International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

**Research Article** **CODEN: IJPNL6**

# DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS, NITRATE AND NITRITE IN IRAQI VEGETABLES BY HPLC AND UV/VIS SPECTROPHOTOMETER

Abdal-Jabbar A. Ali[1], Khalil I. Hussain[2*], Amera H. Hamed[1], Abood H. Moslah[1], Riyadh R. Neama[1]

[1]Ministry of Science & Technology, Environmental & Water Directorate, Iraq
[2]Department of Chemistry, College of Education-Ibn Al-Haitham, University of Baghdad, Iraq

***Corresponding author e-mail:** Khalil-ih-53@yahoo.com

**ABSTRACT**

This research deals with analytical methods for (16) polycyclic aromatic hydrocarbons (PAHs), nitrate and nitrite determination in Iraqi vegetables. Some PAHs are of proven carcinogenic activity; consequently, they exceed the limits of concentrations which approved by the European Food Safety Authority. Nitrate and nitrite also determined in Iraqi vegetables compared with the European specifications. There is no recommendations for the allowable limits of nitrate and Nitric in the fresh vegetables. The analytical determination for PAHs is carried out by high performance liquid chromatography, with ultraviolet (UV) detector, while the analytical determination for nitrate and nitrite is carried out by UV/Vis. Spectrometer.

**Keywords:** Polycyclic aromatic hydrocarbons, Nitrate, Nitrite, Vegetables analysis, HPLC, UV/Vis. Spectrometer

## INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a complex group of chemical compounds present in the environment as result of incomplete combustion, petrochemical activities and certain types of food processing such as smoking and drying. In 2002, the Scientific Committee of Food (SCF) concluded that 15 PAHs may potentially be genotoxic (damaging to DNA) and carcinogenic (cancer causing) to humans and should be prioritised when looking at the dietary intake of PAHs **[1,2]**. A list of the (15) PAHs identified by the SCF, along with their abbreviations, are given in Table 1. The SCF concluded that it was not possible to establish a threshold level below which risk would be insignificant and therefore a Tolerable Daily Intake (the amount of substance that can be ingested daily over a lifetime without appreciable health risk) could not be set. PAHs generally exist as colorless, pale yellow or white solids **[3]**. Because they do not dissolve easily in water and generally do not burn, they can persist in the environment for months to years **[4]**. SCF also concluded that, on the basis of toxicological information, Benzo (a) Pyrene (BaP) was a suitable marker of the occurrence of the carcinogenic PAHs in food and their health effects. Most of the (16) compounds prioritised by the European Food Safety Authority (EFSA) were often undetected, particularly in vegetables and cereal based foods. The highest PAH concentrations were found in smoked fish and shellfish products. PAH concentrations were low in vegetables, vegetable products cereal, and cereal products **[5]**. Local enforcement authorities investigated the companies whose products were non-compliant and advised them to explore the feasibility of changes to raw material sources and production processes in order to reduce PAH levels in their products.

The presence of ionic nitrate ($NO_3$) and nitrite ($NO_2$) normal condition theirs production to absorb large amount of nitrates or ammonia from the soil, and a high concentration of nitrogen in the soil is the main

reason for the increased concentration of nitrate, which is transmitted to the vegetables and then produce toxic levels of nitrite ($NO_2$) at smashing the vegetables in the stomach and intestines by microbiology **[6]**. High concentration of nitrate causes the disease methemoglobinemia in the children aged 6 months **[7]**; Also it causes what is known infected child (baby blue) through the reduction of hemoglobin in the red blood cells or pulling oxygen to make the child's body bluish **[8]**. Ionic nitrate ($NO_3$) is more stable than its output metabolic poison ($NO_2$) and usually nitrate concentration is higher than nitrite in the forms of environmental and biological **[9]**. Nitrate levels vary depending on the type of vegetables origin, as in Table (1).

The addition of nitrate and sodium nitrite to meat products to keep them through:

**1-** Maintaining the red color without switching to brown color.

**2-** Blocking the activity of microorganisms that cause lethal bacterial toxins.

In recent study, Bamtheis in a hospital Aazlanda red, USA, suggested that there is relationship between the increase in the level of nitrates and increased mortality of various diseases, including Alzheimer's, diabetes, Parkinson's disease, as well as cancer, through the pernicious influence of the compound (Nitrosamines) on DNA **[10]**. There is strong evidence that red meat and its products cause bowel cancer and it is preferable not to exceed a daily dose of red meat to 500 grams **[11]**.

