ferric iron (III), rendering it unable to carry oxygen which might lead to generalized lack of oxygen in organ and tissue to cause a condition termed "methemoglobinemia in adults" or " blue baby syndrome in infants" with infants being more vulnerable due to high concentration of nitrate metabolizing triglycerides [2].

High nitrate in water can cause a lot of health challenges like pancreatic cancer. Research has been conducted to see the relationship between eating processed foods and pancreatic cancer and according to *Journal of the National Cancer Institute* and by *Cancer Treatment Centers of America,* it appeared that the strongest association was found among those who have a daily intake of processed meat [3]. N-Nitroso-compounds (NOC) and Polycyclic Aromatic Hydrocarbons (PAH) can cause cancer. The Linus Pauling Institute stated that nitrates may be associated with development of brain tumors, leukemia, nose and throat tumors. Environmental Working Group (EWG) reports that this along with other food additives may be linked to stomach cancers [4]. Research also shows that high nitrate consumption can cause Alzheimer's and diabetes and may also affect brain health and insulin function.

There are many ways to avoid high consumption of nitrates which include but are not limited to avoidance of processed meat and their products, filtration of water to remove impurities and toxins, taking enough organic vegetables and fruits like tomatoes, oranges, bananas, watermelon, peas, mushroom, egg plants, sweet potatoes etc.

Specific Aim and Objectives of the Study

The aim of this study is to quantify the nitrate level in water samples collected from different sources in Enugu metropolis.

Objectives of the study include:

1. To check the quantity of nitrate present in water samples collected from different part of Enugu.
2. To know if the nitrate present in the water sample is fit for human consumption.
3. To discuss the possible health implication of high consumption of nitrate in water.

Hypothesis

Null hypothesis

There is low level of nitrate in water found and used in Enugu metropolis.

Materials

Eppendorf Biophotometer plus, Erlenmeyer flask, micro filters, potassium nitrate solution, concentrated hydrochloric acid (HCl), distilled water, Zinc, Griess reagent (N-1-naphthylethylenediamine dihydrochloride), sulfanillic acid, sodium acetate, purified water.

Methods

Study design

The study was carried out in the city of Enugu, Southeastern Nigeria. Random sampling method was used to collect a total of 37 water samples from 17 different areas in Enugu. This method of sampling ensured that every part of Enugu city was represented. In addition, few water samples packaged for drinking were collected to ascertain its level of safety for human consumption.

Inclusion and exclusion criteria

All possible sources of water supply in Enugu city were collected with the inclusion of stream, rain, well, tap, bore-hole, bottled and sachet water.

The rural and poorly developed parts of Enugu state were excluded from the study.

Laboratory procedures

The water samples collected were safely transported to Safety Molecular and Pathology Laboratory, Enugu for determination of concentration of nitrate in them. Eva water with nitrate level of about 0.2 ppm (0.2 mg/L) was used as control. First, the control standards were prepared at 2.5, 5.0, 10.0 and 15.0 concentrations (Mg/L) and the intensity of the red colour was measured spectrophotometrically at 520 nm using Eppendorf Biophotometer Plus.

The water samples collected for analysis were selected and the determination of nitrate in them were done by adding 0.5 ml of dilute HCl (1:4) and 0.5 ml of sulfanillic acid reagent into 250 ml Erlenmeyer flask containing 25 ml of water sample. The flask was gently vortexed. In a dry tube, 0.5g of Zinc was weighed and transferred into the Erlenmeyer flask and stirred for 7 minutes. Filter with a micro filter tube and add 0.5 ml of N-1-naphthylethylenediamine dihydrochloride reagent and mix for 5 minutes or more. The nitrate present in the water samples is reduced to Nitrite by addition of the Zinc, the nitrite now reacts with the sulfanillic acid and N-1naphthylethylenediamine to produce the red coloured compound.

Spectrophotometric measurement

The intensity of the red colour is measured spectrophotometrically at 520 nm using Eppendorf Biophotometer. The intensity of the red coloured compound is directly proportional to nitrate concentration in the water sample.

