



Potassium Permanganate Alleviates the Potential Effect of Estrogenic Pollutants on Vitellogenin Gene Expression in Male *Oreochromis niloticus*

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ABSTRACT

This study aimed to determine the concentration of some estrogens like 17 β -estradiol (E₂), 17 α -ethynylestradiol (EE₂) and bisphenol A (BPA) in agriculture drainage water that used in Egyptian fish farms and to evaluate the oxidative degradation effect of potassium permanganate on these estrogenic pollutants. In addition to assess vitellogenin gene expression on mature male *Oreochromis niloticus* as a biomarker for estrogenicity. Three groups of males were allocated in three farm ponds filled with agriculture drainage water used in Egyptian fish farms. Water of two of them was treated with potassium permanganate in a concentration of 2.5 and 5 ppm while that of the third pond was kept as untreated control. Other three groups were allocated in three laboratory glass aquariums filled with tap water. Water of two of them was treated with 2.5 and 5 ppm potassium permanganate, while the third was kept as untreated control. The concentrations of E₂, EE₂ and BPA assessed by High Performance Liquid Chromatography (HPLC) were 9.150, 16.655 and 0.371 mg/L respectively in ponds water and only 0.125 mg/L bisphenol in tap water. This concentration declined in agriculture water treated by 2.5 ppm, moreover E₂ and EE₂ were completely eliminated with 5 ppm. As an accurate biomarker for estrogenicity, vitellogenin gene expression was assessed in the livers of all groups. A significant downregulation in farm treated groups was observed compared to control, it was 0.108 and 0.029 fold for 2.5 and 5 ppm, respectively.

Key words: Estrogenic pollution, Potassium permanganate, Vitellogenin, Gene expression, *Oreochromis niloticus*

INTRODUCTION

In spite of industrial sophistication and human made chemicals which have made our lives more convenient, nevertheless at the same times they may also cause unforeseen adverse effects on both humans, wildlife (Nakamura et al., 2015) fish (Hallgren et al., 2014) and their reproduction (Barucca et al., 2006). Many Endocrine Disrupting Chemical substances (EDCs) may behave as estrogen mimics including pesticides, insecticides, surfactants, plasticizers (Denslow et al., 2001) and fertilizers (Verderame et al., 2016). Estrogens disrupt endocrine and other vital systems when present in the aqueous environment and increase the risk of cancer, even at nanograms (Benhamou and Sarasin, 2002). Exposure of mammals to Bisphenol A (BPA), one of estrogenic EDCs, may result in decreased steroidogenesis, inhibition of testis growth with low semen quality, disturbance in follicle growth and oocyte meiotic abnormalities (Zhang et al., 2016). BPA also showed adverse effects on the immune system and lipid metabolism (Rogers et al., 2013). Multiple adverse effects due to exposure to estrogens have been reported such as fish feminization, delayed sexual maturation, reduced gonadal growth and altered steroidogenic capacity (Rodas-Ortiz et al., 2008). The most potent chemicals potentially causing adverse effects on fish species are estrogens in human waste (Nakamura et al., 2015). Egyptian Nile river receives many pollutants like effluents of fertilizer and pesticides factories (Osman et al., 2015). Moreover, it was estimated that the Nile river receives more than three million cubic meters daily of untreated or partially treated domestic wastes and municipal sewage (El Gammal and El Shazely, 2008).

In Egypt the most intensive fish farms receive water from agricultural drains to which industrial outfalls and sewage are discharged. Sewage effluent contains many thousands of chemicals, only some of which have been identified. Some, if not many, of the unidentified compounds will possess estrogenic activity. Fish living in waters contaminated with sewage effluent are therefore probably exposed to a mixture of estrogenic chemicals (Harries et al., 1997) which are absorbed and bioaccumulated in sufficient concentrations to induce adverse physiological responses in fish (Sumpter and Jobling, 1995) that could negatively affect their reproduction (Pereira et al., 2015).

Both females and males have Vitellogenin (VTG) gene but the lack of estrogens in the males prevents the expression of the protein under normal conditions (Sundararaj et al., 1982). The ability of many EDCs to mimic the estrogen can lead to unscheduled hepatic synthesis of VTG in male fish (Arukwe and Goksøyr, 2003). As male fish possess the hepatocyte Estrogen Receptor (ER), it can synthesize VTG when exposed to natural estradiol or other estrogen mimics (Virk et al., 2014). The induction of VTG in male fish is an indicator of exposure to estrogen or estrogen mimics. So, VTG induction has been suggested to be a useful tool for the screening of endocrine disruption and potentially concerning estrogenicity (Gröner et al., 2015).