## EXPERIMENTAL

### Apparatus:

- High Performance Liquid Chromatography (HPLC) Sykam CO. (Germany).
- UV/Vis. Spectrometer, Model (1650), Shimadzu CO. (Japan).
- Rotary Evaporator, Büchi RE 121.
- Soxhlet for Extraction.
- Ultrasonic Hi power-Sus-300, Shimadzu CO.

### Reagents:

− Standards of polycyclic Aromatic Hydrocarbons (99.92%), Greifenberg am aammersee, Flurstrasse CO. D-86926, Germany.
− Standards for ($NO_3$) and ($NO_2$).
− Acetonitril, 99.9% for HPLC (BDH, UK).
− Acetone, 99% (Fluka Chemika, Switzerland).
− n-hexane, 99% (GCC, UK).
− Water, gradient (HPLC grade) D=1, 00 gm/cm$^3$ (Scharlau, Spain).

### A: Procedure for the Extraction of PAHs from the vegetable samples [12]:

**1-** Samples of vegetable were taken from farms of Baghdad and its environs.

**2-** Every sample was dried in a temperature between (60-70 C) for (4-6) hours.

**3-** Grinding every sample to small particles (100 μm) by special mill to powder.

**4-** 25 mg was taken from each sample and put in cellulose thimble and then in the Soxhlet extraction.

**5-** 200 ml was used from the mixture of acetone and normal hexane in the ratio (1:1) as extraction solvents. The process of extraction continued at boiling points of solvents at 50 C for 20 hours.

**6-** Each sample was concentrated by the Rotary Evaporator to dryness and the volume was completed to 1 ml, after that, the sample was analysed by HPLC apparatus.

### B- Procedure for the Extraction of Nitrate and Nitrite from the vegetable samples [13]:

**1-** 500 grams of the sample was dried in oven between (80-100 C) for 5 hours.

**2-** 10 grams of this sample was taken with 400 ml of distilled water and left for 15 minutes in water bath then cooled and filtered.

**3-** The color is bleached by the effective charcoal.

**4-** To examine the nitrate, 50 ml of the solution was taken and added 2 ml (1 N HCl) and then measured by U.V./Visible in two wave lengths (220, 275 nm), the first one for the organic and nitrate, while the second for the organic only.

**5-** To examine the nitrite, 10 ml of the solution was taken and added 1 ml of the reagent and then measured by U.V./Vis. In wavelength (543 nm) which represents the concentration of nitrite.

## RESULTS AND DISCUSSION

HPLC is the most used component for a quantitative determining of PAHs in the vegetable samples. The PAHs were detected by UV detector in series operating at fixed wavelength. The compounds were initially identified by retention times and the concentrations were determined by comparing the peak heights of samples to those of PAHs standards. Table (2) illustrates the retention times and relative standard deviation for the sixteen compounds standard of the polynuclear aromatic hydrocarbons. These dangerous compounds were selected by United States Environment Protection Agency (USEPA) from PAHs family to follow the limits of concentrations in order to restrict its spread and presence in the environment more than others **[14]**. The HPLC peaks of the vegetable samples

corresponding to 16 PAHs were identified by coelution with authentic standard Fig. (1). The peaks are labeled in the chromatogram. The information in Table (3) provides a tabular summary of PAHs in vegetable samples, where it is noticed that some of these compounds increase the allowable limits, which approved by the European Food Safety Authority (EFSA) in a mean (0.14 ppb) to each four compounds **[15]**.

The increase of these compounds is evident in the most sample such as okra, onion, carrot, eggplant, and potatoes. This increase in the presence of PAHs in these vegetables, because they are grown near the quarters of pollution sources, such as oil refineries, electric power station, as well as irrigation with water contaminated with these compounds. Some of these PAHs compounds are carcinogens like Benzo (K) flouranthene, Benzo (a) pyrene and Benzo (b) flouranthene **[16]**, therefore, it is preferred that these vegetables are grown in places far from pollution sources. To determine the concentrations of nitrates and nitrites in Iraqi vegetables by UV/Vis. Spectrometer, made the drawing standard curves for nitrates and nitrites Fig. (3, 4) respectively.

