Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1753-3033

Effect of Methyl Tert-Butyl Ether (MTBE) at in vitro conditions on antioxidant enzyme activities of superoxide dismutase and catalase in *Rutilus caspicus* (Yakovlev, 1870)

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ABSTRACT

Methyl Tert-Butyl Ether (MTBE) increases oxygen consumption and reduces carbon monoxide and also air pollution. It is very active in soil, and its motility in water is a function of water movement in soil. MTBE is resistant to biodegradation, with high half-life and solubility in water and weak absorption in soil particles. The activities of SOD and CAT enzymes were examined in *Rutilus caspicus* in concentrations of 50, 100 and 150 mg L^{-1} MTBE for 7, 14 and 21 days. The mean water temperature and oxygen were 19 ± 1°C and 7.6 ± 0.2 respectively and salinity was maintained zero. 156 *R. caspicus* were stocked in aquariums, 3 specimens were randomly selected from aquarium in the first, second and third weeks. Blood serum was separated to assay the SOD and CAT activities. A strong significant positive relationship was found between MTBE concentration and SOD as well as CAT: [$r(108)=0.73$, $P < 0.01$]. Elevated MTBE increased the activity of SOD and CAT. In addition, there was a mean significant positive relationship between exposure time and SOD: [$r(108)=0.41$, $P < 0.01$]. SOD activity increased over time, and there was a negative weak significant relationship between exposure time and CAT [$r(108)=-0.20$, $P < 0.05$]. CAT activity decreased by prolonged exposure time. Moreover, there was a mean positive significant relationship between SOD and CAT [$r(108)=0.41$, $P < 0.01$].

Key words: Methyl tert-butyl ether (MTBE), *Rutilus caspicus*, antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT).

INTRODUCTION

MTBE is usually used as octane booster to increase the oxygen in gasoline. It causes increased fuel oxygen levels and also reduces the amount of carbon monoxide and air pollution (USPEA 1998).

Although, the use of MTBE improves public health and the environment, its world top production (26 million tons per year) and widespread use in Iran (900 thousand tons) cause its release in large quantities which can have detrimental effects on human health and the environment.

The threshold of taste and smell of MTBE are 2 and 2.5 µg L^{-1} respectively, thus, MTBE at very low concentrations make the consumption of drinking water unsuitable. The Environmental Protection Agency (EPA) recommended daily allowance of 20 to 40 µg L^{-1} based on the thresholds of smell and taste of MTBE. It is believed that at this concentration, the undesirable smell and taste of MTBE will not be felt and appropriate immune distance of its potential carcinogenic effects will be formed (US Environmental Protection Agency, 1998). MTBE contamination of water resources is caused by leaking underground fuel tanks, transmission lines, filling tanks above ground level and mess at the gas stations (Johnson *et al.* 2000).

Caspian J. Environ. Sci. Vol. 17 No. 1 pp. 55~62
DOI: 10.22124/cjes.2019.3344
Received: Sep. 04. 2018 Accepted: Jan. 07. 2019
Article type: Research

