Monitoring Of Methyl Bromide Residues In Dry Plants Collected From Some Egyptian Governorates

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ABSTRACT

A total of 220 samples of 94 cereals and pulses, and 126 herbs and aromatic plants samples were collected from different Egyptian local markets located in eight governorates (Cairo, Giza, Gharbiya, Minufiya, Sharkiya, Benisuef, Ismalia, and Qalyubiya) during 2007 and subjected to inorganic bromide residues analysis. The detected residues were compared with maximum residue limits (MRL's) established by Codex Alimentarius Commission (1993b). Overall, 69.6% of 220 total number of samples analyzed were contaminated with inorganic bromide residues, of which 6.8% exceeded the MRL's. In spite of the herbs and aromatic medicinal plants recorded the highest contamination percentage (94.4%), no exceeding of the levels of inorganic bromide above the MRL's established by the Codex Alimentarius Committee (400 mg kg^{-1}). The mean concentration ranged from 2.93 to 44.5 mg kg^{-1}. The highest mean concentration observed in basil samples, while the lowest was in the caraway. However, in cereals, 36.2 % of 94 samples analyzed were contaminated, while 16% was violated with inorganic bromide , while 16% was violated. The mean concentration varied from 2.8 – 89.2 mg kg^{-1}. Results showed that cowpea recorded the highest contamination percentage (88%). The bromide detected levels exceeded the MRL's in fifteen samples of 25 analyzed cowpea samples, where the violation percentage was 60%. These results showed decline in the contamination and violation levels in both of herbs and cereals samples during different years, which is in accordance with Montreal Protocol issued at 1997 for banning the use of Me – Br.

Keys words: methyl bromide, residues, herbs and aromatic plants, cereals

INTRODUCTION

Methyl bromide (MB) is a fumigant gas used to protect crops from pests in the soil and to fumigate grain bins and other agricultural storage areas. It has been widespread used for more than 30 years. It is used for all types of stored dry foodstuffs, particularly for that products in bags, cases or other packages. Its use for large loose bulks of foodstuff, when it may be used alone or in admixture with ethylene dibromide or with carbon tetrachloride, is more limited. Considerable attention has been given to the determination of this inorganic bromide occurring in food after fumigation and many data are available for amounts in foodstuffs moving in commerce as well as from supervised trials. Williamson *et al.* (1986) reported that MB could be used successfully to fumigate grapefruit against *Anastrepha suspense* and *A. ludens*. However, when MB is used, possibilities of MB residues exist [Getzendaner *et. al.*, (1968) , Dumas (1973), Austin *et. al.*, (1985), Jessup *et. al.* (1994), and Hansen *et. al.* (2000). It is necessary to determine the bromide residue (BR) resulting from fumigation because of the safety concern. Furthermore, to protect the health of the consumers, bromides resulting from MB fumigation must be quantified to make sure that total bromide.

Methyl bromide affects human health both directly and indirectly. Environmental Protection Agency (EPA) classified methyl bromide as a category I acute toxin. It is a toxic primarily to the central nervous system and damage lungs, kidneys, eyes and skin. Workers involved in the manufacture and use of methyl bromide run the greatest risk of toxic exposure and resulting injury.

In general, the monitoring programme is considered as a key of ensuring compliance with the regulations and also to create a database to assess the residue levels in the commodities and the level of bromide residue intake (Gad Alla *et al.*, 2000) and (Nabil, 2004). The current study was conducted as a part of aim-monitoring program of central laboratory of residue analysis of pesticides and heavy metals in foods to access the levels of pesticide residues and other contaminants in food. This survey involved investigation of methyl bromide analysis as inorganic bromide in cereals & pulses and spices and aromatic plants samples collected from eight Egyptian local markets during 2007. Such monitoring program can contribute in improving safety of food, warning of actual and potential food contamination via food and evaluations of possible health hazards throughout providing continuous information on levels of environmental pollution in Egypt.