Table (4) clarifies the concentrations of nitrate and nitrite in Iraqi vegetables in comparison with the European specifications. The results of this table and Fig. (5, 6) for Iraqi vegetables varies from one to another and this agrees with the observed results of the European vegetables. Also this table illustrates the highest value for the concentration of nitrates in lettuce (2600 ppm), while the lowest value in watermeion (100 ppm). As for the concentrations of nitrites, the highest value in the lettuce (120 ppm) while the lowest value in the carrot (0.552 ppm). The high concentrations of nitrate and nitrite in Iraqi vegetables is due to the following reasons:-

**1-** The nature of soil and nitrate content inside.
**2-** The concentration of nitrate in the irrigation water.
**3-** The kind and the quantities of chemical fertilizer that used.

**Table (1) nitrate and nitrite concentrations in the vegetables**

| Year/ Source | nitrate concentration ppm | | | Country | Type of food |
|---|---|---|---|---|---|
| | high | average | low | | |
| *[1]EFSA 2008 | 810 | 392 | 6 | Europe | beans |
| EFSA 2008 | 3670 | 1370 | 110 | Europe | beet root |
| EFSA 2008 | 833 | 311 | 47 | Europe | cabbage |
| *[2] FSA 2008 | ---- | 158 | ---- | Australia | carrot |
| EFSA 2008 | 1574 | 296 | 21 | Europe | carrot |
| EFSA 1982 | ---- | 204 | ---- | Australia | cauliflower |
| EFSA 2008 | ---- | 148 | 7 | Europe | cauliflower |
| EFSA 2008 | 3391 | 1130 | 18 | Europe | cclery |
| EFSA 2008 | 161 | 69 | 8 | Europe | Garlic |
| EFSA 2008 | 1573 | 875 | 210 | Europe | ice berg lettuce |
| EFSA 2008 | 340 | 168 | 10 | Europe | potato |
| EFSA 2008 | 4617 | 894 | 8 | Europe | squash |
| EFSA 2008 | 3048 | 1066 | 64 | Europe | spang |
| EFSA 2005 | ---- | 2138 | ---- | England | spangorgenic |
| EFSA 2008 | 975 | 345 | 5 | Europe | Leek |

***[1] European Food Safety Agency, *[2] Food Safety Agency UK.**

**Table 2: Retention times and relative standard deviation for the standards of (16) PAHs.**

| No. | PAHs | R.T. Min. | RSD% |
|---|---|---|---|
| 1 | Naphthalene | 12.16 | 0.58 |
| **2** | Acenaphthlene | 12.66 | 0.65 |
| **3** | Fluorene | 13.33 | 0.72 |
| **4** | Acenaphthene | 13.44 | 0.38 |
| **5** | Phenanthrene | 13.64 | 0.44 |
| **6** | Anthracene | 13.81 | 0.41 |
| **7** | Fluoranthene | 14.20 | 0.27 |
| **8** | Pyrene | 14.49 | 0.53 |
| **9** | Chrysene | 14.97 | 0.16 |

| | | | |
|---|---|---|---|
| **10** | Benzo (a) Anthracene | 14.98 | 0.26 |
| **11** | Benzo (b) Fluoranthene | 15.11 | 0.37 |
| **12** | Benzo (k) Fluoranthene | 15.80 | 0.26 |
| **13** | Dibenzo (a,h) anthracene | 16.24 | 0.24 |
| **14** | Benzo (a) Pyrene | 16.42 | 0.43 |
| **15** | Indeno (1,2,3-cd) Pyrene | 17.23 | 0.57 |
| **16** | Benzo (g,h,i) Perylene | 17.50 | 0.44 |

