# Quantitative Evaluation of Malachite Green Residues in Fish and Their Cooking Impact

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***Abstract***

Malachite green was classified as a class II health hazard and showed a significant health risk to human through consumption of fish that contain (MG) residues. In addition, MG was heat stable and thus may not be degraded during routine fish processing. Due to effectiveness of malachite green and relatively low cost, it was a procurable agent for freshwater fish farmers; these compounds might influence the immune and reproductive systems. It was also carcinogenic, mutagenic and teratogenic agent so; sixty fish samples, 20 tilapia fish, 20 mullet, 10 bass and 10 shrimp were randomly collected from various regions at Damietta governorate (Egypt). All samples were evaluated for malachite green (MG) residues. Mullet samples fish were significantly ($P < 0.05$) had the highest concentrations of malachite green residues. The mean ± S.D values of malachite green residues levels were as 1.558 ± 0.165ppb in mullet, 1.374 ± 0.326 ppb in tilapia, 0.719 ± 0.148 ppb in bass, as well as 1.213± 0.130 ppb in shrimp, while the minimum values were < 0.3 ppb and the maximum residues levels were as 2.61, 2.76, 1.18, 1.43ppb respectively. Fish samples positive for MG were treated by different heat treatment (microwaving, roasting and boiling). The reduction percentage for malachite green residues were 81.80%, 32.90%, and 100%, consecutively.

Comparing the results of malachite green residues in samples with Commission Regulation **(EU, 2004)** for maximum residues limits, it was clear that 6 (10%) of examined samples were more than MRLs which was 2 μg/kg.

**Keywords:** Malachite Green - ELISA - microwaving, roasting, boiling

## Introduction

Malachite green is a commercially available cationic triphenyl methane dye. It has a metallic appearance and quickly dissolves in water, yielding a blue-green solution, commonly used as a parasiticide in aquaculture and other industries for one or more objectives, because of its controlling effect on fungal attacks, protozoan infections and helminthes on a diverse range of fish and aquatic organisms ***(El-ghayaty et al., 2016).*** Leucomalachite green, a reduced colorless chemical, was rapidly converted from malachite green. Due to its lipophilic nature, the primary metabolite, leucomalachite green, was kept in fish muscle and fat for much longer and hence the majority of malachite green consumption would be in the leuco form ***(Mitrowska et al., 2008).***

Malachite green was highly toxic to mammalian cells. Malachite green and leucomalachite indicated follicular adenoma or carcinoma of the thyroid gland, adenomas of the mammary gland or carcinomas, hepatocellular adenomas, reduction of proliferation potential and loss of mitochondrial function, and even interstitial adenoma of the testes ***(Culp et al., 2006).***

## Materials and Methods

**A. Collection and preparation of the Samples:**

Sixty fish samples were randomly collected from various regions at Damietta governorate (Egypt) consisted of 20 tilapia fish, 20 mullet,10 bass and 10 shrimp, which individually packed in a clean polyethylene bags marked and stored frozen at approximately - 20˚C until transferred to laboratory.

**B. Preparation of Samples**:

Fish samples were washed several times with deionized water to clean them from sediments and other adhesive materials. The fish samples were identified scaled and the heads were removed using a stainless-steel knife, the flesh and other edible portions removed from the bone and entrails. One gram from the dorsal muscle were ground in meat chopper and frozen until analysis.

**C. Analytical procedure:**

According to ***FSIS-USDA ELISA, (2016)***

**Calculations:**

$$\frac{O.D.standard\ (or\ sample)\ x\ 100}{O.D.zero\ standard} = \%$$

maximal absorbance

**Experimental work:** The purpose of this experiment is to keep malachite green residues in fish under control. The positive fish samples contaminated with MG treated by different methods of heat treatment (microwaving at

220 °C for 20 minutes, roasting at 200°C for 15 minutes and boiling at $100^0$C for 20 minutes) then extracted and pass through the steps as mentioned before in raw fish. Reduction rate percentage (R.R) due to the effect of heat on MG residues in fish muscles were calculated according to the following equation:

$$R. = \frac{\text{mean conc. of MG in raw fish} - \text{mean conc. of MG in heat treated fish}}{\text{mean conc. of MG in raw fish}} x100$$

**Results& Discussion**

**Part one: Malachite green residues in fish samples:**

In Table (1) and figure (1) Incidence of malachite green residues was 55%,50%, 50%, and 30% from mullet, tilapia, bass and shrimp samples respectively and in all examined samples 48.33%.