Natural and synthetic estrogens are not completely broken down by current wastewater treatment processes and achieve minimal levels of removal (Ternes et al., 1999). Therefore, effective treatment approaches are very essential for the decomposition of the estrogenic EDCs from wastewaters. Chemical oxidation can convert hazardous pollutants to nonhazardous or less toxic compounds, and may be a good choice to eliminate EDCs in wastewaters (Shao et al., 2010). The common chemical oxidants applied during water treatment include ozone, chlorine and permanganate but both ozone and chlorine can react with bromide to form brominated by-products which have potentially worse health effects (Guan et al., 2010), while permanganate compared to other oxidants is sometimes preferred because of several advantages, it has a relatively low cost, ease of handling, comparative stability and effectiveness over a wide pH range (Zhang et al., 2013) and a wide range of temperature (Shao et al., 2010). Permanganate oxidation is an effective method for clearing waters containing phenolic EDCs. Some researchers reported the kinetics and mechanism of EDCs degradation using permanganate in aqueous solution like BPA (Jiang et al., 2010) and progesterone (Fayad et al., 2013). The study aims to assess the level of estrogenic contaminants specially 17 β -estradiol (E₂), 17 α -ethynylestradiol (EE₂) and Bisphenol A (BPA) levels in water of agricultural drains that are used in intensive fish farms in Egypt and tap water also to evaluate the efficiency of potassium permanganate in degradation of these substances and using VTG gene expression in male *O. niloticus* as a monitor for change in estrogenicity.

MATERIAL AND METHODS

Fish and rearing conditions

All procedures and investigations were reviewed and approved by the animal ethical committee of faculty of veterinary medicine, Kafrelsheikh University and were performed in accordance with the guiding principles for the care and use of laboratory animals. Seventy two sexually mature male *O. niloticus* were randomly selected. Thirty six were kept in three fish farm hatchery in outdoor concrete ponds for 12 days with natural photoperiod (14 hours light and 10 hours dark) in one of Kafr el-Sheikh governorate fish farms during May, the reproductive season. These ponds used agricultural drainage water, their temperature was 25 \pm 2 $^{\circ}$ C and the pH value was in the range of 7.5 to 8.5. Fish were fed twice daily on commercial fish food (28% crude protein). The other fish were kept in three glass aquariums (100 \times 30 \times 50 cm) which were filled with aerated and de-chlorinated tap water (Place the water outside in a sunny location, the chlorine will escape the water in the form of a gas) under laboratory conditions, the same like above.

Experimental design

Three groups of male *O. niloticus* were allocated in three farm ponds. Potassium permanganate was added to water of two ponds, the 1st and 2nd had a concentration of 2.5 and 5 ppm respectively according to Xiao-Yan et al. (2015) while the 3rd was kept as untreated control. At the same time three groups were allocated in three laboratory glass aquariums. Water of two of them was treated with potassium permanganate in a concentration of 2.5 and 5 ppm while that of the 3rd was kept as untreated control. E₂, EE₂ and BPA were determined in both treated and untreated water using HPLC. VTG gene expression was assessed in livers of males in all groups using real time PCR.

Samples

Water samples were collected for determination of E₂, EE₂ and BPA from both the three farm ponds and the three laboratory aquariums. Liver samples from randomly collected males were obtained, preserved in RNA later solution (preserve RNA in tissue from damage) then stored at -20 $^{\circ}$ C until RNA extraction.

Chemicals

E₂ with a purity \geq 98%, EE₂ with a purity \geq 98% and BPA with a purity = 97% were purchased from Sigma-Aldrich and used without further purification. Acetonitrile and methanol (HPLC grade) were also obtained from Sigma-Aldrich. Potassium permanganate, organic solvents and the other chemicals used were analytical grade.