**R. T. = Retention times.**
**RSD= Relative standard deviation.**

**Table 3 : The concentrations (ppm) of polycyclic aromatic hydrocarbons in vegetables samples.**

| No. | Sample | PAHs | | | | | | | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | |
| 1 | Okra | 0.176 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.032 | ---- | ---- | ---- | ---- | 0.208 |
| 2 | Hot Pepper | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.004 | ---- | ---- | ---- | ---- | 0.004 |
| 3 | Onions | 0.048 | 0.215 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.263 |
| 4 | Carrot | ---- | 0.284 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.007 | ---- | ---- | ---- | ---- | ---- | 0.006 | 0.297 |
| 5 | Melon | ---- | 0.131 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.022 | ---- | ---- | 0.153 |
| 6 | Watermelon | 0.0043 | ---- | ---- | ---- | ---- | ---- | ---- | 0.083 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.0873 |
| 7 | Squash | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| 8 | Lettuce | ---- | 0.014 | ---- | ---- | ---- | ---- | ---- | 0.047 | ---- | ---- | ---- | ---- | 0.004 | ---- | ---- | ---- | 0.065 |
| 9 | Pepper | ---- | ---- | ---- | ---- | ---- | ---- | 0.029 | ---- | ---- | ---- | ---- | 0.028 | ---- | ---- | ---- | ---- | 0.057 |
| 10 | Eggplant | ---- | ---- | ---- | ---- | 2.377 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 2.377 |
| 11 | Potato | 0.539 | ---- | ---- | ---- | ---- | ---- | 0.030 | 0.077 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.646 |
| 12 | Tomato | 0.102 | 0.111 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.205 | ---- | ---- | 0.418 |
| 13 | Cucumber | 0.176 | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.176 |

**Table 4: The concentrations of nitrate and nitrite in the vegetable samples.**

| NO. | Vegetables Kind | Nitrate concent. ppm | Nitrite concent. ppm | Nitrate concent. according to the European specification |
|---|---|---|---|---|
| **1** | Potato | 1040 | 89.2 | 80 |
| **2** | Onions | 1872 | 3.28 | 60 |
| **3** | Squash | 937 | 114.12 | ---- |
| **4** | Pepper | 1013.7 | 93.15 | 1300 |
| **5** | Cucumber | 1100 | 10.8 | 150 |
| **6** | Eggplant | 643.3 | 72 | ---- |
| **7** | Hot pepper | 859.2 | 15.74 | ---- |
| **8** | Melon | 100 | 27.52 | 45 |
| **9** | Okra | 1464 | 1.68 | ---- |
| **10** | Watermelon | 667.2 | 12.84 | ---- |
| **11** | Tomato | 1382 | 6.92 | 60 |
| **12** | Carrot | 722.8 | 0.552 | 300 |
| **13** | Lettuce | 2600 | 120 | 2800 |