MATERIALS AND METHODS

1. Sampling:

A total of 220 samples of 94 cereals and pulses, and 126 herbs and aromatic plants samples were collected from different Egyptian local markets located in eight governorates (Cairo, Giza, Gharbiya, Minufiya, Sharkiya, Benisuef, Ismalia, and Qalyubiya), during 2007. All samples were subjected to methyl bromide analysis as inorganic bromide according to Codex Alimentarius Guidelines (1993 a) of sampling relative to methyl bromide residues analysis, 2 kg of cereals and pulses and 0.25 kg of herbs and aromatic medicinal plant samples were collected. Sub samples were taken according to Codex Alimentarius Guidelines (1993 a) for residue analysis, mixed well and homogenized thoroughly. One g of the homogenized sample was taken for analysis.

2. Standard preparation :

2.1. Spiking solution:

Bromide standard solution (1000 µg/ml) was prepared by dissolving 149 mg of potassium bromide in 100 ml deionized water. A solution was diluted from the stock solution 2.5 ml in 50 ml of deionized water was prepared as spiking solution (50 µg /ml).

2.2. Bromoethanol standard solution (100ug/ml):

Exactly 0.0103 g of 2-bromoethanol (97.5%) was diluted into 100 ml ethyl acetate to prepare 100 µg/ml as stock standard solution . intermediate solutions were prepared from 2- bromoethanol stock standard solutions such as 0.1, 0.3, 0.5, 0.75, 1.0, 1.25 µg/ml .

3. Method of analysis:

A method of analysis CEN, 1992 described by Greve and Grevenstuk (1976) & (1979), Arbeitsgruppe "Pestizide" (1981), and Stijve (1977), (1981) was adopted. The method determined bromine – containing fumigants as total inorganic bromide. 1 g of homogenized dry sample was put in bottle suspended in an aqueous solution with 10 ml ethylene oxide reagent acidified with 1 ml 6 N H_2SO_4. The mixture was swirled 1min and let to stand for 30 min. Ammonium sulphate (4 g) was added to 50 ml ethyl acetate and swirled gently for 1 min by hand and let for 20 min (occasionally shaking). Whereupon, organic bromide is converted to 2 bromoethanol (BE) and simultaneously partitioned into ethyl acetate. Ethyl acetate layer (10 ml) was taken into a 10 ml test tube and 4 g anhydrous sodium sulphate (4 g) was added as dehydrating agent. Finally, Gas Chromatograph with Electron Capture Detector was used for analyzes the extract of 2 bromoethanol.

3.1. Determination of methyl bromide as bromide ion:

Gas chromatograph HP 5890 was equipped with an electron capture detector (GC-ECD) from Hewlett Packard. Capillary column for gas chromatography was provided with Hewlett Packard fused silica capillary column HP-FFAP cross linked Polyethylene glycol-TPA 25 m x 0.32 mm i.d. x 0.52 µm. The injector temperature was 200 °C, and the detector temperature was 300 °C. The Oven temperature program for bromide ion analysis was as follows: 80 °C hold for 2 min, then ramp rate at 5 °C /min to 150 °C, then ramp rate at 15 °C/ min to 200 °C and hold for 2.5 min. Carrier gas was nitrogen at flow rate of 2 ml/min, make up gas for ECD was N_2 at a flow rate of 30 ml/min. injection was in split less mode, purge was set on after 0.75 min and purge flow was 15 ml/min. The external standard mode used for detecting of bromide ion in samples by manual injection of standard and samples on GC ECD.

5.2. Sample analysis and calculations:

The retention time and peak area obtained for the sample solution were compared with those obtained for standard solutions of 2-bromoethanol in ethyl acetate. The amount of 2-bromoethanol present in the injected solution was calculated. The blank determination by following the entire procedure without the sample was carried out. The amount of 2-bromoethanol present in the injected solution was also calculated for the blank determination. The bromide content of the sample by means of the formula:

$$\text{Content of inorganic bromide (mg/kg)} = \frac{F_a \cdot V_{end} \cdot W_{st}\,(0.639)}{Fst \cdot Vi \;\; G}$$

Where: F_a = Peak area obtained from V_i.

V_{end} = Volume of ethyl acetate used for extraction (ml).

W_{st} = Amount of 2-bromoethanol injection with standard (ng).

0.63 = Factor for conversion of 2-bromoethanol to bromide.

F_{st} = Peak area obtained from W_{st}.

V_i = Portion of volume V_{end} injected into the gas chromatograph (ul).

G = Sample weight (g).