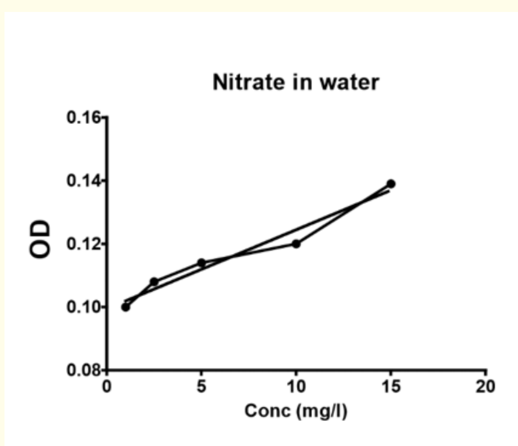
Statistical analysis

The average of the absorbance readings was computed and the test for significant difference between the nitrate levels in water and the accepted safe levels were noted using statistical tool (Linear Regression).

Result

Standard concentration

All 37 samples of water collected had varying degrees of nitrate contamination by quantitative tests using Eppendorf Biophotometer.



Along the line of collection, few samples were collected severally due to high consumption rate from different location. The nitrate concentration found in the water samples ranged from 1.3 to 30.6 (ppm) mg/L with a median value of 2.3 mg/L which is still within the range mapped out to be fit for human consumption.

S/N	Description	Site of sample collection	Nitrate level (mg/L)
1	Aqua Rapha Sachet water	Ind. Layout	30.6
2	Rain water	Ind. Layout	2.1
3	Well water	Ind. Layout	1.3
4	Well water 1	Maryland	2.3
5	Well water 2	Maryland	2.1
6	Sachet water	Agbani Road	19.8
7	Well water	Agbani Road	23.4
8	Well water	Awkunanaw	3.0
9	Annex sachet water	Awkunanaw	2.3
10	Well water	Achara layout	3.8
11	Well water	Uwani	7.0
12	Well water	Gariki	13.4
13	Tap water	G.R.A	2.4
14	Tanker water	G.R.A	7.0
15	Well water	Emene	6.6
16	Aqua Rapha water sachet	Emene	12.6
17	Stream water	Emene	4.2
18	Aqua Rapha water sachet	Trans-Ekulu	14.2
19	Well water 2	Trans-Ekulu	13.4
20	Tanker water	Trans-Ekulu	2.0
21	Rain water	Trans-Ekulu	2.3
22	Well water 1	Trans-Ekulu	1.8
23	Tanker water	T. Corner	2.2
24	Well water 1	T. Corner	2.3
25	M & SONS Sachet water	T. Corner	2.2
26	Well water 2	T. Corner	1.9
27	Well water	Abakpa	2.2
28	Aqua Rapha sachet water	Abakpa	2.4
29	Zikko sachet water	New Haven	2.1
30	Well water	New Haven	2.4
31	Stream water	New Haven	2.2
32	Well water	Asata	2.0
33	Tap water	Asata	11.4
34	Well water	Ogui Road	2.3
35	Well water	Coal camp	2.2
36	Rain water	Coal camp	2.2
37	Exalted bottle water	Ind. Layout	2.2

Using the Wilcoxon Signed Rank Test for one sample population, the P value was < 0.0001 at an α level of 0.05. The nitrate levels found were significantly lower than the internationally accepted levels fit for human and animal consumption.

Discussion

Considering most of the studies to date, the strongest evidence for a relationship between drinking water nitrate ingestion and adverse health outcomes (besides methemoglobinemia) is for colorectal cancer, thyroid disease, and neural tube defects. Four of the five published studies of colorectal cancer found evidence of an increased risk of colorectal cancer or colon cancer associated with water nitrate levels that were mostly below the respective regulatory limits [5-8].

Conclusion

In summary, most adverse health effects related to drinking water nitrate are likely due to a combination of high nitrate ingestion and factors that increase endogenous nitrosation. Some of the recent studies of cancer and some birth defects have been able to identify subgroups of the population likely to have greater potential for endogenous nitrosation.

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