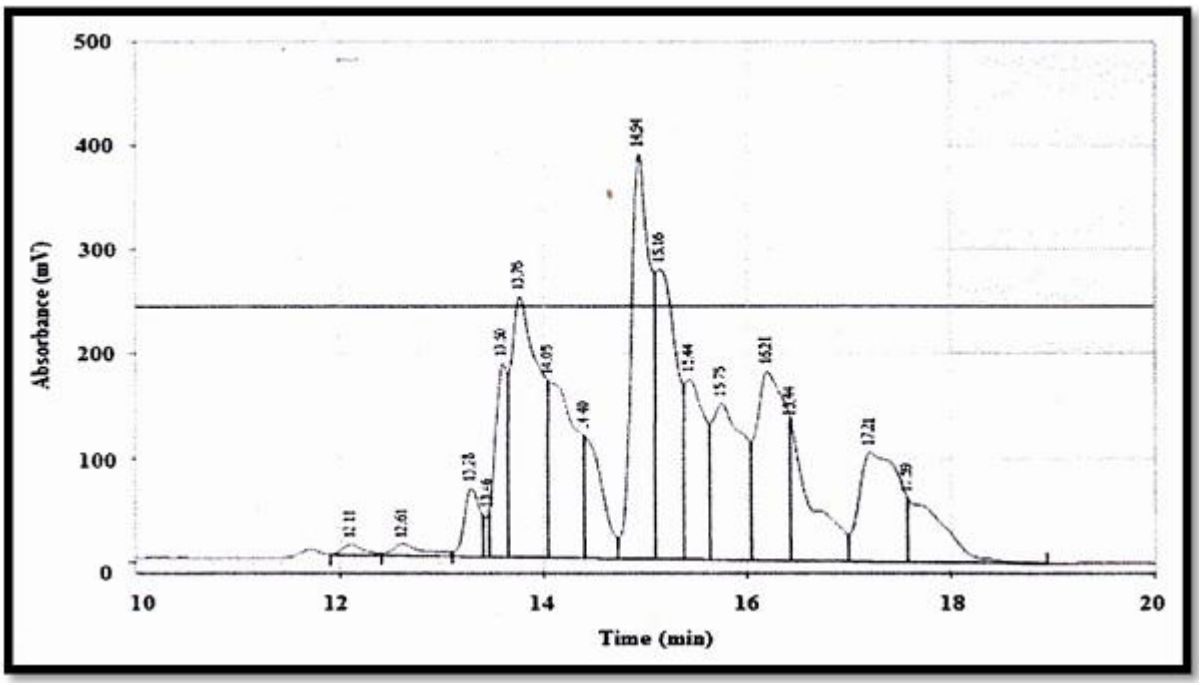


**Fig (1): The chromatogram of separation for 16 PAHs of the mixture standard: 1-Nephthalene, 2-Acenaphthylene, 3-Fluorene, 4-Acenaphthene, 5-Phenanthrene, 6-Anthracene, 7-Fluoranthene, 8-Pyrene, 9-Chrysene, 10-Benzo (a) Anthracene, 11-Benzo(b) Fluoranthene, 12-Benzo(k) Fluoranthene, 13-Dibenzo(a,h) Anthracene, 14- Benzo(a) Pyrene, 15- Indeno(1,2,3-cd) Pyrene, 16- Benzo(g,h,i) Perylene.**