Analytical method

High performance liquid chromatography (HPLC) was used. The separation conditions were carried out according to Naimi and Bellakhal (2012) with some modifications to determine the concentrations of E₂, EE₂ and BPA in water

samples. The separations were carried out by HPLC system (YL9300 series), which consisted of a quaternary pump, manual sample injector with a 20 μ L loop and a UV/VIS detector. Analytical separations were achieved on by reversed phase C-18 (Agilent, 5 μ m; 4.6 mm \times 250 mm) column, column temperature was 40°C and UV detection was performed at 230 nm. The mobile phase was a mixture of acetonitrile, methanol and acetic acid 0.1% (55:5:40 (v/v)), with constant flow rate 1 mL/min. Individual standard solutions of E₂, EE₂ and BPA were prepared in acetonitrile. The concentrations of the analytes were calculated by measuring the peak area for each compound and comparing it with those obtained from a standard solution.

RNA extraction and real time PCR

Total RNA was extracted using RNeasy mini kit (QIAGEN) according to manufactures protocol. All primers of real time PCR were synthesized by Bio Basic Canada Inc. oligonucleotide primers used in SYBR Green real time PCR (Gröneret al., 2015) were illustrated in table 1. Preparation of PCR Master Mix was carried out according to Quanti Tect SYBR green PCR kit (QIAGEN). Cycling conditions for Elongation Factor 1 α (EF-1 α) and VTG gene were illustrated in table 2. Real time PCR data were analyzed using MxPro software (Stratagene) version 4.10 by means of the comparative Cycle Threshold method ($\Delta\Delta C_T$), C_T values are equal to the number of cycles required to reach fluorescence above the threshold level (Pfaffl, 2001). EF-1 α was used as an internal standard by performing each PCR of the target gene and EF1- α from the same samples. Expressions of target gene were normalized to the corresponding level of EF1- α mRNA.

Table 1. Primer sequences position, product lengths, annealing temperature (Ta) and GenBank accession numbers of primers used for gene expression quantification of reference gene elongation factor 1 α (EF-1 α) and VTG gene of *O. niloticus*

Target gene	Primer sequences (5'-3')	Position	Product lengths (bp)	Ta (°C)	GenBank accession numbers
EF-1 α	Forward GCTTCAACGCTCAGGTCATC	1050	86	62	AB075952.
	Reverse TGTGGGCAGTGTGGCAATC	1136			
VTG	Forward CTTCCATCCAGCCACCAAG	71	160	60	FJ709597.1
	Reverse CTGCAGGAGGTTGATGATGC	231			

Table 2. Cycling conditions for EF-1 α and VTG SYBR green real time PCR

Stage	Temperature	Time	Cycles
Reverse transcription	50° C	30 min	1
PCR initial activation step	94° C	10 min	1
Amplification	94° C	30 sec	45
a) denaturation			
b) Annealing	60° C for VTG & 62° C for EF1 α	30 sec	
c) Extension	72° C	45 sec	
Dissociation curve	95° C	1 min	1
a) Secondary denaturation			
b) Annealing	60° C for VTG & 62° C for EF1 α	1 min	
c) Final denaturation	95° C	30 sec	

Data analysis

Statistical significances among the groups were performed using GraphPad Prism 6 software. PCR data are presented as mean \pm SE, group differences of gene expression levels were determined by one-way ANOVA. For statistical significance between groups, one way ANOVA was followed by the unpaired t-test, the significance level was set to $P \leq 0.0002$ in farm groups and $P \leq 0.005$ for laboratory groups.

RESULTS

Concentration of E₂, EE₂ and BPA and effect of potassium permanganate on their degradation

HPLC analysis for fish farm water revealed that the concentration of E₂, EE₂ and BPA were 9.150, 16.655 and 0.371 mg/L respectively. While in tap water, analysis showed absence of E₂ and EE₂ while 0.125mg/L BPA was detected. Treatment of agriculture water with potassium permanganate at a concentration of 2.5 ppm reduced the concentrations to be 8.457, 4.776 and 0.094 mg/L respectively. The increase of potassium permanganate concentration to 5ppm resulted in complete elimination of E₂ and EE₂ and slight decrease in BPA concentration to 0.090 mg/L (Figure 1). Treatment of tap water with 2.5 ppm potassium permanganate reduced the concentration of BPA to be 0.112 mg/L. Increase of potassium permanganate concentration to 5ppm resulted in the complete elimination of BPA (Figure 2).

