original article

Determination of malachite green in trout tissue and effluent water from fish farms

Abbas Khodabakhshi, Mohammad Mehdi Amin[1]

Department of Environmental Health Engineering, Shahrekord University of Medical Sciences, Shahrekord, Iran, [1]Environment Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Address for correspondence:
Dr. Mohammad Mehdi Amin,
Environment Research Center,
Isfahan University of Medical Sciences, Hezar-Jerib Avenue, Isfahan, Iran.
E-mail: amin@hlth.mui.ac.ir

ABSTRACT

Aims: The objective of this study was to determine the malachite green (MG) concentration in trout tissue and the effluent water of fish farms, at one of the largest trout fishery industries in Iran.

Materiels and Methods: Twelve samples of water and fish tissue were collected from fish farms placed at the upstream ends of two large rivers in the study area. The samples, after extraction, were analyzed with liquid chromatography–mass spectrometry (LC–MS). The effluent water samples were also analyzed by the spectrophotometric method after cloud point extraction using the anionic surfactant Triton X-100.

Results: The concentration of malachite green in the fish samples ranged from 265.2 to 1663 μg/kg, which is more than the recommended maximum allowable concentration by the Codex standards. MG in the water samples ranged from 5.65 ng/L to 384 μg/L. The equivalent concentrations of MG in the two large rivers in the study area were 1.78 and 0.62 ng/L, and the total MG load for these two rivers, with a fish production rate of 10,000 tons per year, was around 644 kg/d.

Conclusion: We concluded that the concentration of MG used as an antimicrobial chemical in trout fish tissues and water samples in this study were out of compliance with the existing standards. Therefore, the fish products of these farms could cause serious public health hazards, and the discharge of the effluent from these farms, without treatment, posed potential environmental problems.

Key words: Effluent water, fish farm, liquid chromatography–mass spectrometry (LC–MS), malachite green, Triton X-100, trout tissue

INTRODUCTION

Aquaculture is one of the fastest growing food-producing sectors, supplying approximately 40% of the world's fish food. Fish from farming constitute a significant component of the total diet, particularly for those consumers who eat fish in preference to other meats. Although this does benefit society to some extent, the industry does have its problems.[1]

Malachite green (MG) is an extensively used biocide in aquaculture and fisheries worldwide. It is highly effective against important protozoans and fungal infections. Aquaculture industries have been using malachite green as a topical treatment by bath or flush methods extensively, without taking into consideration the fact that topically applied treatments might also be absorbed systemically and produce significant internal effects. Additionally, it is used as a food coloring agent, food additive, medical disinfectant, and antihelminthic, as well as a dye in the silk, wool, jute, leather, cotton, paper, and acrylic industries.[2]

Access this article online

Quick Response Code:

Website: www.ijehe.org

DOI: *****

Malachite green is a popular, but potentially carcinogenic, mutagenic, and teratogenic ectoparasiticide, fungicide, and antiseptic used in fish farming. The public health threat from its use (illegal) in edible fish species, such as trout and eel, has been recognized since 1933.[3]

Malachite green is a highly controversial compound due to the risks it poses to the consumers of treated fish, including its effects on the immune system, reproductive system, and its genotoxic and carcinogenic properties.[2]

Malachite green belongs to the group of triphenylmethane dyes. This compound is normally present in chromatic forms, but it can be easily reduced to leuco (i.e., colorless) forms. It has recently been seen that some members of this group of compounds are linked to an increased risk of cancer. MG is highly cytotoxic to mammalian cells and acts as a liver tumor–enhancing agent.[4]

At present, the use of MG in fish farms has become a matter of concern, as MG metabolites have been reported to cause human carcinogenesis and mutagenesis. Several studies have shown that exposure to malachite green increases the risk of cancer and mutagenesis, chromosomal fractures, teratogenecity, and respiratory toxicity. Thus, the use of MG in aquaculture has been banned in many countries. However, due to its low cost and high efficacy, this harmful dye is still used and will probably continue to be used for aquaculture in some parts of the world.[2]

