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Risk Assessment of Malachite Green in Fish Produced in Armenia

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ABSTRACT

This study aimed to investigate malachite green residues in farmed fish and to assess its dietary exposure in Armenia. Quantitative measurement of malachite green in fish samples was done by the enzyme-linked immunosorbent assay method. The results were approved by the LC-MS/MS method. The malachite green residues were found only in 20 % of the investigated fish samples and exceeded the minimum required performance limit (MRPL) of 0.002 mg/kg set by the EU legislation. The obtained results highlighted the issues concerning the illegal use of malachite green in some fish farms in Armenia.

Introduction

Aquaculture has a great development potential in Armenia. Since 2010 the strategy for export-oriented production has been established by the government of Armenia. Besides, since 2011 the Russian market has opened for Armenia, but taking into consideration the high demand for fish in EU countries, efforts are being also made to enter the European markets. As a mandatory requirement, the EU regulations for monitoring food residue of animal origin must be implemented by the third countries (FAO 2005). In the scope of the residue monitoring program, not only the allowed but also the banned substances must be included. Among the banned substances, the malachite green is one of the illegally used chemicals.

Malachite green (MG) is a triphenylmethane dye that has been used worldwide as an effective and inexpensive therapeutic technique for ectoparasitic and fungal infections in fish farming since the 1930s. Due to several toxic effects of MG and its major metabolite leucomalachite green (LMG) on mammalian cells, MG has never been authorized as veterinary medicine in the EU. The US Food and Drug Administration does not approve the use of this dye either (Ali, et al., 2016, Bajc, et al., 2011, Bilandžić, et al., 2012).

Despite the ban, residues of malachite green and leucomalachite green have systematically been found in fish and fish products in the past decade, causing great concern for consumers in many countries, including European Union, Australia, Canada, the United States, Indonesia, and Vietnam (Chi, et al., 2017, Conti, et al., 2015, Renwick, et al., 2010). Moreover, several adverse toxic effects have been reported including carcinogenesis, mutagenesis, chromosomal fractures, teratogenicity and respiratory toxicity (EFSA 2016).