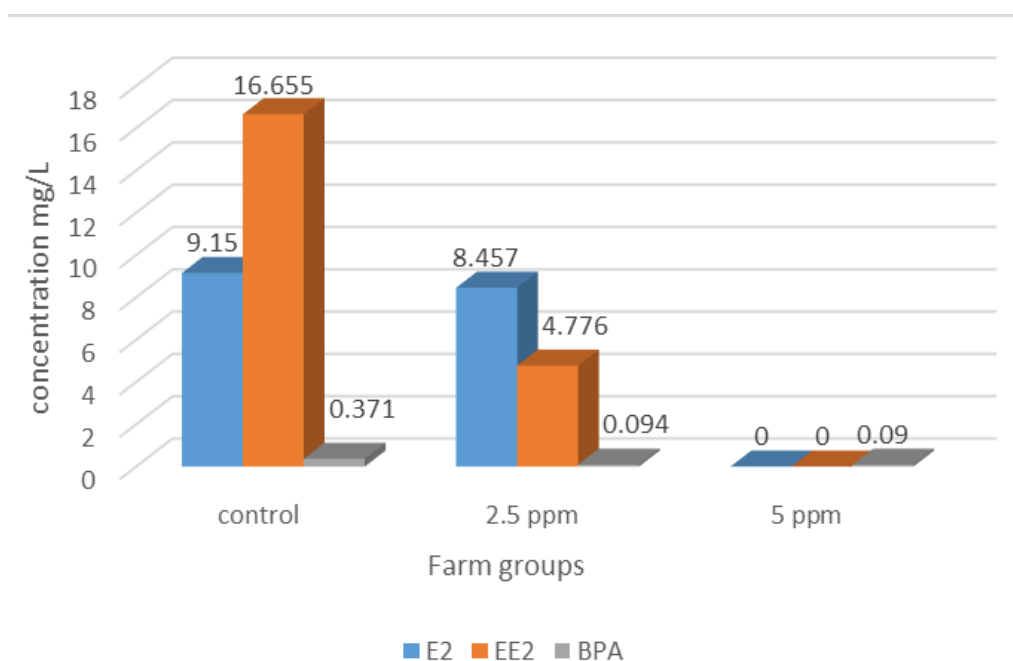


Figure1. Effect of 2.5 and 5 ppm potassium permanganate in reduction of E₂, EE₂ and BPA concentrations in farm water

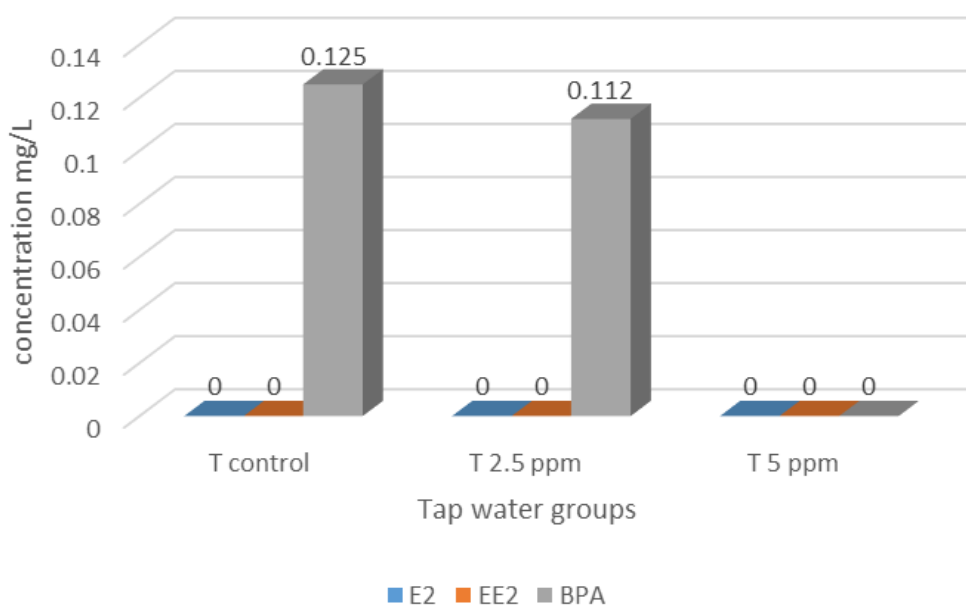


Figure 2. Potassium permanganate effect in reduction of bisphenol A (BPA) to be 0.112 mg/L after treatment of tap water by 2.5 ppm while increase the concentration to 5 ppm resulted in complete elimination of BPA