Findings from MG residues in aquaculture products have also been frequently reported in Rapid Alert System for Food and Feed (RASFF) notifications of the European Commission[5] MG has never been registered as a veterinary medicine in the European Union.[6]

Studies have also shown that a high concentration of malachite green (more than 0.1 mg/L) causes severe damage to the internal organs of the fish and to growth of fish eggs. About 20% of the dyestuff produced in the world is discharged into streams without any pretreatment.[7]

The toxicity of malachite green is such that many countries, including the United States (U.S.), Canada, and the European Union Member States, have banned the use of this dye in fish raised for human consumption.[8] Therefore, because of its industrial importance and possible exposure to humans, malachite green poses a potential health hazard and is an environmental concern. Thus, development of sensitive and reliable methods is necessary for the determination of malachite green in foodstuffs, such as fish samples, and in environmental samples, such as wastewaters.[7]

The assessed fish farms in this study were placed at the upstream ends of two large rivers of Iran, Zayanderood and Karun, as they were two sources of drinking water for the cities downstream, in the center and south of Iran. The effluents of the fish farms are discharged without treatment to these precious water resources and could have serious health risks for people.[9]

The objective of this study was to determine the MG concentration in trout tissue and the water of the fish farms in Chahar-Mahal and Bakhtiari, one of the largest fishery industries in Iran, and to compare the results with the existing standards. In addition, we present an MG mass balance to highlight the importance and magnitude of pollution loading from MG in the study area.

MATERIALS AND METHODS

Instrumentation

Water (effluent)

A UV-visible spectrophotometer, Model: DR-5000 (Hatch-Lange, England) was used for recording the absorption spectra and absorbance measurements using 1-cm glass cells for the analysis of malachite green in water. A Metrohm digital pH meter, Model 632, with a combined glass electrode, measured the pH. A thermostat bath, model Colora, maintained the desired temperature for the cloud point temperature experiments.

Fish tissue

The LC-MS system used in this study was of a Shimadzu 2010EV Class, equipped with a quaternary pump, degasser, column heater, and a ultraviolet (UV) and quadruple detector using a computerized system controller (with the LC solution software). Water/acetonitrile (10/90) was used as the mobile phase, at a flow of 0.2 ml/minute, and the mass spectra were acquired in the positive ion mode (ESI^+). The selective ion monitoring (SIM) mode was applied for quantification and generation of the calibration curve.

Reagents

Water analysis

All reagents were of analytical grade and were used without further purification. A stock solution of 1000 µg/mL of malachite green (Merck) was prepared by dissolving 0.1 g of the reagent in water and diluting it to 100 mL in a volumetric flask. The desired concentrations were obtained by successive dilutions. A citrate buffer, pH 2.5, was prepared by dissolving 2.1015 g of citric acid (Merck) in 100 mL of water and adding 0.1 mol/L NaOH (Merck) to adjust the pH to 2.5 using a pH meter. Next, 0.5 mol/L of Triton X-100 (Aldrich) was prepared by dissolving 80.8590 g in water and diluting to 250 mL in a volumetric flask. A solution of benzoic acid (1 mol/L) was prepared by dissolving 30.5309 g of benzoic acid (Merck) in ethanol and it diluting to 250 mL in a volumetric flask.[7]

Fish tissue assays

Acetic acid, acetonitrile, ascorbic acid, citric acid monohydrate, lead (IV) oxide, and di-sodium hydrogen phosphate dehydrate were purchased from Merck. Ammonium hydroxide 25% (m/v), dichloromethane, methanol (HPLC-grade), sodium acetate, sodium perchlorate monohydrate, para-toluenesulfonic acid (p-TSA), brilliant green, and N, N, N, N-tetramethyl-1,4-phenylenediamine dihydrochloride (TMPD) were purchased from Aldrich, and malachite green oxalate from the Veteran reference standard. Aromatic sulfonic-acid-bonded SPE columns (3 mL, 500 mg) of Milli-Q quality were used.