Table (2) and figure (2) showed the mean values of malachite green residues (±S. E) were 1.558 ± 0.165, 1.374± 0.326, 0.719 ± 0.148, and 1.213± 0.130 ppb in mullet, tilapia, bass and shrimp respectively, while the minimum values were < 0.3 ppb and the maximum residues levels were 2.61,2.76, 1.18, 1.43ppb respectively. These results were similar to that detected by ***Halme et al. (2007)*** who found that the concentration range of MG was 0.35–1.54 ppb in 34 fish muscle samples**.** Meanwhile, ***Olesen (2007)*** found in four fish samples the MG was more than 4 ppb and one sample was 2.7 ppb in 2005**.** On the other hand, ***Huang et al. (2008)*** estimated the mean residues contents of MG in first fish sample as 4.89, 5.46 ppb, in second fish sample as 3.24, 2.86 ppb, as well as ***Faraget al. (2012)*** estimated the MG mean in fresh tilapia as 2.20 ± 0.50 ppb,

Higher findings were obtained by ***Andersen et al. (2006)*** who found the mean± SD for MG contents was by 31.3 ±8.7 ppb, 28.6 ±3.8, and 27.4 ±7.3ppb. ***Shalaby et al. (2017)*** analyzed the mean residues contents of MG in raw tilapia tissues by 63.8 ppb.The lower finding were obtained by ***Guo et al. (2011)*** estimated the MG in five Chinese fish samples were as 0.0685 ±0.0072 ppb, 0.535 ± 0.029 ppb, 0.131 ± 0.0095 ppb.

The variations between the obtained results and other investigations could be explained by differences in applicable methods time, purity of chemical substances, concentration of MG, and the presence of remaining contaminants in varied concentrations ***(Sudova et al., 2007),*** Water temperatures influence the persistence of MG and LMG residues in fish, as well as the warm climate in the tropical countries. Fish had a greater metabolic rate in warm water, which could hasten the elimination of MG and LMG residues from the fish. ***(Bajc et al., 2011).***

Table (3) showed the incidence of

malachite green residues in studied fish samples (n=60) that less than the maximum residue limit (2 µg/kg=ppb) were 90%, 80%, 100%,and 100% from mullet, tilapia, bass and shrimp samples respectively, and in all examined fish samples was 90%, while the levels that was more than the maximum residue limit as 10%, 20%, 0%,and 0% consecutively, and in all examined fish samples 10%. Table (4) illustrated the frequency distribution of malachite green levels (ppb) for examined fish samples

**Part two experimental part**:

Experimental work was done to investigate the effect of different cooking methods (microwaving, roasting, and boiling) on the concentration of malachite green in fish meat. The results illustrated in table (5) showed the variance between mean values of malachite green residues before and after microwaving as 1.31±0.75, 0.24±0.13 ppb with 81.80% reduction percentage, There were highly significant differences (P<0.01).This result was nearly similar to ***Shalaby et al. (2017b)*** as 80.8 % and less than the results recorded by ***Mitrowska et al. (2007)*** as 97 % and more than the data provided by ***Farag et al. (2012)*** who found Microwaving had a reduction rate of 59.98.

Table (6) showed the variance between mean values of malachite green residues before and after roasting was as 1.31±0.75 and 0.88±0.50 ppb, with 32.90% reduction percentage. There were highly significant differences (P<0.05). ***Shalaby et al. (2017)*** found higher reduction percent from roasting as 48.4 %.

Table (7) illustrated the correlation between the different malachite green means value were 1.31±0.14, 0.24±0.02, and 0.88±0.09 ppb from raw, microwaved, and roasted samples. There were highly significant differences (P<0.01) between different means. Malachite green residues were completely reduced by boiling (100%).

**Public health hazard from Malachite green residues in fish samples:**

Malachite green is seriously destructive to mammalian cells. caused necrosis in the liver, kidney, intestine and degenerative changes in gonadsincrease in the occurrence of micronucleated normochromatic erythrocytes in peripheral blood was observed, thyroid gland follicular cell adenoma or carcinoma, chromosomal fractures, increased risk of human bladder cancercarcinogenesis, mutagenesis, and teratogenicity, ***(Culp et al., 2006).***

**Table (1):** *Incidence of malachite green residues in examined fish samples:*

| Type of sample | Total number | Positive | | Not Detected | |
|---|---|---|---|---|---|
| | | No | % | No. | % |
| Mullet | 20 | 11 | 55 | 9 | 45 |
| Tilapia | 20 | 10 | 50 | 10 | 50 |
| Bass | 10 | 5 | 50 | 5 | 50 |
| Shrimp | 10 | 3 | 30 | 7 | 70 |
| Total | 60 | 29 | 48.33 | 31 | 51.67 |