Oil is the most important pollutant in aqueous environments (Pacheco & Santos 2002). The close Caspian field also contains this pollution. *Rutilus caspicus* is one of the commercially important fish species and its reservoirs have significantly decreased for various reasons in recent years. The fish is considered as one of the threatened species in this region (Kiabi *et al.* 1999). One of the most important factors in reducing its reservoirs is water pollution from various chemicals.
Naturally, reactive oxygen species (ROS) are made in metabolic processes that react with important molecules such as lipids, proteins and nucleic acids in the body. All living organisms have protective systems against free radical reactions such as antioxidant enzymes and oxidative stress. Actually, these protective systems can cause balance in natural condition between production and removal of ROS. Imbalance in this process causes imbalance in body homeostasis and oxidative stress in various cells of living organisms. Antioxidant defense systems encompass enzymatic and non-enzymatic ones, the former includes SOD and CAT (Ahmad *et al.* 2000; Kohen & Nyska 2002; Wong *et al.* 2007).
Therefore, living organism generally has antioxidant defense system to prevent oxygen active species that control the molecules surface during metabolism in common physiological conditions in the body (Farombi *et al.* 2007; An *et al.* 2008).
Defense response to the antioxidant enzymes in all aquatic and terrestrial organisms is generally discussed (Zikic *et al.* 1996; Livingstone 2001; Valavanidis *et al.* 2006; Miller *et al.* 2009). Furthermore, evaluation of the amount and response of enzymes in living organisms gradually necessitates environmental purification plans. DOS and CAT assays are used in most studies (Akcha *et al.* 2000; Regoli *et al.* 2002; Sole *et al.* 2007) to determine activity of these enzymes in cell and tissue levels, e.g., they were assayed in a study entitled "toxic effects of lead and zinc on antioxidant activities of young *Clarias gariepinus* (Joseph *et al.* 2012). In addition, antioxidant responses and biochemical features in rainbow trout were assessed after exposure to propiconazole fungicides (LI *et al.* 2011). Furthermore, antioxidant enzyme activities in *Tilapia zillii* from QARUN Sea were considered as a bio-indicators (Desoky *et al.* 2016). Moreover, biochemical changes due to antioxidant enzymes in catfish, *Heteropneustes fossilis* were examined by exposure to fluoride (Shankar Yadav *et al.* 2015). Antioxidant enzyme activities were also examined in blood serum of *Cyprinus carpio* once assessing water pollution in Ataturk Dam Lake (Karadag *et al.* 2014). The effect of burnt oil on antioxidant enzymes and safety response were also examined in *Thalassophryne maculosa* (Marcano *et al.* 2006). So, this study was conducted to assess the effect of sub-lethal MTBE concentrations on CAT and SOD antioxidant activities of *R. caspicus*.

MATERIALS AND METHODS

A total number of two hundred and twenty *R. caspicus* with an average body length of 150 ± 30 mm and weight of 15 ± 3 g were obtained from Syjuval hatchery located in Bandar Turkman, Golestan Province, Iran and transferred to the laboratory by bags containing water (1/3) and oxygen (2/3) to the laboratory in Islamic Azad University, Science and Research Branch of Tehran, Iran. They were kept for 10 days in an aquarium with 150 L water and oxygen pump, for adaptation of fish to their environment before the experiments. Biomar floating food (Sera Granulat Company, China) were used for feeding fish during adaptation period (once every 24 h). Fifteen fish were then transferred to twelve 50-L aquaria (3 aquaria as control and 9 for treatments). All aquaria were aerated with oxygen pumps, salinity = 0 and water temperature = 19 ± 1 °C. Based on preliminary tests and previous results, sub-lethal concentrations of 50, 100 and 150 mg L^{-1} MTBE were used in this experiment. The LC_{50} (96 h) of MTBE measured for *R. caspicus* was about 600 to 900 ppm. Therefore, approximately 1/4 (750 mg L^{-1}) was taken up during the experiment. Fish were placed under semi-static conditions for 21 days (70% of water was changed every 48 h and then the desired concentration of MTBE was replaced), while the control fish were exposed to fresh water.

Sampling

Fish blood was obtained in order to measure CAT and SOD antioxidant enzyme activities. The fish were anesthetized using 150 ppm clove extract (Sharifpour *et al.* 2002). Blood was obtained from the fish caudal peduncle and then centrifuged for 5 min at 10000 rpm (Vesely *et al.* 2006). Special kits for SOD and CAT (Kit Zellbio, Germany) were used to assay these enzymes in blood serum and then evaluated by Sandwich ELISA method (Moss & Henderson 1999).

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Statistical analysis

Data normality and homogeneity of variance were measured using Kolmogrov-Smirnov and Levene's tests, respectively. Repeated measures test was used to check the existence of significant difference between SOD and CAT enzymes as well as time and MTBE concentration. Results were presented as mean ± standard error. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, ver. 21).

RESULTS

SOD enzyme activity in *R. caspicus* blood serum was elevated with increase in the MTBE concentration and prolonged exposure time to it. The minimum activity of this enzyme was found using 50 mg L^{-1} MTBE in the 7th day, while the maximum activity was observed by 150 mg L^{-1} in the 21st day. Consequently, increase in MTBE concentration and its exposure time induced elevating in SOD activity. Noteworthy, SOD activity in all the treatments were higher than those of all controls.