5.3. Quality Assurance:

The analytical method and instrument were fully validated as a part of the quality assurance system in Central Lab. of Residue Analysis of Pesticides and Heavy Metals in Food and accredited by FINAS, Center for Metrology and Accreditation - Finland, according to (ISO / IEC 17025, 2005). The criteria of quality assurance of Codex Committee are followed to determine the performance of the analytical method. Recovery percentages, accuracy, limit of determination and coefficient of variation (CV %) were determined for inorganic bromide using dry samples. Blank samples were fortified with 50 mg/kg of (Br-ion) level and analyzed with every set of samples. The recovery % ranged between 80-120 %. The relative standard deviation (CV %) is 2.2 % for dry samples. The limit of determination is 1 mg/kg. The measurement uncertainty on 95 % confidence was found to be within ±21%.

RESULTS AND DISCUSSION

A total of 220 samples of 94 cereals and pulses and 126 herbs and aromatic medicinal plants were collected from eight Egyptian governorates, i.e. Cairo, Giza, Gharbiya, Minufiya, Sharkiya, Benisuef, Ismailia, and Qalyubiya, during 2007. All samples were subjected to methyl bromide analysis as inorganic bromide.

Table (1) showed the minimum, maximum, and mean in (mg/kg) of detected inorganic bromide, number and the percentages of contaminated samples, and violate compound in samples collected from different Egyptian governorates during 2007. The results showed that overall 69.6% of all analyzed samples (220) were contaminated with inorganic bromide, of which 6.8% were violated. The herbs and aromatic medicinal plants recorded the highest contamination percentage. However, 94.4 % of 126 total samples analyzed were contaminated with inorganic bromide. No exceeding of levels of inorganic bromide above the MRL's established by the Codex Alimentarius Committee (2005b) 400 (mg/kg). The dry coriander and fennel recorded the highest contamination percentage (100 %), followed by basil (96.9%), anise seed (94.7%). The contamination percentage in herbs varied from (87-100%) and the mean concentration ranged from 2.93 to 44.5 (mg/kg). The highest mean concentration was recorded in basil samples, while the lowest was in the caraway.

In cereals, the total contamination percentage was 36.2 % of which 16 % was violated. The mean concentration varied from 2.8 (mg/kg) for dry beans to 89.2 (mg/kg) for cowpea. However, the levels of detected bromide exceeded the MRL's in 15 samples out of 25 cowpea samples analyzed, representing 60% violation. The high violation of methyl bromide for cereals was due to the violation of cowpea samples. Such high levels of violation indicate that excessive levels of fumigation of cowpea. So, cowpea should be kept stored in a well ventilation after fumigation to avoid the violation of methyl bromide in such kind of plant commodities.

Table (2) shows the comparison between these results obtained during (2006 – 2007) with another similar studies conducted in the laboratory in the previous years by (Gadd Alla, *et. al.* 2000) and (Nabil, 2004). The results show the trend of the contamination levels in the cereals and pulses and herbs and aromatic medicinal plants samples collected from different Egyptian local markets during different years, where during the period from 1996 to 1997, the contamination level of cereals and pulses with inorganic bromide was high (76.6%) (Gadd Alla, *et. al.* 2000). This contamination percentage declined to 46% and finally to 36.2 % during the periods from (2002 to 2003) (Nabil, 2004) and from (2006 to 2007), respectively. Also, the violation percentage showed a gradual decline from 24.8% to 21% and finally to 16% during the periods from (1996 to 1997) , from (2002 to 2003) and from (2006 to 2007), respectively. Such decline in the contamination and violation levels with Me –Br is in accordance with Montreal Protocol issued at 1997 for banning the use of Me – Br.

Table (2) also showed that in (1996-1997), the herbs and aromatic medicinal plants recorded the highest contamination (100 %) and violation (2.6 %) percentages (Gadd Alla, *et. al.* 2000). However, the contamination percentage was declined to 80 % during the period from 2002 to 2003 (Nabil, 2004) and slightly increased up to 94.4% during (2006 and 2007). However, no exceeding of limits of inorganic bromide above the MRL's in all samples analyzed during (2002 and 2003) and (2006 and 2007).

The decline of the violated samples collected from Egyptian governorates during 2006-2007 agrees with Montreal Protocol Commitments 1997. According to this Protocol, the developing countries should freeze the level of methyl bromide at (1995- 1998) average levels by the year 2002, while by 2003 it should review the reduction schedule. However, it should be phased out by the year 2015.