**Table (2):** *Statistical analytical results of malachite green residues (ppb) recovered from examined fish samples:*

| Items | Samples (n=60) | | | |
|---|---|---|---|---|
| | Mullet (n=20) | Tilapia (n=20) | Bass (n=10) | Shrimp (n=20) |
| Min. | < 0.3 | < 0.3 | < 0.3 | < 0.3 |
| Max. | 2.61 | 2.76 | 1.18 | 1.43 |
| Mean | 1.558* | 1.374* | 0.719** | 1.213** |
| S.E. | 0.165 | 0.326 | 0.148 | 0.130 |
| Calculated F | 1.59* | | | |
| P- value | 0.22* | | | |

* There are no significant differences (P>0.05) between the means from different analyzed samples.

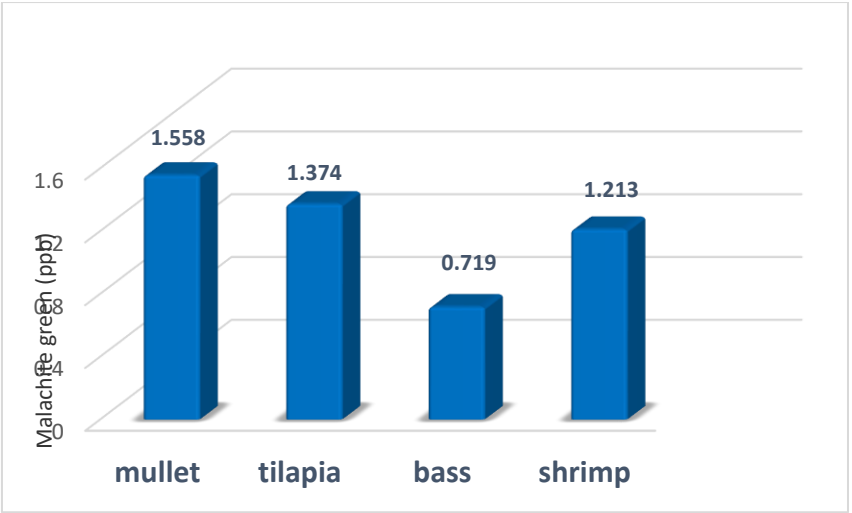


**Figure (2):** Mean values of malachite green residues (ppb) in fish samples

**Table (3):** *Incidence of malachite green residues in examined fish samples (n=60).*

| Examined samples | Less than MRLs | | More than MRLs | |
|---|---|---|---|---|
| | No | % | No | % |
| **Mullet** | 18 | 90 | 2 | 10 |
| **Tilapia** | 16 | 80 | 4 | 20 |
| **Bass** | 10 | 100 | 0 | 0 |
| **Shrimp** | 10 | 100 | 0 | 0 |
| **Total** | 54 | 90 | 6 | 10 |

*Maximum Residue Limits (MRLs) in µg/kg: according to Commission Regulation (EU) (2004) (2µg/kg=ppb).*

**Table (4):** *Frequency distribution of malachite green levels (ppb) for examined fish samples (n=60).*

| Levels range (ppb) | Mullet | | Tilapia | | Bass | | Shrimp | |
|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % |
| **< 0.3 ppb** | 9 | 45 | 10 | 50 | 5 | 50 | 7 | 70 |
| **0.3 to< 1 ppb** | 1 | 5 | 6 | 30 | 4 | 40 | 1 | 10 |
| **1 to< 2 ppb** | 8 | 40 | 0 | 0 | 1 | 10 | 2 | 20 |
| **≥ 2 ppb** | 2 | 10 | 4 | 20 | 0 | 0 | 0 | 0 |

**Table (5):** *Variance between mean values of malachite green residues before and after microwaving of analyzed samples (n=29).*

| Items | Microwaving | |
|---|---|---|
| | Before | After |
| **Min.** | 0.33 | 0.06 |
| **Max.** | 2.76 | 0.50 |
| **Mean** | 1.31* | 0.24* |
| **S.D** | 0.75 | 0.13 |
| **Reduction%** | 81.80% | |
| **t- test** | 9.4326** | |
| **p-value** | 0.000 | |

** Highly significant

** There are highly significant differences (P<0.01) between means before and after microwaving.

**Table (6):** *Variance between mean values of malachite green residues before and after roasting of analyzed samples (n=29).*

| Items | Roasting | |
|---|---|---|
| | Before | After |
| **Min.** | **0.33** | **0.22** |
| **Max.** | **2.76** | **1.85** |
| **Mean** | **1.31**** | **0.88**** |
| **S. D** | **0.75** | **0.50** |
| **Reduction%** | **32.90%** | |
| **t- test** | **2.33**** | |
| **p-value** | **0.014** | |

