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Effect of Montmorillonite on Nonylphenol Enrichment in Zebrafish

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Abstract: To investigate the effect of montmorillonite on nonylphenol enrichment in a zebrafish model. The AB strain zebrafish were used as the animal subjects and three concentration gradients were set for both nonylphenol and montmorillonite, according to their actual concentrations in aquaculture water in Huzhou city. A group treated with nonylphenol alone was also set, adding up to 12 experimental groups.

Concentrations of nonylphenol enriched in the liver, muscle, and gill of zebrafish were detected by solid phase microextraction–high performance liquid chromatography at Day 7, 15, and 30, respectively. Besides, the relative enzymatic activity of Superoxide dismutase (SOD) and the Glutathione S-transferase (GST) were also been detected, and the data were statistically analyzed. The results showed that the concentrations of nonylphenol in zebrafish peaked at Day 7 and gradually decreased afterwards for all the experimental groups. And the montmorillonite reduces short-term accumulation of nonylphenol in gills, and the high concentration of nonylphenol facilitates its enrichment in liver and muscle while the low concentration of nonylphenol doesn't. Meanwhile, the low concentration of nonylphenol in liver exerts an influence on the inductive effect of SOD and GST while the high concentration of nonylphenol shows the inhibiting effect of SOD and GST.

Key words: Nonylphenol; Montmorillonite; Zebrafish; Enrichment; Enzyme activity.

Abbreviations: Montmorillonite (MMT); Nonylphenol (NP).

Introduction

Environmental endocrine disrupting compounds (EDCs) interfere with the synthesis, release, transport, metabolism, binding, action, or elimination of endogenous hormones, and then impact the normal endocrine system of organisms,

leading to reproduction and immune dysfunctions[1]. In addition to the reversible or irreversible biological effects on the organisms, the offspring, or the population, EDCs also compromise the disease resistance of the body[2,3] and even cause diseases and cancer[4-7]. For instance, nonylphenol (NP), a common industrial raw material, is a typical phenolic environmental hormone and mainly accumulates in water bodies with a solubility of 5.43 mg/L[8]. This chemical presents genotoxicity, developmental toxicity, immunotoxicity, and neurotoxicity[9-13]. And it may deposit in living organisms and exhibit biological effects via the water body as well as through the food chain, and the effect of environmental EDCs might be more harmful after enrichment by the food chain[14,15].

The dose of environmental EDCs is generally low in nature and the correlation between their effect and dose is complex, for example, the toxicity of bisphenol A is stronger at low dose than at high dose[16,17]. The application of biomarkers is a common method to evaluate and analyze toxic effects of toxicants. The antioxidant enzymes of zebrafish are commonly used biomarkers[18-20]. However, the dose used in current study of the dose-effect relationship is basically the dose of toxicants exposed to the environment, and the study of the concentration-effect relationship between toxicant concentrations and markers in tissues or organs of zebrafish has rarely been reported. The situation is more complicated in actual nature environment, where a variety of substances, especially some nanoparticles in the water, modify the biological effects of environmental EDCs, and impact the adsorption, transport, enrichment, and even the toxicity of EDCs[21-23]. Montmorillonite (MMT) is a

typical layered aluminosilicate mineral that is adsorptive, hydrophilic, electrically charged, dispersedly suspended, and swells in water[24-26], therefore it is widely used in medicine, aquaculture, and sewage treatment[27-32]. MMT, as a common nanoparticle in water body, has the potential to enhance the toxicity of harmful substances and meanwhile reduces the accumulation of harmful substances and exhibits detoxification function in aquatic animals[33-36]. Few studies have reported the role of MMT in specific water environment. In the present study, the effect of MMT on NP accumulation in zebrafish was investigated in water environment using NP as a specific toxic substance, in addition, the relationship between the concentration of NP in liver and the enzyme activity of SOD and GST had also been analyzed.

1. Materials and methods:

1.1 Instruments and experimental materials

HPLC (high performance liquid chromatograph, LC-20AT, Shimadzu Corporation), solid phase microextraction (Supelco, 75 μm PDMS/DVB). Zebrafish (*Danio rerio*) AB strain (purchased from local fish market), both sex, weighing approximately 1.5–2 g and having the body length of 2.5–3.5 cm, were kept in recirculating water at 28 °C under standard laboratory conditions for two weeks. Nonylphenol (NP, analytically pure, 98 %. Purchased from Shanghai Ziyi Reagent Company). The pharmaceutical grade montmorillonite (MMT) was purchased from

Gaoyu Bentonite Company (Anji, China). The SOD and GST Assay Kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China).

All animal care and experimental procedures were approved by the Committee on Animal Care and Use and the Committee on the Ethic of Animal Experiments of Huzhou University and Zhejiang Sci-Tech University. And all methods were performed in accordance with the relevant guidelines and regulations.

1.2 Experimental methods

1.2.1 HPLC parameter settings

Chromatographic column: Waters Symmetry C18 (4.6×150 mm,5 μm); Mobile phase: Methyl alcohol:H_2O=26:74; Detection wavelength: 225 nm; Flow velocity: 1.0 $mL \cdot min^{-1}$; The column temperature was at 35 °C; Inlet sample quantity: 20 μL.