VTG gene expression

In comparison with the control farm group, treatment of water by 2.5 and 5 ppm potassium permanganate caused significant down regulation in expression of VTG gene in *O. niloticus* livers for the two concentrations. Treatment by 2.5 ppm showed a decrease of 0.108 fold while 5 ppm leads to a 0.029 fold decrease (Figure 3). While in tap water, the group treated with 2.5 ppm potassium permanganate showed slight change to be 1.103 fold in comparison with control while in 5 ppm group the significant decline was 0.507 fold (Figure 4).

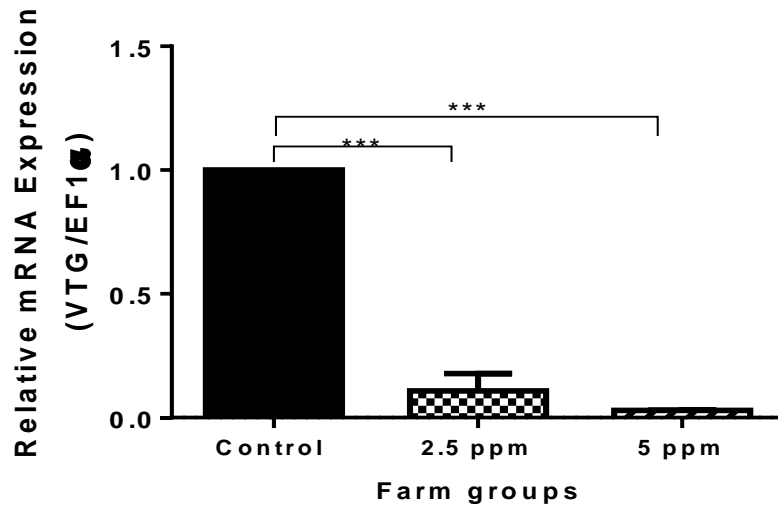


Figure 3. The fold changes (means \pm SE) in mRNA expression of Vitellogenin (VTG) gene relative to Elongation Factor 1- α (EF1- α) gene in the farm groups. Asterisks indicate statistically significant differences to control. $P \leq 0.0002$ (t-test for unpaired values).

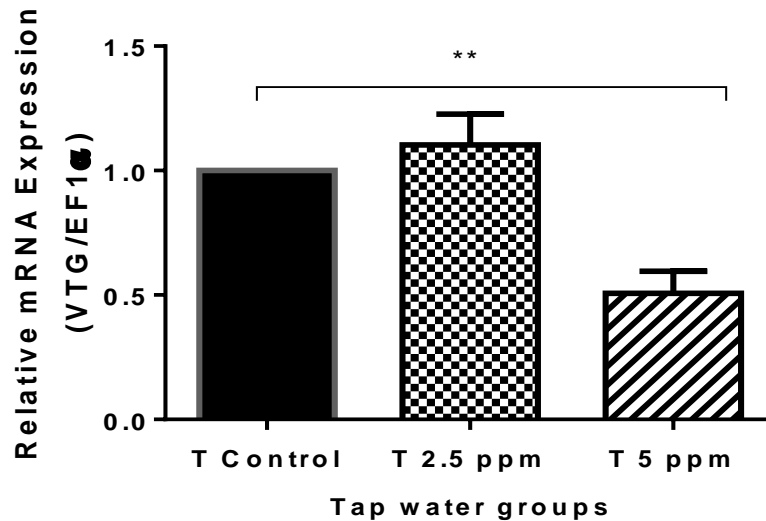


Figure 4. The fold changes (means \pm SE) in mRNA expression of Vitellogenin (VTG) gene relative to Elongation Factor 1- α (EF1- α) gene in tap water groups. Asterisks indicate statistically significant differences to control. $P \leq 0.005$ (t-test for unpaired values).