**Highly significant

** There are highly significant differences (P<0.05) between means before and after roasting.

**Table (7):** *Correlation between means value of malachite green residues recovered from raw, microwaved and roasted samples.*

| Item | Raw | Microwaved | Roasted |
|---|---|---|---|
| **Mean** | 1.31 | 0.24 | 0.88 |
| **S. E** | 0.14 | 0.02 | 0.09 |
| **LSD** | 0.23 | | |
| **Calculated F** | 30.78** | | |
| **p-value** | 0.000 | | |

LSD: Least significant difference
** Highly significant by one-way ANOVA test.
** There are highly significant differences (P<0.01) between different means.

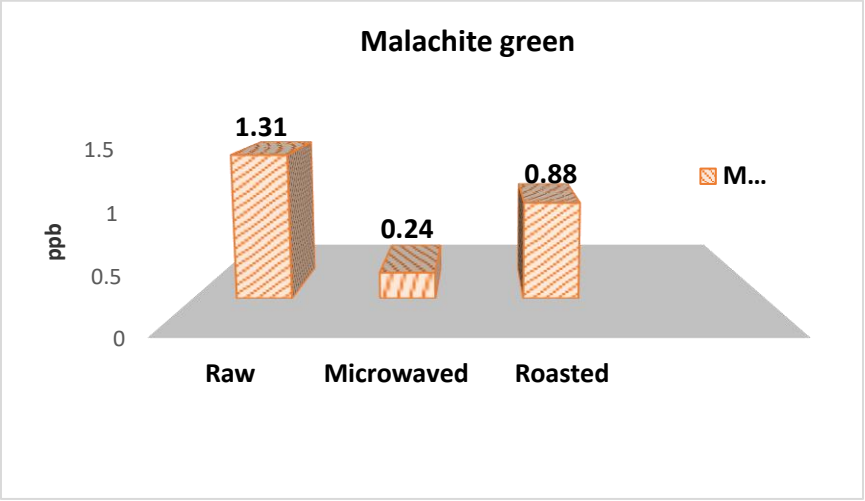


**Figure (2): Correlation between means value of malachite green residues recovered from raw, microwaved and roasted samples**

## Conclusions and Recommendation

A proportion of fish had been detected of containing some residual of the malachite green element, which was higher than the standard specifications 2µg/kg that was to have detrimental effects on the consumers' health. The varieties of cooking processes had a powerful impact on breaking the malachite green and eliminate its toxicity. The following steps should be recommended: Malachite green should be banned and completely prohibited from use in farmed fish due to carcinogenicity and their potential harmful effect on human health. Hygienic practice should be

strictly followed and enforced to make the fish meat safer for human consumption. Much more concerns must be given to the cooking regime by efficient cooking of fish meat immediately before eating. Good manufacturing practice (GMP) should be followed in order to assure safety and quality of fish and fish products. Educational programs should be improved to raise the awareness for workers, processors and handler.

### *References*

**Andersen W. C., Turnipseed S. B., and Roypal J. E. (2006):** Quantitative and Confirmatory Analyses of Malachite Green andLeucomalachite Green Residues in Fish and Shrimp. J. Agric. Food Chem. 54, 4517-4523.

**Bajc Z., Jen cic V., and Gacnik K. S. (2011):** Elimination of malachite green residues from meat of rainbow trout and carp after water-borne exposure. *Aquaculture, **(321) (**1-2), p: 13-16*

**Culp S., Mellick P., Trotter R., and Kodell R. (2006):** Carcinogenicity of malachite green chloride andleucomalachite green in B6C3F1 mice and F344 rats. *Food Chem.Toxicol.**44**: 1204–1212.*

**El-ghayaty, H. A.; Salwa, M. S. and Asmaa M. S. F. (2016):** Malachite green residues in farmed fish and the effect of some different cooking ways on it. Egypt. J. Chem. Environ. Health 2(1): 37-47.

**Farag, H.; EL-Tabiy, A. A. and Hassan, H. M. (2012):** Effect of thermal processing on the level of malachite green residues in Oreochromisniloticus with special references to its public health significance. Assiut Vet. Med. J. 58 (132), 1-19.

**FSIS-USDA ELISA (2016):** Food Safety and Inspection Service U.S. Department of Agriculture, Chemistry Laboratory Guidebook, available online at: http://www.fsis.usda.gov/science/ Chemistry-Lab-Guidebook/index.asp.