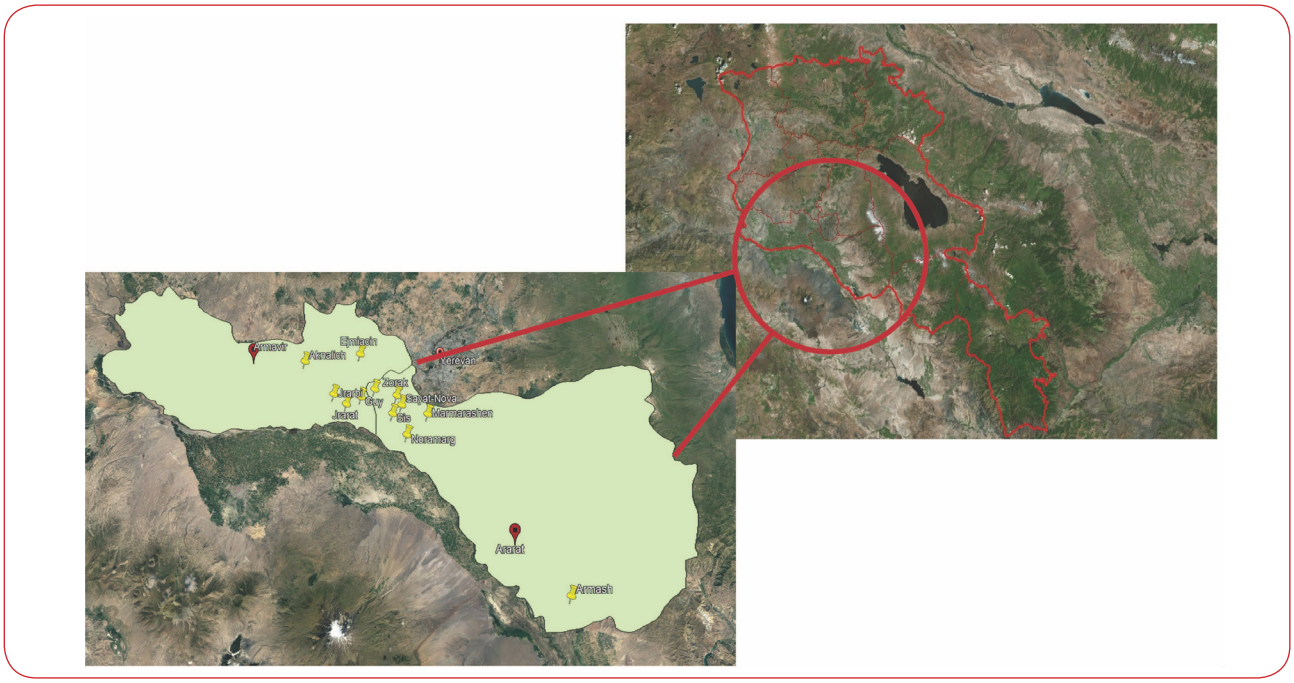

Figure 1. Fish sampling points (fish farms) in Armenia

The collection of detailed information on the use of malachite green in aquaculture is crucial for the evaluation of its toxic effects and potential health risks (Benford, et al. 2010). Therefore, this study aimed to investigate malachite green residues in farmed fish and assess its dietary exposure in Armenia.

Materials and methods

The sampling of fish produced in Armenia was done in the frame of the state residue monitoring program (2016-2017), according to the government decree on food sampling (RA Government 2012). The sampling points (fish farms) in Armenia are presented in Figure 1. In total, 15 samples (containing at least 3 sub-samples) of farmed fish (FM-1 - FM-15) were sent to the international accredited laboratory ‘‘RVSPCLS” SNCO SSFS MA RA for malachite green residue determination.

Quantitative measurement of malachite green in fish samples was done by the enzyme-linked immunosorbent assay (ELISA) method. The results were approved by the LC-MS/MS method (Ding, et al., 2007, Liang, et al., 2006). This method is intended to rapidly and precisely determine residue levels of malachite green and leucomalachite green in fish. Using positive mode electrospray ionization (ESI+) and multiple reaction monitoring (MRM) technique, the LC/MS/MS method shows the detection limit of 10 ppt. The analytical method was validated by analyzing fortified roast eel samples at 1, 2 and 5 µg/kg levels, corresponding to 0.5×, 1×, and 2.5× MRPL (Minimum Required Performance Limit) respectively. Seven replicates were performed at each level. Excellent recovery values of 90 %-106 % were obtained with RSD % ranging from 3.7 % to 11 %.

All chemicals were of reagent grade. MG oxalate salt and LMG were from Sigma-Aldrich (St Louis, MO, USA), and d6-LMG from WITEGA (Berlin, Germany).

Additionally, dietary exposure of malachite green through daily fish intake was estimated by the following equation.

$$EDI = C \times IR / Bw,$$

where EDI - estimated daily intake, mg/kg bw/day, C - malachite residue green content, IR - daily fish consumption (kg/day), according to Statistical Committee of Armenia (Armstat 2017), Bw - body weight (70 kg).

Results and discussions

The table data indicate the malachite green residues in farmed fish produced in Armenia.

According to data presented in the table it can be noted that for the majority (80 %) of investigated fish samples malachite green residues were not detected. Malachite green residues detected in 3 fish samples (FM-2, FM-5, FM-10) were in the range of 0.47 mg/kg -1.7 mg/kg.

Table. Malachite green residues in fish samples*

№	Sample Code	Malachite green residues, mg/kg
1	FM -1	N/D
2	FM-2	0.47
3	FM-3	N/D
4	FM-4	N/D
5	FM-5	0.87
6	FM-6	N/D
7	FM-7	N/D
8	FM-8	N/D
9	FM-9	N/D
10	FM-10	1.7
11	FM-11	N/D
12	FM-12	N/D
13	FM-13	N/D
14	FM-14	N/D
15	FM-15	N/D

Note: N/D – not detected.