A McIlvaine solution at pH 3.0 was prepared by mixing 18.9 mL of 0.2 M sodium hydrogen phosphate and 81.1 mL of 0.1 M citric acid, with volumes of 62.5 and 37.5 mL, respectively, to obtain a McIlvaine solution at pH 6.0. Fish were caught from the fish farm.[3]

This study was on aquaculture in the province of Chahar Mahal and Bakhtiari in the year 1988–1989.

Experimental

Water

Six effluent samples, with a given volume (10 L), from the fish farm output were collected, and then the collected samples were concentrated to a volume of 1 L. Next, an aliquot of the malachite green solution, 6 mL of citrate buffer pH 2.5, 2.5 mL of 0.5 mol/L of Triton X-100, and 6 mL of 1 mol/L benzoic acid were added to a 50-mL volumetric flask and diluted to the mark with water. The resultant solution was transferred to a 50-mL tube and equilibrated at 40°C in a thermostat bath, for 20 minutes. To separate the phases completely, the solution was cooled in an ice bath. The removal of the aqueous phase was carried out by decantation. The surfactant-rich phase was diluted with ethanol in a 2-mL volumetric flask. The absorbance of the solution was measured at 630 nm. For the standard addition procedures, 35-mL aliquots of the sample were added to the standards before addition of the above-mentioned reagents, and the same cloud point extraction procedure, introduced by Pourreza and Elhami, was followed.[7]

Fish tissue

Six samples of fish were collected from different fish farms and then some of the tissue without the skin of each fish was homogenized, Then 2 g of homogenized fish tissue material was weighed in a 50 mL tube, and 2 mL McIlvaine buffer pH 3, 100 µL of 1 M para-toluenesulfonic acid, 50 µL of 1 mg/mL N, N, N-, N-tetramethyl-1, 4-phenylenediamine dihydrochloride, and 12 mL acetonitrile were added. Extraction was supported on a platform shaker at 500 rpm for 10 minutes. After centrifugation at ×3500 g at 15°C for 10 minutes, the supernatant was collected and the residue extracted with 2 mL McIlvaine buffer pH 6.0 and 12 mL acetonitrile. Following an additional centrifugation with the same parameters, the supernatants were combined and mixed with 6 ml of dichloromethane to remove the water. The organic phase was passed through an aromatic sulfonic acid SPE column (J.T. Baker, 500 mg, 3 mL), which was conditioned with 2 mL of acetonitrile/dichloromethane 80/20 (v/v) prior to use. The analyte-containing column was washed with 1.5 mL methanol and dried in a stream of nitrogen gas. Elution of the analytes was obtained with 4 mL of a mixture containing 90% (v/v) methanol, 5% (v/v) of 1 mg/mL ascorbic acid, and 5% (v/v) of 25% (m/v) aqueous NH_4OH, which was prepared just before use. The collected elute was dried under a stream of nitrogen gas, at ambient temperature. Finally, the residual material was dissolved in 500 µL of a mixture of 50 mM acetate buffer pH 4.5 and acetonitrile 40/60 (v/v).[3]

RESULTS

Table 1 indicates the comparison between the tonnage of fish products and dosage of MG consumption in the sampling fish farms in this study.

Table 2 shows the analytical results of the MG concentration in fish tissues and fish farm effluents.

Table 3 indicates the results of the accumulated MG in the fish tissues that were observed in this study.

The effluent quality parameters, such as temperature, pH, electrical conductivity (EC), total dissolved solids (TDS), total suspended solids (TSS), nitrate, phosphate, biochemical oxygen demand, and chemical oxygen demand, have been measured, and the results are presented in Table 4.

Table 5 indicates the environmental conditions that influence the LC_{50} values of MG for fish trout, and shows the comparisons with the parameters of this study.