DISCUSSION

The usage of agriculture drain water carrying many EDCs, in aquaculture may cause environmental hazards on the fish populations in these farms. Scientific research in this area must focus on how to mitigate or eliminate these hazards. EDCs including natural steroid and synthetic estrogens have been verified as the common form of estrogenic activity in water (Kolpin et al., 2002 and Snyder et al., 2001). The actual concentrations of EDCs in environmental samples varies from ng/L to μ g/L (Shao et al., 2010). In the present study, the concentration of E_2 , EE_2 and BPA in fish farm water

samples were 9.150, 16.655 and 0.371 mg/L respectively and the concentration of BPA in tap water was 0.125 mg/L. The concentrations are high in farm water because it receives numerous nonpoint and point sources of pollution like untreated industrial, municipal and agricultural wastes in addition to sewage which contain many chemicals that possess estrogenic activity (Flammarion et al., 2000).

Chemical oxidation may be a good choice to eliminate EDCs in natural waters. Permanganate has been described as an inexpensive, easy and effective oxidant for degradation of EDCs (Abe et al., 2003). Permanganate oxidation has already been used in drinking water treatment processes (Shao et al., 2010). Besides, Potassium permanganate has been used for many years in aquaculture as an effective oxidizer for control of many bacterial, parasitic and fungal agents, eliminating the need for antibiotic therapy (Francis-Floyd and Klinger, 2009). Permanganate may have a high selectivity for EDCs oxidation in water (Guan et al., 2010). Permanganate mainly reacts with double bonds by donating oxygen, but it can also accept electrons or hydrogen atoms (Stewart, 1964). The mechanism for the oxidation of phenolic EDCs by permanganate was a single electron transfer and aromatic ring cleavage (Guan et al., 2010). In the farm experiment, treatment of water with potassium permanganate at a concentration of 2.5 ppm reduced the concentration of E₂, EE₂ and BPA from 9.150, 16.655 and 0.371 mg/L to 8.457, 4.776 and 0.094 mg/L respectively. Increase of potassium permanganate concentration to 5 ppm resulted in the complete elimination of E₂ and EE₂. In other words the efficiency of degradation of these EDCs is correlated with permanganate concentration. This is in agreement with results of Xiao-Yan et al. (2015) who stated that E₂ concentration decreased with increasing permanganate dosage and the removal efficiency of E₂ was greater than 99.6% when the reaction reached 30 min with 5 mgL⁻¹ permanganate. Xiao-Yan et al. (2015) also compared between the E₂ removal efficiency of ultrasound/potassium permanganate, potassium permanganate and ultrasound water treatment processes, the results showed that the ultrasound/potassium permanganate process was the most effective process among the three, followed by potassium permanganate and then ultrasound. It can be concluded that in the process of ultrasound/potassium permanganate, potassium permanganate played a promotional action in the degradation of E₂. The results of our study showed that addition of permanganate alone was as efficient as to bring the concentration of estrogens in agriculture drained water to nearly the same concentration in the very expensive tap water.

Biomarkers have the ability to improve accuracy, reliability and scientific basis for the quantitative assessment of environmental health risks (Arukwe and Goksøyr, 2003). VTG in male fish is an ideal biomarker to study the estrogenicity of EDCs on fish (Gröner et al., 2015; Virket al., 2014; Yamaguchi et al., 2015). Measurement of VTG mRNA expression in male fish liver is a rapid accurate method for detecting changes in VTG in fish exposed to estrogens and for monitoring estrogenic exposure (Barucca et al., 2006). Levels of VTG mRNA increase after exposure to estrogenic pollutants (Scholz et al., 2004). VTG mRNA in fish continuously exposed to estrogens is upregulated in a dose dependent manner also VTG mRNA transcription is induced immediately and its half life is short as it is quickly degraded in the absence of estrogen (Bowman et al., 2000). The results of our study showed down regulation of the estrogenic biomarker VTG gene expression in the livers of male *O. niloticus* that reared in water treated with potassium permanganate, revealed elimination of estrogenic potential via oxidation of E₂, EE₂ and BPA by potassium permanganate and decrease their adverse effect on fish.

CONCLUSION

E₂, EE₂ and BPA are present with a relatively high concentration in agriculture drainage water which used in fish farms in Kafr El-Sheikh governorate. VTG gene expression in male *O. niloticus* may be considered a valuable biomarker for exposure to environmental estrogens. Contamination of fish farm water with estrogenic material can activate VTG gene expression in male fish which is normally inactive. The changes in mRNA level can be used as fingerprints to characterize an estrogenic exposure. Adding potassium permanganate in a dose 2.5 and 5 ppm can induce oxidative degradation of these estrogens and this lead to downregulation VTG expression in male fish.