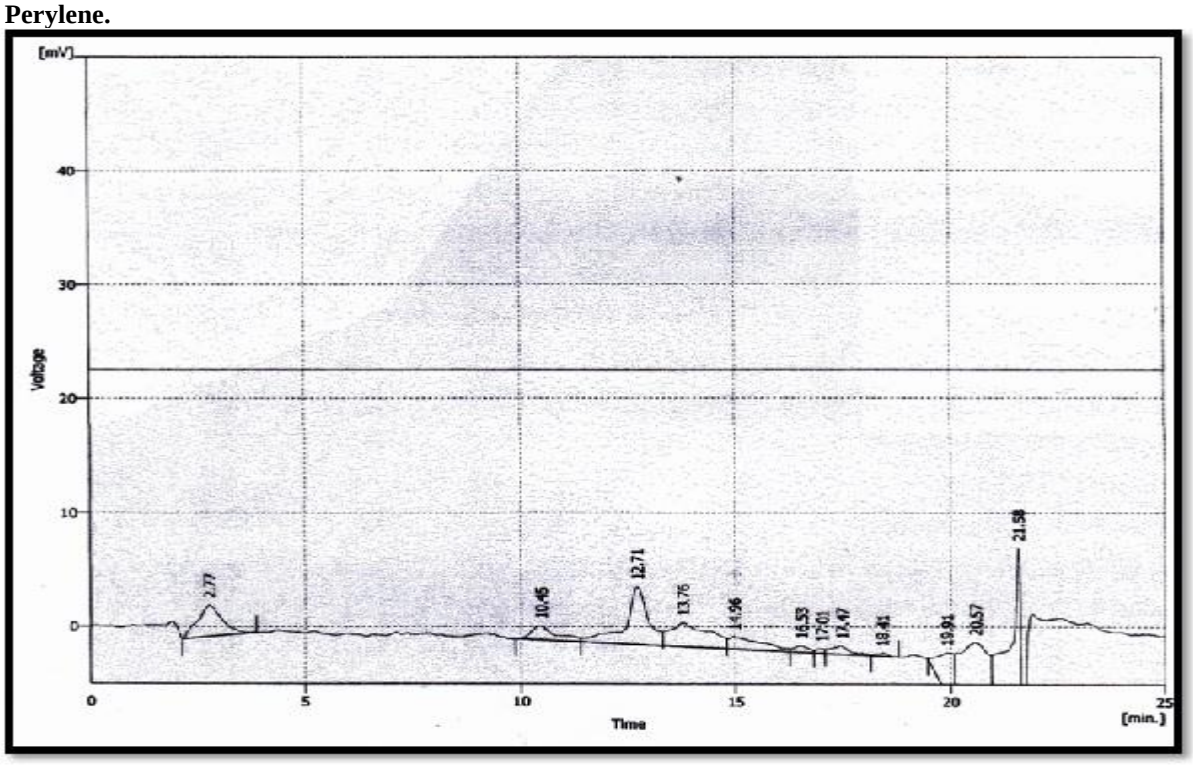


**Fig (2): The chromatogram of carrot sample.**

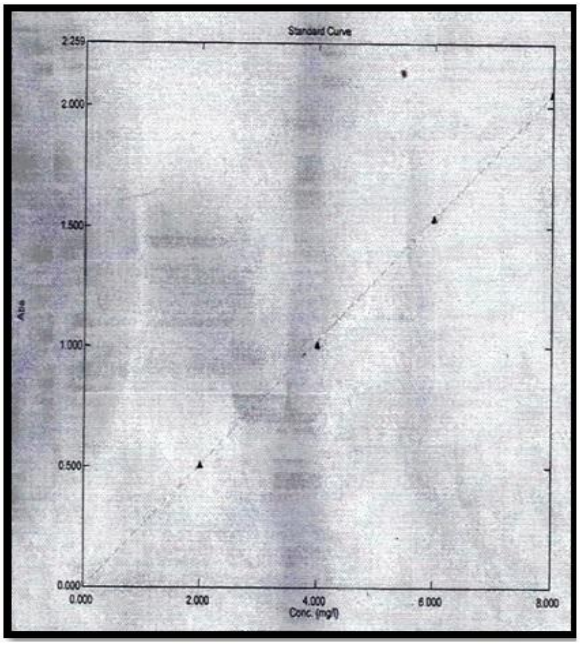


**Fig (3): The Standard Curve of $NO_3$**

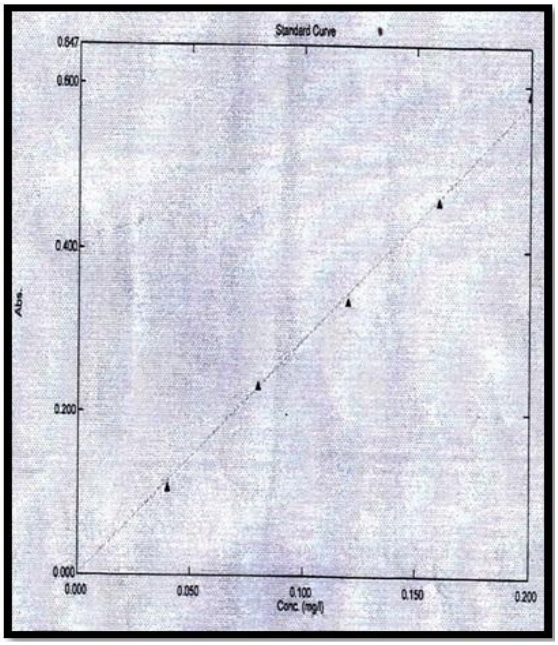


**Fig (4): The Standard Curve of $NO_2$**

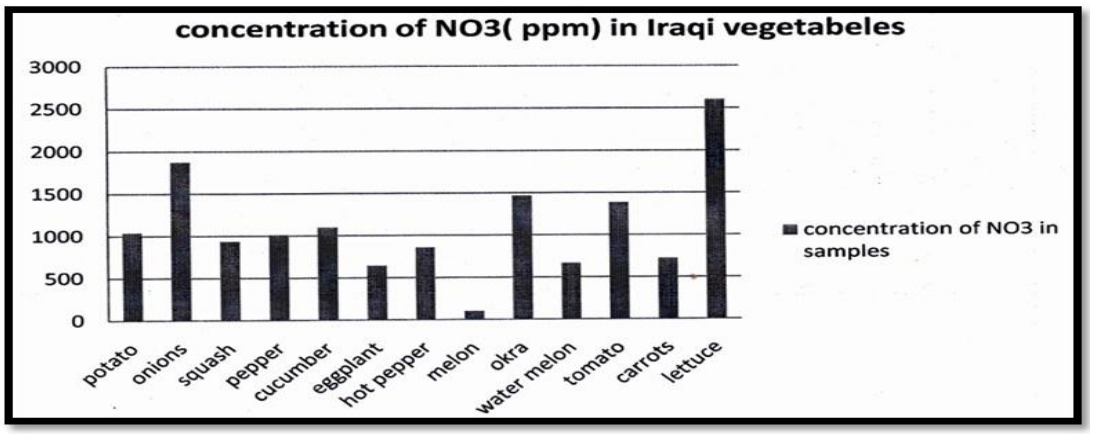


**Fig (5): Concentrations of nitrate in Iraqi vegetables**

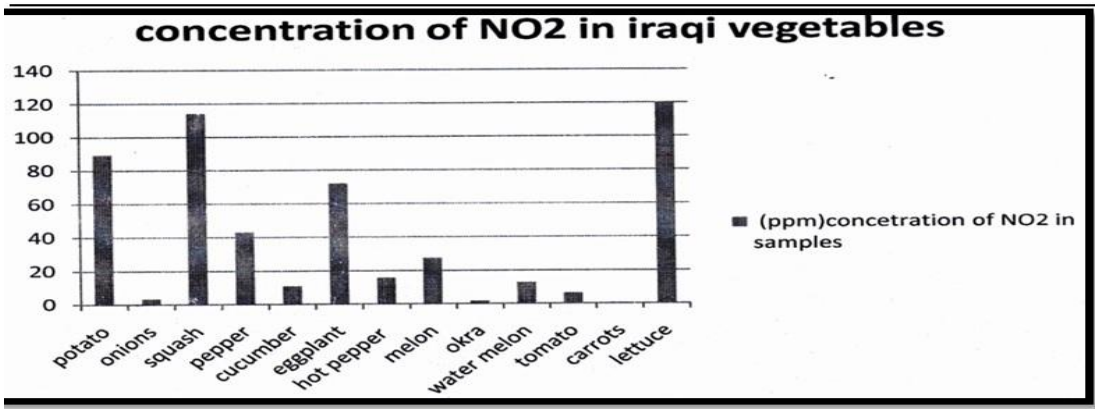


**Fig (6): Concentrations of nitrite in Iraqi vegetables**

**REFERENCES**


1. Scientific Committee on Food, 2002; Opinion of the Scientific Committee on the Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food. SCF/CS/CNTM/PAH/29 Final.
2. Barid W, Hooven L, and Mahadevan B, Environ. Mol Mutagen, 2005; 45(2-3): 106-114.
3. Casarett and Daull's, Toxicology, the Basic Science of Poisons, 5th edition, McGraw– Hill, New York: 1995.
4. Awata H, Bates S, Knaub D, and Popelka, R, 1998; EE & S 45 Environmental Engineering Chemistry 11: Environmental Organic Chemistry.