Table (1): Minimum, Maximum, and Mean in mg/kg of inorganic bromide, number and percentages of contaminated samples, and violated compound in samples collected from different Egyptian governorates during 2007.

Governorate	Total no. of samples	Contaminated samples with inorganic bromide		Min. mg/kg	Max. mg/kg	Mean mg/kg	MRL's (mg/kg)	Violative samples	
		No.	%					No.	%
Herbs:									
Anise seed	19	18	94.7	6	18	10.84	400	-	-
Basil	32	31	96.9	14	205	44.5	400	-	-
Caraway	16	14	87.5	2	9	2.93	400	-	-
Dry coriander	18	18	100	5	132	18.1	400	-	-
Fennel	18	18	100	2	32	15.3	400	-	-
Marjoram	23	20	87	2	102	17.55	400	-	-
Total herbs	126	119	94.4	-	-	-	-	-	-
Cereals :									
Caw peas	25	22	88	14	232	89.2	50	15	60%
Corn	12	4	33.3	1	23	9.5	50	-	-
Dry beans	12	6	50	2	4	2.83	50	-	-
Wheat	32	1	3.1	4	4	4	50	-	-
Maize	13	1	7.7	3	3	3	50	-	-
Total cereals	94	34	36.2	-	-	-	-	15	16
Total	220	153	69.6	-	-	-	-	15	6.8

Table (2): Trend of Me – Br in cereals and herbs during different Years:

Year	commodities	Contaminated		Violated	
		No.	%	No.	%
1996- 1997[a]	Cereals and pulses (145 samples)	111	76.6	36	24.8
	Herbs and aromatic medicinal plants (77 samples)	77	100	2	2.6
2002-2003[b]	Cereals and pulses (113 samples)	52	46	24	21
	Herbs and aromatic medicinal plants (86)	69	80	-	-
2006-2007	Cereals and pulses (94 samples)	34	36.2	15	16
	Herbs and aromatic medicinal plants (126 samples)	119	94.4	-	-

[a] Gad Alla et al (2000)
[b] Nabil (2004)

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المخلص العربي

تقصى لتحليل متبقيات بروميد الميثيل فى النباتات الجافة المجمعة من بعض محافظات مصر

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المعمل المركزى لتحليل متبقيات المبيدات والعناصر الثقيلة فى الأغذية – مركز البحوث الزراعية – وزارة الزراعة – الدقى – مصر.

تم جمع عينات بلغت ٢٢٠ عينة من ٩٤ نوع من الحبوب و١٢٦ أعشاب ونباتات عطرية من أسواق محلية مصرية مختلفة (القاهرة – الجيزة – الغربية – المنوفية – الشرقية – بنى سويف – اسماعيلية والقليوبية) خلال ٢٠٠٧ بغرض تقصى مستويات متبقيات بروميد الميثيل.

وقد تم مقارنة النتائج تبعا للجنة الكودكس (١٩٩٣١٥) العالمية والحدود القصوى المسموح بها. وبدراسة نسبة تلوث ميثيل البروميد فى العينات التى تم تحليلها وجد أن فوق ٩٦,٦% من ٢٢٠ عينة كانت ملوثة بمثيل البروميد وأن ٦,٨% كانت تتعدى قيمة الحدود القصوى المسموح بها.

وكانت أعلى نسبة تلوث فى النباتات الطبية والعطرية التى مثلت ٩٤,٢% لم يكن هناك مستويات من التلوث تتعدى الحدود القصوى المسموح بها.

وقد وجد أعلى تركيز من التلوث كان فى عينات الريحان وأقل تلوث كان فى عينات الكراوية وعلى الرغم أن فى الحبوب كانت نسبة التلوث ٣٦,٢% و ١٦% تم تجاوزها الحدود المسموح بها. وكانت أعلى نسبة تلوث فى اللوبيا (٨٨%). و١٥ عينة من اللوبيا تم تجاوزها الحدود القصوى المسموح بها من ٢٥ عينة أى بنسبة ٦٠% حيود. وبمقارنة النتائج مع الدراسات الأخرى أظهرت النتائج تقليل نسبة التلوث والحيود فى عينات الأعشاب والحبوب باختلاف السنين.