DISCUSSION

As shown in Table 2, the MG concentrations in the

Table 1: Fish products and MG used in the fish farms[a]

Sampling fish farm no.	Flow rates of effluent (L/s)	Tonnage of trout fish products (Ton/year)	MG consumption (Kg/using period)[a]	MG consumption (kg/year)[b]
1	1000	250	129.6	1555
2	700	150–200	90.7	1089
3	50	10	6.5	78
4	300	60	38.9	467
5	500	100	64.8	778
6	250	50	32.4	389

[a]Zamani[9] [b]MG concentration used in the fish farms of this study was about 1.5 mg/L and the 'using period' of MG was once a month. In addition, the complete growth period for fish was six months. During every six-month period, MG addition to farms was repeated six times

effluent of fish farms were in the range of 5.7 to 384 ng/L. In addition, the concentration of MG obtained in the fish tissue was between 265 and 1663 µg/kg. Therefore, the MG concentrations in the fish tissue of this study were outside the Codex, Europe, and US standards of zero. This was because, in the Iranian trout production industry, MG has been used for many years as a general hatchery disinfectant, to treat fungal conditions on trout eggs and for the control of certain ectoparasites. In addition, the other reasons for the high utilization of MG by fish farmers were, its low price, availability, high efficiency, lack of supervision to prevent consumption by legal agencies, such as, the Department of Environment (DOE) in Iran, and the fact that they were being prescribed by veterinarians.

Table 2: MG concentration in fish tissues and fish farm effluents

Sampling fish farm no.	MG concentration in fish farm effluent water (ng/L)	MG found in fish tissue (µg/kg)
1	73.2	1663
2	384	273
3	5.7	265
4	nd[(a)]	452
5	nd[(a)]	280
6	nd[(a)]	320
Europe standard	0	2[(b)]
USA standard	-[(c)]	-[(c)]
Australia standard[(d)]	0	0
Codex standard	-[(c)]	-[(c)]
Ireland standard[(e)]	100 µg/L	-

[(a)]non-detectable. [(b)]Scherpenisse and Bergwerff.[3] [(c)]The US and Codex standards for MG were not found in the literature. [(d)]Zealand.[10] [(e)]Safarik and Safarikova[4]

Table 3: Mass balance of accumulated MG in the fish tissues

Sampling fish farm no.	MG concentration in fish tissue (µg/kg)	Tonnage of trout fish products (Ton/year)	Accumulated MG in the fish tissue (g/year)
1	1663	250	416
2	273	175	48
3	265	10	3
4	452	60	27
5	280	100	28
6	320	50	16
Sum	-	645	537

However, MG as an endocrine disrupter (EDC) is highly cytotoxic to mammalian cells and carcinogenic to the liver, thyroid, and other organs of experimental animals. Incidences of tumors in lungs, breast, and ovary, have also been reported from rats exposed to malachite green. In the thyroid gland, MG results in a blockade of hormone synthesis, a decrease of T4 and increase in TSH concentrations causes tumors in the thyroid follicle cells of rats.[2]

As we know, the Zayanderood River generates 40 m^3/s of water and is the main source for the production of 12 L/s of treated drinking water for more than three million people in Isfahan. In addition, the Karoon River, with a flow rate of 432 m^3/s, is one of the largest sources of water supply in the Khuzestan area. MG concentrations in the effluent of fish basins [Table 2] for sampling farm No. 1, placed in the upstream of Zayanderood River, is about 73 ng/L, and for sampling farms Nos. 2 and 3 in the upstream of Karoon river, around 384 and 6 ng/L, respectively. Therefore, the MG mass balance in the effluent water from the fish farms demonstrates that the maximum MG pollution loadings from Farm No.1, with a flow rate of 1000 L/s [Table 1], for Zayanderood River, is around 6.3 kg/d, and the MG load from Farm No. 2, with a flow rate of 700 L/s [Table 1] for Karoon River, is about 23.2 kg/d (Eq.1).

$$\text{MG Loading}(L_{MG}) = (\text{MG Concentration}) \times (\text{Effluent flowrate}) \quad (1)$$

Table 4: Physicochemical characteristics of fish farm effluents

Sampling fish farm no.	BOD5 mg/L	COD mg/L	PO4 – mg/L	NO3- mg/L	TSS mg/L	pH	EC Ms/cm	TDS mg/L	T°C
1	7.6	20.64	0.14	1.3	90	7.6	035	0.19	13
2	7	23.67	0.11	1.4	90	7.6	0.43	0.23	18
3	6.5	16.2	0.06	1	80	7.5	0.27	0.16	14.4
Std.[(a)]	≤30	-	≤0.3	≤3[(b)]	≤50	6–9	-	-	-