**Guo Z., Gai P., Hao T., and Wang S. (2011):**Determination of malachite green residues in fish using ahighly sensitive electrochemiluminescence method combinedwith molecularly imprinted solid phase extraction. *J. Agric. Food Chem. **59**: 5257–5262.*

**Halme K., Lindfors K., and Peltonen K. (2007):** A confirmatory analysis of malachite green residues in rainbow trout withliquid chromatography–electrospray tandem mass spectrometry. *J. Chromatogr. B **845**:74–79.*

**Huang W., Yang CH., and Zhang SH. (2008):**Voltammetric determination of malachite green in fish samples based on the enhancement effect of anionic surfactant. *Russian J. of Electrochemistry. Vol. **(44).**No. 8.*

**Mitrowska K., Posyniak A., and Zmudzki J. (2007):** The effects of cooking on residues of malachite green and leucomalachite green in carp muscles. *AnalyticaChimicaActa, **586(1-2),**p: 420-425*.

**Mitrowska, K.;Posyniak, A. and Zmudzki, J. (2008):** Determination of malachite green and leucomalachite green residues in water using liquid chromatography with visible and fluorescencedetection and confirmation by tandem mass spectrometry. Journal of Chromatography A, 1207: 94-100.

**Olesen P. T. (2007):** Risk assessment of malachite green in food. Soeborg, Denmark: National Food Institute, Technical University of Denmark.

**Shalaby A. R., Abdelmaguid N. M. and Emam W. H. (2017):** Impact of cooking on Malachite Green and Leucomalachite Green residues existing in Tilapia fish. *American J. of Food Technology. ISSN 1557-4571.*

**Sudova E., Machova J., Svobodova Z,and Vesely T. (2007):**Negative effects of malachite green and possibilities of its replacement in the treatment of fish eggs and fish: a review, *Vet. Med. **(52)**, p: 527-539.*

## الملخص العربي

## التقدير الكمي لبقايا الملاكيت الاخضر في الأسماك وأثر الطهي عليها

**سعاد احمد سليمان اسماعيل [1] ، الدسوقي حسن ابراهيم دره[2] ، نهله حماده مجد خليل[2]**
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تم تجميع ستون عينة عشوائيا من الأسماك المحلية المباعة بواقع 20 سمكة بلطي، 20 بوري، 10 قاروص و10 جمبري من مناطق مختلفة بمحافظة دمياط (مصر). وتم تحليل تلك العينات للكشف عن بقايا مركب الملاكيت الأخضر بواسطة اختبار الاليزا. وأظهرت النتائج ان عينات أسماك البوري بها فرقا معنويا ($P < 0.05$) الاعلى تراكيز لبقايا الملاكيت الاخضر. كانت قيمة المتوسط الحسابي ± الخطأ القياسي لمحتوي مركب الملاكيت الاخضرهي 1.558 ± 0.165 جزء في البليون في اسماك البوري، و 1.374 ± 0.326 جزء في البليون في اسماك البلطي، و 0.719 ± 0.148 جزء في البليون في اسماك القاروص، وكذلك 1.213 ± 0.130 جزء في البليون في الجمبري، بينما كان الاحد الادني<0.3 جزء في البليون والحدالأقصى 2.61، 2.76 ، 1.18 ، 1.43 جزء في البليون على التوالي. الحدود المسموح بها 2 جزء في البليون (ميكروجرام/لكل كيلو جرام) قد تم اجراء جزء تجريبي:بتعريض العينات لطرق طهي مختلفة مثل الطهي بالمايكرويف والشواء والغليان أظهرت أن نسبة الاختزال لبقايا مركب الملاكيت الاخضر كانت 81.80٪، 32.90٪، 100٪ على التوالي. وبالتحليل الاحصائي وجد فروق معنويه كبيره بين المتوسط الحسابي للملاكيت الاخضر قبل وبعد الطهي بالميكروويف والشواء. التوصيات الواجب اتباعها: عمليات الطهي المختلفة لها تأثير قوي على تكسير ماده الملاكيت الاخضر وتقليل سميتها كما يجب اتباع الممارسة الصحية بدقه وتطبيقها لإنتاج لحوم اسماك أكثر امانا.

- منع اصحاب المزارع السمكية من استخدام ماده الملاكيت الاخضر ومحاوله توفير بديل امن لها لتجنب مخاطرها على صحة الانسان من احداث سرطانات وتشوهات.
-لابد من انظمه أكثر صرامة وعقوبات لمستخدمي هذه المواد الخطيرة.