**Composed by the authors.*

fish health management, and farm environment management (Kamaruddin & Baharuddin, 2015).

Since malachite green is a toxic substance, the consumption of contaminated fish may, therefore, pose adverse health effects on consumers. So, dietary exposure was estimated by combining malachite green contents' data and fish intake data. The estimated daily intakes for malachite green are presented in Figure 2.

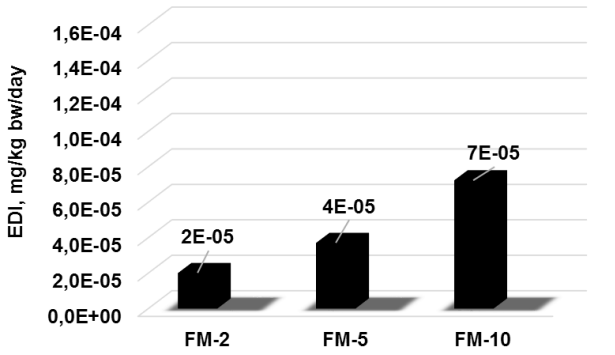

Figure 2. Estimated daily intake (EDI) of malachite green via consumption of fish

The Joint FAO/WHO Export Committee on Food Additives (JECFA) did not recommend MRLs for malachite green, as it did not support the use of malachite green for food-producing animals. Nevertheless, it can be emphasized that detected contents of malachite green were much higher than the established MRPL level (0.002 mg/kg) set by the EU (EC 2004).

Malachite green concentrations in fish muscle in Croatia were lower than the established MRPL (0.002 mg/kg). The concentration of malachite green ranged from 0.0003 mg/kg to 0.4872 mg/kg, measured in eel originating from the Netherlands in 2006 (Bilandžić, et al., 2012).

Sudova et al. stated that malachite green is widely used due to the lack of a proper alternative. Pyceze, however, is a pharmaceutical alternative to malachite green, with bronopol as its active ingredient (Srivastav & Roy, 2015).

It should be noted that success in fish farming is related to the possibility of providing an adequate supply of good-quality water with minimum contamination by organic substances and on the use of good-quality feeds. This will keep fish in the best health condition and increase their resistance to infections (Sudova, et al., 2007). From this point of view, the Good Aquaculture Practices (GAP) should be implemented in fish farming. GAP helps fish farms improve in key areas such as farm infrastructure management, farm husbandry,

The obtained EDI values are in the range of 2E-05 to 7E-05 mg/kg bw/day. These values were much lower than the WHO's best estimate: 1.5E-04 - 6.9E-04 (sum vet. Drug + contaminant) mg/kg bw/day (WHO 2008). In comparison to this, mean dietary exposure across different European dietary surveys and age groups ranged from 0.1E-6 to 5E-6 mg/kg bw/day. For high and frequent fish consumers, the exposure ranged from 1.3E-6 to 11.8E-6 mg/kg bw/day (EFSA 2016).

Conclusion

Taking into consideration the obtained results, it may be concluded that the concentrations of malachite green were identified only in 20 % of investigated fish samples. Overall, the measured malachite green residues exceeded the minimum required performance limit (MRPL) of 0.002 mg/kg set by the EU legislation. According to the EFSA CONTAM Panel, it is unlikely that exposure to food contaminated with malachite green at or below the reference point for action (RPA) of 0.002 mg/kg represents a health concern (EFSA 2016). Nevertheless, the obtained results of this investigation highlighted the issues concerning the illegal use of malachite green in some fish farms in Armenia. Hence, the national surveillance programs are required for the prevention of the illegal use of malachite green. Moreover, further investigations with a large number of fish samples

need to be carried out to perform more comprehensive risk assessment not only for malachite green residues but also for its metabolite- leucomalachite green.

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