[(a)]Water Quality Standards for Shrimp Farm Effluents Recommended by the Global Aquaculture Alliance, Boyd.[11] [(b)]Total ammonia nitrogen (mg/L)

Table 5: Influence of the environmental conditions on the LC_{50} values of MG for fish trout, and comparison with the parameters of this study

In this study				Toxicological effects of MG Srivastava *et al*.[2]			
Sampling fish farm no.	pH	T°C	MG concentration in water, ng/L	LC50 mg/L	pH	T°C	Time, H
1	7.6	13	73.2	1.4	7.5	12	3
2	7.6	18	384	2.35	8.0	12	3
3	7.5	14.4	5.7	6.8	8.0	12	6

$$\left[\begin{array}{l}\left(73\frac{ng}{L}\right)\times(1000)\frac{L}{s}\times\left(10^{-9}\frac{kg}{ng}\right)\times\left(86400\frac{s}{d}\right)\\=\left(6.3\frac{kg}{d}\right)\left(384\frac{ng}{lit}\right)\times\left(700\frac{L}{s}\right)\times\\\left(10^{-9}\frac{kg}{ng}\right)\times\left(86400\frac{s}{d}\right)=\left(23.2\frac{kg}{d}\right)\end{array}\right]$$

Therefore, the equivalent concentration of MG in the Zayanderood River (C_{eqv-Z}), with the flow rate (Q_r) of 40 m^3/s downstream of the effluent discharge from Farm No. 1, with the flow rate of 1 m^3/s (Q_e) and MG concentration (C_e) of 73 ng/L, would be around 1.78 ng/L(Eq.2). The equivalent concentration of MG for the Karoon River (C_{eqv-K}) was around 0.62 ng/L (Eq.3). We assumed that the MG concentration in both rivers (Cr), in upstream of the discharge point of the farm effluent was zero.

$$C_{eqv}=\frac{(C_e\times Q_e)+(C_r\times Q_r)}{(Q_e+Q_r)} \quad (2)$$

$$C_{egv-z}=\frac{\left[\left(73\frac{ng}{L}\right)\times\left(1\frac{m^3}{s}\right)+\left(0\frac{ng}{L}\right)\times\left(40\frac{m^3}{s}\right)\right]}{\left[\left(1\frac{m^3}{s}\right)+\left(40\frac{m^3}{s}\right)\right]}=1.78\frac{ng}{L} \quad (2)$$

$$c_{eqv-k}=\frac{\left[\left(384\frac{ng}{L}\right)\times\left(0.7\frac{m^3}{s}\right)+\left(0\frac{ng}{L}\right)\times\left(432\frac{m^3}{s}\right)\right]}{\left[\left(0.7\frac{m^3}{s}\right)+\left(432\frac{m^3}{s}\right)\right]}=\left(0.62\frac{ng}{L}\right) \quad (3)$$

Therefore, the MG loading in Zayanderood (L_{MG-Z}) River due to Farm No.1 will be 6.15 kg/d, and MG loading in Karoon (L_{MG-k}) due to Farm No. 2 will be 23.14 kg/d.(Eq. 4).

$$L_{MG-Z}=\left[\begin{array}{l}\left(1.78\frac{ng}{L}\right)\times\left(40\frac{m^3}{s}\right)\times\left(10^3\frac{L}{m^3}\right)\\\times\left(10^{-9}\frac{kg}{ng}\right)\times\left(86400\frac{s}{d}\right)=\left(6.15\frac{kg}{d}\right)\end{array}\right] \quad (4)$$

$$L_{MG-k}=\left[\begin{array}{l}\left(0.62\frac{ng}{L}\right)\times\left(40\frac{m^3}{s}\right)\times\left(10^3\frac{L}{m^3}\right)\\\times\left(10^{-9}\frac{kg}{ng}\right)\times\left(86400\frac{s}{d}\right)=23.14\frac{kg}{d}\end{array}\right]$$