CAT activity increased with time and concentration until 14 days, but suddenly decreased in the 21st day. 150 mg L^{-1} in the 14th day exhibited the maximum activity, while 50 mg L^{-1} in the 21th day displayed the minimum compared to other treatments.

It is noticeable that CAT enzyme activity was as compared to other control samples. Pearson correlation test was used to determine the significant relationship between MTBE concentration and SOD as well as CAT. Results showed the strong positive and significant relationship between MTBE concentration and SOD [$r(108) = 0.73$, $P < 0.01$]. There was also strong positive and significant relationship between MTBE concentration and CAT [$r(108) = 0.73$, $P < 0.01$].

Table 1. Mean SOD enzyme activity in *Rutilus caspicus* in different concentrations (u mL^{-1}).

Time / Concentration(mg l^{-1})	7th day	14th day	21st day
0	14.74 ± 1.30	14.22 ± 1.73	14.46 ± 1.88
50	17.37 ± 1.84	20.31 ± 2.96	22.38 ± 3.07
100	19.87 ± 2.09	23.35 ± 4.02	28.67 ± 4.63
150	22.17 ± 2.26	27.13 ± 4.45	37.89 ± 5.43

Table 2. Mean CAT enzyme activity in *Rutilus caspicus* in different concentrations (u mL^{-1}).

Time / Concentration (mg L^{-1})	7th day	14th day	21st day
0	28.92 ± 4.90	26.90 ± 4.80	27.39 ± 4.92
50	39.48 ± 9.00	43.85 ± 7.29	33.52 ± 8.08
100	51.54 ± 12.79	50.93 ± 13.54	40.86 ± 8.52
150	62.87 ± 15.72	71.44 ± 12.49	47.61 ± 8.04

Pearson correlation test was used to assess the significant relationship between time and SOD as well as CAT. Results showed the mean positive and significant relationship between exposure time and SOD [$r(108) = 0.41$, $P < 0.01$]. In addition, weak negative and significant relationship was observed between time and CAT [$r(108) = -0.2$, $P < 0.05$]. In addition, Pearson correlation test was used to assess the significant relationship between SOD and CAT enzymes. The mean positive and significant relationship was found between SOD and CAT enzymes [$r(108) = 0.41$, $P < 0.01$]. Repeated measures test was used to assess the SOD and CAT at various times in each MTBE concentration

Table 3. Pearson correlation coefficient between MTBE concentration and exposure time to different variables.

Variables	1	2	3	4
MTBE Concentration	1			
Exposure time	0.00	1		
SOD	0.73**	0.41**	1	
CAT	0.73**	-0.20*	0.41**	1

*P < 0.05 **P < 0.01

Caspian J. Environ. Sci. Vol. 17 No. 1 pp. 55~62 Received: Sep. 04. 2018 Accepted: Jan. 07. 2019
DOI: 10.22124/cjes.2019.3344 Article type: Research

Table 4. Comparison the mean activity of SOD and CAT enzymes (u mL^{-1}) in various times in each MTBE concentrations.

Concentration (mg L^{-1}) Enzyme	0	50	100	150
CAT	1.04 ± 27.74	1.83 ± 38.95*	3.07 ± 47.78	0.92 ± 60.63**
SOD	0.44 ± 14.47	0.43 ± 20.02*	0.73 ± 23.96**	3.18 ± 29.06**

Values are shown in mean ± standard deviation.
Except ** P < 0.01 and *P < 0.05 other differences weren't significant.

Results showed that there were no significant differences in CAT and SOD activities at different times and in zero concentration of MTBE [X(2)=0.47, P > 0.05 and X(2)=0.43, P > 0.05 respectively]. Significant differences were observed in CAT and SOD activities at various times and in 50 mg L^{-1} MTBE [X(2) = 0.007, P < 0.05 and X(2) = 0.02, P < 0.05 respectively]. Besides, the difference in CAT activity at various times and in 100 mg L^{-1} MTBE was not significant [X(2) = 0.06, P > 0.05], while SOD activity at various times and in 100 mg L^{-1} MTBE exhibited significant differences [X(2) = 0.001, P < 0.01]. Furthermore, significant differences were observed in CAT and SOD activities at various times and in 150 MTBE [X(2) = 0.001, P < 0.01 and X(2)=0.001, P < 0.01 respectively].