Competing interests

The authors have no competing interests to declare.

REFERENCES

- AbeY, Takigami M, Sugino K, TaguchiM, Kojima T, Umemura T and Tsunoda KI (2003). Decomposition of Phenolic Endocrine Disrupting Chemicals by Potassium Permanganate and GAMMA.-Ray Irradiation. Bulletin of the Chemical Society of Japan, 76: 1681-1685.
- Arukwe A and Goksøyr A (2003). Eggshell and egg yolk proteins in fish: hepatic proteins for the next generation: oogenetic, population, and evolutionary implications of endocrine disruption. Comparative hepatology, 2: 4.

- Barucca M Canapa A, Olmo E and Regoli F (2006). Analysis of vitellogenin gene induction as a valuable biomarker of estrogenic exposure in various Mediterranean fish species. *Environmental research*, 101: 68-73.
- Benhamou S and Sarasin A (2002). ERCC2/XPD gene polymorphisms and cancer risk. *Mutagenesis*, 17: 463-469.
- Bowman CJ, Kroll KJ, Hemmer MJ, Folmar LC and Denslow ND (2000). Estrogen-induced vitellogenin mRNA and protein in sheepshead minnow (*Cyprinodon variegatus*). *General and comparative endocrinology*, 120: 300-313.
- Denslow ND, Lee HS, Bowman CJ, Hemmer MJ and Folmar LC (2001). Multiple responses in gene expression in fish treated with estrogen. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 129: 277-282.
- El Gammal H and El Shazely H (2008). Water quality management scenarios in Rosetta river Nile branch, Egypt. In *Twelfth International Water Technology Conference, IWTC12 edition*: Citeseer.
- Flammarion P, Brion F, Babut M, Garric J, Migeon B, Noury P, Thybaud E, Palazzi X and Tyler C (2000). Induction of fish vitellogenin and alterations in testicular structure: preliminary results of estrogenic effects in chub (*Leuciscus cephalus*). *Ecotoxicology*, 9: 127-135.
- Fayad PB, Zamyadi A, Broseus R, Prévost M and Sauvé S (2013). Degradation of progestagens by oxidation with potassium permanganate in wastewater effluents. *Chemistry Central Journal*, 7: 84.
- Francis-Floyd Rand Klinger R (2009). Use of potassium permanganate to control external infections of ornamental fish.
- Gröner F, Ziková A and Kloas W (2015). Effects of the pharmaceuticals diclofenac and metoprolol on gene expression levels of enzymes of biotransformation, excretion pathways and estrogenicity in primary hepatocytes of Nile tilapia (*Oreochromis niloticus*). *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 167: 51-57.
- Guan X, He D, Ma J and Chen G (2010). Application of permanganate in the oxidation of micropollutants: a mini review. *Frontiers of Environmental Science & Engineering in China*, 4: 405-413.
- Hallgren P, Nicolle A, Hansson LA, Brönmark C, Nikoleris L, Hyder M and Persson A (2014). Synthetic estrogen directly affects fish biomass and may indirectly disrupt aquatic food webs. *Environmental Toxicology and Chemistry*, 33: 930-936.
- Harries JE, Sheahan DA, Jobling S, Matthiessen P, Nea IIP, Sumpter JP, Tylor T and Zaman N (1997). Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. *Environmental Toxicology and Chemistry*, 16: 534-542.
- Jiang J, Pang SY and Ma J (2010). Role of ligands in permanganate oxidation of organics. *Environmental Science & Technology*, 44: 4270-4275.
- Jobling S, Sumpter JP, Sheahan D, Osborne JA and Matthiessen P (1996). Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environmental Toxicology and Chemistry*, 15: 194-202.
- Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB and Buxton HT (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. *Environmental Science and Technology*, 36: 1202-1211.
- Naimi I and Bellakha IN (2012). Removal of 17 β -estradiol by electro-fenton process.
- Nakamura A, Tamura I, Takanobu H, Yamamuro M, Iguchi T and Tatarazako N (2015). Fish multigeneration test with preliminary short-term reproduction assay for estrone using Japanese medaka (*Oryzias latipes*). *Journal of Applied Toxicology*, 35: 11-23.
- Osman AG, Abouelfadl KY, Krüger A and Kloas W (2015). Screening of multiple hormonal activities in water and sediment from the river Nile, Egypt, using in vitro bioassay and gonadal histology. *Environmental monitoring and assessment*, 187: 1-16.
- Pereira TSB, Boscolo CNP, da Silva DGH, Batlouni SR, Schlenk Dand de Almeida EA (2015). Anti-androgenic activities of diuron and its metabolites in male Nile tilapia (*Oreochromis niloticus*). *Aquatic Toxicology*, 164: 10-15.
- Pfaffl MW (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic acids research*, 29: e45-e45.
- Rodas-Ortíz JP, Ceja-Moreno V, Chan-Cocom ME and Gold-Bouchot G (2008). Vitellogenin Induction and Increased Plasma 17 β -Estradiol Concentrations in Male Nile Tilapia, *Oreochromis niloticus*, Exposed to Organochlorine