These loadings are related to fish production rates of around 450 (250 + 200) ton/year for Farms No. 1 and 2 [Table 1]. However, with a trout production rate of 10,000 tons per year in all farms in the study area (2007), all the loadings should be multiplied by a ratio of 22 (10000 ÷ 450, Table 1). Thus, the estimated total MG loading ($L_{MG-Total}$) due to all farms in this study, for the two rivers of Karoon and Zayanderood, would be 644 kg/d (Eq.5).

$$\left(L_{MG-Total}\right)_{Estimation-1}=\left[(6.15+23.14)\frac{kg}{d}\times 22\right]=644\frac{kg}{d} \quad (5)$$

In other words, for this study area, the water with a flow rate of 50 m^3/s is cycled from river to fish farms and then recycled to the river as a raceway type of fish farm (open-type farm), with a fish production rate of 10,000 tons per year. Therefore, taking into account the 154 ng/L [(73.2 + 384 + 5.7)/3 = 154.3] [Table 2], of average concentration of MG in the effluent from the fish farms, the mass loading of MG produced via all fish farms would be 665 kg/d (Eq.6).

$$\left(L_{MG-Total}\right)_{Estimation-2}=\left(154\frac{ng}{L}\right)\times\left(50\frac{m^3}{s}\right)\times\left(10\frac{L}{m^3}\right)\times\left(10^{-9}\frac{kg}{ng}\right)\times\left(86400\frac{s}{d}\right)=665\frac{kg}{d} \quad (6)$$

The $L_{MG-Total}$ in estimation – 1 was 644 kg / d. It is comparable with the estimation - 2 of 665 kg / d (Eq.6)

In recent years, based on the government policy in Iran, the production rate of fish in cold water for aquaculture industries has increased to 50,000 tons as of 2007. The share of fish production in the study area related to whole trout production of the country is about 20% and is equivalent to 10,000 tons per year.

Globally, farmed fish production more than doubled from 1987 to 1997 at a rate of 9% per year and aquaculture is becoming a major industry that provides approximately 43% of seafood to consumers.[1]

Table 3 shows a mass balance for MG, using the results of the MG analysis on the fish tissue, with liquid chromatography equipped with a mass spectrometry detector, in the six fish farms in the study area. Considering the production

capacity of 645 tons of fish per year in the six observed farms, the accumulated MG in the fish tissue will be around 537 g/year [Table 3]. Therefore, the total accumulated MG for the entire fish tissues of the study area, with a production rate of 10,000 tons, would be around 8326 g/year. Therefore, the average concentration of the MG in the fish tissue of the studied farms was 832 μg/kg. A considerable part of this MG pollution load eventually entered the human body.

According to the European Commission, methods that can be used for the determination of MG residues in fish muscles should meet a minimum required performance limit (MRPL) of 2 μg/kg for the sum of MG and Leucomalachite green (LMG).[6]

In Germany, the use of malachite green is not allowed as an animal drug because of the possible carcinogenic, mutagenic, and teratogenic risks to human health. A zero tolerance of 0.01 mg/kg for the sum of malachite green and LMG in edible fish has been established. In the fish tissue, MG accumulates in the serum, liver, kidney, muscle, skin, and gut.[2]

The physicochemical characteristics of fish farm effluents in Table 4 reveal that except for TSS, all the other parameters meet the environmental requirements. Environmental degradation from aquaculture practices has been reported. The negative effects include organic pollution and eutrophication, a buildup of excess nutrients (primarily organic nitrogen and phosphorus), and wastes in an ecosystem. These problems, together with chemical pollution, can cause algal bloom, depletion of oxygen, reduction in water quality, death of corals, and habitat destruction.[12, 13]

In Table 5, the influence of the MG concentrations on LC_{50} in this study are compared with the studied data by Srivastava *et al*.[2] This comparison shows that the higher concentration of MG and the temperature of water in this study could cause a higher toxicity of MG.