DISCUSSION

The most important antioxidant enzymes include catalase, SOD and glutathione peroxidase (GPX) with the following characteristics:

CAT degrades hydrogen peroxide to oxygen and water molecule, DOS degrades super oxides to hydrogen peroxide, and glutathione peroxidase with glutathione oxidase reduces fatty hydro peroxidase and hydrogen peroxide (Almedia *et al.* 2007).

When organisms are exposed to pollutants such as hydrocarbon, ROS system is activated, and antioxidant enzymes particularly, CAT and SOD increases (Niyogi *et al.* 2001). The DOS enzyme had incremental trend in fishes exposed to hydrocarbon pollutant (Digiulio 1989). In the present study, it was observed that after exposure of fish to the treatments in 7, 14 and 21 days and also 50, 100 and 150 mg L^{-1} MTBE, SOD activity increased. The treatments showed a significant difference in comparison with the control group. Yilmaz *et al.* (2006) in a study on *Cyprinus carpio,* found that by increasing in pollutants, CAT dropped while SOD elevated. They concluded that the various tissues exhibit different responses to pollution. So that, some tissues may not respond to the oxidative stress, hence enzymatic system may not well activate, resulting in tissue damage.

Organic and metal pollutants and also poisons were reported to agitate ROS production process and reduce antioxidant enzyme activities. Consequently, oxidant damages are acute in aquatic organisms in experiments. On the other hand, prevention or reduction of ROS toxicity is an effective function of antioxidant enzymes (Pinto *et al.* 2003).

It was observed in the present study that after exposure of the fish to the treatments, CAT activity elevated with increase in MTBE concentration, but dropped with increased time of producing the enzyme; the maximum activity was found by the 7th and 4th days, while the minimum by the 21st day, compared to the other treatments. The treatments showed significant differences in comparison with the control group.

In a study, the toxic effects of lead and zinc on *Clarias gariepinus* enzyme antioxidant activities were examined using sub-lethal concentrations of these elements. GST and GSH activities elevated once exposing to $ZnCL_2$, while SOD and CAT activities dropped.

In another group, SOD, GST, GSH and CAT activities dropped when exposing to $Pb(NO_3)_2$ as compared to the control (Saliu *et al.* 2012). It can be pointed out that in the present study, SOD sensitivity was more than CAT in the blood serum of *R. caspicus.* SOD showed more reaction to MTBE when its exposure time increased by elevating both concentration and time. However, CAT blood serum of *R. caspicus* first quickly reacted, increased, and then decreased after the 21st day. Noteworthy, antioxidant enzyme changes are different according to the type of aquatic pollutant. So that, the pollutant effect on the antioxidant levels depends on organic materials to some extent (Oruce *et al.* 2004).

Reproduction time, available food, temperature, season, etc. also influence this effect (Almedia *et al.* 2007; Ferenzilli *et al.* 2004; Manduzio *et al*. 2004). In addition, notably, some antioxidant enzymes do not react to some pollutants and somehow act more specialized (Tetiana *et al.* 2005).

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As MTBE concentration increase due to pollutant increase, enzymes activity show more stimulation and intensity of these antioxidant activities against stress factors, because these enzymes prevent damages of the body cells, diseases and free radicals. In the present study, the MTBE changed the SOD and CAT activities in *R. caspicus*. Increase in MTBE elevated SOD and CAT activities in comparison with the control. SOD activity had incremental trend with time, but CAT activity showed decreasing trend. SOD activity had a positive significant relationship with time and concentration. CAT activity showed also a positive significant relationship with concentration, but a negative significant relationship with time. In other words, MTBE in water containing *R. caspicus* can cause oxidative stress and consequently activate antioxidant enzymes such as SOD and CAT to fight with stressful and pathogenic agents. Repeated measures test was used to check the existence or non-existence of significant difference between CAT and SOD in various MTBE concentrations and times.

Results showed that there were significant differences in CAT and SOD activities in various concentrations during the first week ($p < 0.01$). Significant differences were also observed in CAT and SOD activities in various concentrations during the second week ($P < 0.01$). It was also true for CAT and SOD activities in various concentrations during the third week ($P < 0.01$).