- Pollutants and Polycyclic Aromatics Hydrocarbons. *Bulletin of Environmental Contamination and Toxicology*, 81: 543-547.
- Rogers JA, Metz L and Yong VW (2013). Review: Endocrine disrupting chemicals and immune responses: a focus on bisphenol-A and its potential mechanisms. *Molecular immunology*, 53: 421-430.
- Scholz S, Kordes C, Hamann J and Gutzeit HO (2004). Induction of vitellogenin in vivo and in vitro in the model teleost medaka (*Oryziaslatipes*): comparison of gene expression and protein levels. *Marine environmental research*, 57: 235-244.
- ShaoX, Ma J, Wen G and Yang J (2010). Oxidation of estrone by permanganate: Reaction kinetics and estrogenicity removal. *Chinese Science Bulletin*, 55: 802-808.
- Snyder SA, Villeneuve DL, Snyder EM and Giesy JP (2001). Identification and quantification of estrogen receptor agonists in wastewater effluents. *Environmental Science & Technology*, 35: 3620-3625.
- Stewart R (1964). *Oxidation mechanisms: applications to organic chemistry*: New York: WA Benjamin.
- Sumpter JP and Jobling S (1995). Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environmental health perspectives*, 103: 173.
- Sundararaj BI, Goswami SV and Lamba VJ (1982). Role of testosterone, estradiol-17 β , and cortisol during vitellogenin synthesis in the catfish, *Heteropneustes fossilis* (Bloch). *General and comparative endocrinology*, 48: 390-397.
- Ternes TA, Stumpf M, Mueller J, Haberer K, Wilken RD and Servos M (1999). Behavior and occurrence of estrogens in municipal sewage treatment plants—I. Investigations in Germany, Canada and Brazil. *Science of the Total Environment*, 225: 81-90.
- Verderame M, Limatola E and Scudiero R (2016). Estrogenic contamination by manure fertilizer in organic farming: a case study with the lizard *Podarcis sicula*. *Ecotoxicology*, 25: 105-114.
- Virk P, Al-Sakran AAM and Elobeid MA (2014). Effect of Bisphenol A on the Levels of Vitellogenin and Metallothionein in Adult Male Carp, *Cyprinus carpio* L. *Tropical Journal of Pharmaceutical Research*, 13: 1107-1112.
- Xiao-Yan M, Kai T, Qing-Song L, Ya-Li S, Yong-Jiong N and Nai-Yun G (2015). Degradation of 17 β -estradiol by combined ultrasound/KMnO₄ in an aqueous system. *Desalination and Water Treatment*, 53: 493-500.
- Yamaguchi A, Ishibashi H, Arizono K and Tominaga N (2015). In vivo and in silico analyses of estrogenic potential of bisphenol analogs in medaka (*Oryziaslatipes*) and common carp (*Cyprinus carpio*). *Ecotoxicology and environmental safety*, 120: 198-205.
- Zhang J, Sun B and Guan X (2013). Oxidative removal of bisphenol A by permanganate: Kinetics, pathways and influences of co-existing chemicals. *Separation and Purification Technology*, 107: 48-53.
- Zhang Y, Tao S, Yuan C, Liu Y and Wang Z (2016). Non-monotonic concentration –response effect of bisphenol A on rare minnow *Gobiocypris rarus* ovarian development. *Chemosphere*, 144: 304-311.