In several studies, the LC_{50} values of many commercial dyes on fish have been estimated at different time intervals. These studies indicate that the toxicity of MG increases with a rise in temperature. They have evaluated the mortality rate of MG-exposed eggs and fries of Largemouth Bass, *Micropterus salmonides*. A two-fold increase in MG concentration resulted in an increase in the mortality rate of eggs and fries to more than 20 times. This observation led researchers to conclude that MG is extremely toxic and should not be used for any purpose involving Largemouth Bass eggs or fries.[2]

We concluded that the concentration of MG used as an antimicrobial chemical in trout fish tissues and water samples in this study were outside the existing standards. Therefore, the fish products of these farms could cause serious public health hazards, and discharge of the effluent from these farms, without treatment, posed potential environmental problems.

The total accumulated MG for the entire fish tissues in the study area, with production rates of 10,000 tons, was around 8326 g/year. Therefore, the average concentration of the MG in the fish tissue of the studied farms was 832 μg/kg. This is much higher than the European and Australian standards for MG in fish tissue.

The equivalent concentrations of MG in the two large rivers in this survey were in the range of 0.62 and 1.73 ng/L. Considering 154 ng/L to be the average concentration of MG in the effluent of fish farms, the mass loading of MG produced via all fish farms would be 665 kg/d. This was also much higher than the European, Australian, and Irish standards.

ACKNOWLEDGMENTS

The authors are grateful for the financial support for the research approved by Vice-Chancellery of Research, Isfahan University of Medical Sciences; Research Projects: #287213 and #286142.

REFERENCES

1. Cole DW, Cole R, Gaydos SJ, Gray J, Hyland G, Jacques ML, *et al*. Aquaculture: Environmental, toxicological, and health issues. Int J Hyg Environ Health 2009;212:369-77.
2. Srivastava S, Sinha R, Roy D. Toxicological effects of malachite green. Aquat Toxicol 2004;66:319-29.
3. Scherpenisse P, Bergwerff AA. Determination of residues of malachite green in finfish by liquid chromatography tandem mass spectrometry. Anal Chim Acta 2005;529:173-7.
4. Safarik I, Safarikova M. Detection of low concentrations of malachite green and crystal violet in water. Water Res 2002;36:196-200.
5. Halme K, Lindfors E, Peltonen K. A confirmatory analysis of malachite green residues in rainbow trout with liquid chromatography–electrospray tandem mass spectrometry. J Chromatogr B 2007;845:74-9.
6. Mitrowska K, Posyniak A, Zmudzki J. Determination of malachite green and leucomalachite green in carp muscle by liquid chromatography with visible and fluorescence detection. J Chromatogr A 2005;1089:187-92.
7. Pourreza N, Elhami Sh. Spectrophtometric determination of malachite green in fish farming water samples after cloud point extraction using nonionic surfactant Triton X-100. Anal Chim Acta 2007;596:62-5.
8. Andersen WC, Turnipseed SB, Karbiwnyk CM, Lee RH, Clark SB, Rowe WD, *et al*. Multiresidue method for the triphenylmethane dyes in fish: Malachite green, crystal (gentian) violet, and brilliant green. Anal Chim Acta 2009;637:279-89.
9. Zamani F. Agricultural and Natural Resources Research Center of Chahar-Mahal and Bakhtiari province, 2009 [In Persian].
10. Zealand FS. Report on a Survey of Chemical Residues in Domestic and Imported Aquacultured Fish, 2005.
11. Boyd C. Guidelines for aquaculture effluent management at the farm-level. Aquaculture 2003;226:101-12.
12. Aubin J, Papatryphon E, Van der Werf HM, Petit J, Morvan YM. Characterisation of the environmental impact of a turbot (Scophthalmus maximus) re-circulating production system using Life Cycle Assessment. Aquaculture 2006;261:1259-68.
13. Boesch DF, Burroughs RH, Baker JE, Mason RP, Rowe CL, Siefert RL. Marine pollution in the United States, The pew oceans commission, 2001.

How to cite this article: ???

Source of Support: Vice-Chancellery of Research, Isfahan University of Medical Sciences; Research Projects: #287213 and #286142. **Conflict of Interest:** None declared.