ACKNOWLEDGMENT

The authors would like to acknowledge Razi laboratory, Science and Research Branch, Islamic Azad University, Tehran, for cooperation.

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Caspian J. Environ. Sci. Vol. 17 No. 1 pp. 55~62 Received: Sep. 04. 2018 Accepted: Jan. 07. 2019
DOI: 10.22124/cjes.2019.3344 Article type: Research

بررسی اثر متیل ترشیو بوتیل اتر (Methyl tert-butyl ether) بر میزان فعالیت آنزیم‌های آنتی‌اکسیدانی سوپر اکسید دیسموتاز و کاتالاز ماهی کولمه دریای خزر *Rutilus caspicus* در شرایط آزمایشگاهی

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(تاریخ دریافت: ۹۷/۰۶/۱۳ تاریخ پذیرش: ۹۷/۱۰/۱۷)

چکیده

MTBE سبب افزایش میزان اکسیژن سوخت می‌شود و میزان مونواکسید کربن و آلودگی هوا را کاهش می‌دهد. MTBE در خاک بسیار متحرک است و حرکت آن در آب تابع قوانین حرکت آب در خاک است. MTBE مقاومت زیادی به تخریب زیستی دارد و نیمه عمر آن در آب بالاست، جذب آن توسط ذرات خاک ضعیف است، حلالیت بالایی در آب دارد و بسیار متحرک است. این تحقیق به منظور بررسی میزان فعالیت آنزیم‌های سوپر اکسید دیسموتاز (SOD) و کاتالاز (CAT) ماهی کولمه دریای خزر که در معرض غلظت‌های ۵۰،۱۰۰،۱۵۰ میلی‌گرم بر لیتر MTBE به مدت ۱۴،۷و۲۱ روز قرار گرفته بودند، انجام شد. میانگین درجه حرارت آب ۱±۱۹ ،اکسیژن آب ۰/۲ ± ۷/۶ میلی‌گرم بر لیتر و شوری صفر در نظر گرفته شد. تعداد ۱۵۶ عدد ماهی در این آزمایش مورد بررسی قرار گرفت. در هفته اول ، دوم و سوم آزمایش ،۳ ماهی به صورت تصادفی از هر آکواریوم برداشته شد. خون ماهیان گرفته شد و سرم خون برای بررسی فعالیت آنزیم‌های SOD و CAT جدا شد. مطابق با نتایج بین غلظت MTBE با آنزیم SOD و CAT رابطه قوی مثبت و معنی‌داری وجود دارد.($r(108)=0/73$ P<۰/۰۱) به این صورت که با افزایش MTBE میزان فعالیت آنزیم SOD و CAT افزایش می‌یابد. همچنین بین زمان در معرض‌گذاری با آنزیم SOD رابطه متوسط مثبت و معنی‌داری وجود دارد (r(108)=0/41,P <۰/۰۱). به طوری که با طولانی‌تر شدن زمان، میزان فعالیت آنزیم SOD افزایش می‌یابد و بین زمان در معرض‌گذاری با آنزیمCAT رابطه ضعیف منفی و معنی‌داری وجود دارد. (r(108)=-0/20,P <۰/05) به این صورت که میزان فعالیت آنزیم CAT با طولانی‌تر شدن زمان کاهش می‌یابد. هم‌چنین بین آنزیم‌های SOD و CAT رابطه متوسط مثبت و معنی‌داری وجود دارد (r(108)=0/41 P <۰/۰۱).

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Bibliographic information of this paper for citing:

Mohammadzadeh Baran, S, Mashinchian Moradi, A, Sharifpour, I, Jamili, S, Ghavam Mostafavi, P 2019, Effect of Methyl Tert-Butyl Ether (MTBE) at in vitro conditions on antioxidant enzyme activities of superoxide dismutase and catalase in *Rutilus caspicus* (Yakovlev, 1870). Caspian Journal of Environmental Sciences, 17: 55-62

Caspian J. Environ. Sci. Vol. 17 No. 1 pp. 55~62 Received: Sep. 04. 2018 Accepted: Jan. 07. 2019
DOI: 10.22124/cjes.2019.3344 Article type: Research