5. Fernandes A, Holland J, Petch R, Miller M, Stewart S and Rose M, 2011; FD 10/04. Food and Environment Research Agency.
6. Tanak A, Iwasaki N, Analyst, 1982; 107: 190-194.
7. Sanchez J, Benito J, Mintegui S, Pediatrics, 2001; 107, 5: 1024-1028.
8. European Food Safety Agency (EFSA Survey), EFSA Journal, 2008; 689, 1-79.
9. Horowitz W, Nitrogen (nitrate and nitrite) in animal feed, official methods of analysis of AOAC, 17th Edn, Washington: 2000, pp. 430-431.
10. Tong M, Neusner A, and Longato L, Alzheimer Dis; 2009; 17 (4), 827-844.
11. Okafor P and Ogbonna U, J. Food. Compos. Anal, 2005;16: 1105.
12. Zohair A, World J. of Agricultural Science, 2006; 2(1): 90-94.
13. British Standards Institution, 1997; Food stuffs determination of nitrate and nitrite content, Part 2, BSEN 12014-2 London.
14. Marce R and Borrull F, J. of Chromatography A, 2000; 885: 273-290.
15. Food Standards Agency, 2012; Food Survey Information Sheet, No. 01/12.
16. Clark J, "Hazardous Waste Management Plan", EE 4S Student hand book, (1997-1998).