1.2.2 The methodology of NP detection based on HPLC method

(1) Accuracy: 6 parallel samples of NP with the identical concentration, the concentration of each sample was 2.092×10^3 μg/L according to the HPLC detection. The RSD (relative standard deviation) was also been calculated.

(2) The confirmation of quantitation limit (LOQ) and detection limit (LOD): The standard NP samples were diluted, then the LOQ and LOD were set as S/N=10:1 and S/N=3:1, respectively.

(3) The recoveries of NP: The zebrafish tissue samples of liver, muscle and gill, as well as water sample, were added the NP to the final concentration of 2.092×10^1,

107 2.092×10^2, 2.092×10^3 μg/L, respectively. The water sample was processed in
108 accordance with chapter 1.2.3, the zebrafish tissue samples were processed in
109 accordance with chapter 1.2.5. The processed samples were analysized by HPLC and
110 the recoveries of NP were acquired.

111 (4) Standard curve: The zebrafish tissue samples of liver, muscle and gill, as well
112 as water sample, were added the NP to the final concentration of 2.092, 2.092×5^1,
113 2.092×5^2, 2.092×5^3, 2.092×5^4 and 1.046×5^5 μg/L, respectively. The water sample was
114 processed in accordance with chapter 1.2.3 (Under the optimum condition), the
115 zebrafish tissue samples were processed in accordance with chapter 1.2.5. The
116 processed samples were analysized by HPLC and the absorption peak areas were
117 measured. Next, the linear equation between the concentration and the absorbance of
118 NP has been established.

119 **1.2.3 The conditions of solid phase microextraction (SPME)**

120 The water samples derived from aquaculture water was filtered by microfiltration
121 membrane (0.45 μm), and assembly of the adsorption time (60, 40, 30 and 20 min) of
122 SPME and the resolution time (40, 30, 20, 10, 9, 7, 5 and 3 min) of SPME can be
123 confirm the optimal adsorptional analytical conditions through the HPLC analysis,
124 and the experimental procedure of SPME was according to the introductions..

125 **1.2.4 Exposure measurement and grouping**

126 (1) Determination of exposure concentration of NP

127 A total of 10 typical aquaculture water samples in Huzhou area were selected,

with the average concentration of NP detected by high performance liquid chromatography regarded as 1× exposure concentration of NP .

(2) Determination of MMT concentration

The accumulation in 7 consecutive days was calculated as 1× exposure concentration of MMT on the basis that the depth of the aquaculture water system was 1.2-1.7m, the annual input of commercial feed per mu was 350-500kg and 2-5 kg of MMT in aquatic feed per ton was added, and the result was 2.949×10^{-5}g/L.

(3) Exposure test grouping

The samples were divided into 17 experimental groups, respectively 1×, 10× and 100× NP exposure group, 1/100, 1× and 100× MMT exposure group, 9 pairwise combinations between 1/100, 1× and 100× MMT exposure concentration and 1×, 10× and 100× NP exposure concentration, organic solvent group (with 1ml ethanol added) and test water group, with 3 parallel tests in each group. NP with different amounts in the experimental groups were dissoluted with 1ml ethanol.

1.2.5 The treatment of zebrafish tissue samples

Each of 25 zebrafish were raised in a 20L-water-filled tank, with the pH 7.0±0.5 (adjusted by $NaHCO_3$). Fluorescent lamp was chosen to simulate the natural light, replace half of the aquaculture water in every 24 h. Fed the zebrafish with the commercial feeds (without MMT), fish maintenance and the feeding protocol have been described by Lee et al[37]. After raised for 7, 15 and 30 d, 6 fish were randomly selected from each tank, respectively. The tissue samples of liver, muscle and gill

were extracted and storage at -20°C.

Tissue samples derived from 2 fish were classified into one group, the tissue homogenates were added 10 mM/L HCl up to 9 mL, storage at 4°C for 24 h, then each group was centrifuged for 10 min (6000 rpm at 4°C). The supernate was filtered by 0.45 μm filter membrane and was diluted by ultrapure water to 15 mL. Then the diluent was processed in accordance with chapter 1.2.3 (Under the optimum condition).

1.2.6 Determination of the concentrations of NP in tissues and data analysis

The concentrations of NP in treated samples were detected by HPLC in accordance with chapter 1.2.1. Statistical evaluations of the significant differences among the means of experimental groups were performed using Student's *t* test (MS Excel 2010).

1.2.7 Measurements of enzymatic activity

The liver samples were derived from 2 fish of each experimental groups, the sampling and the enzymatic activity determinations of SOD and GST were according to the Kit instructions.

2. Results

2.1 Parameters of NP testing methodology

2.1.1 LOQ and LOD of NP

LOQ: NP concentration of 1.046 μg/L; LOD: NP concentration of 0.4184 μg/L.

2.1.2 Accuracy

NP concentration of 2.092×10^3 μg/L with the RSD 2.25% (n=6).

2.1.3 Recovery

The recovery rate of NP were among 77.797 %–89.274 % (Table 1).

Table 1 Average recovery rate of NP

Samples	Content (μg/L)	Average recovery rate % (n=3)
Water	2.092×10^1	77.797%
	2.092×10^2	78.359%
	2.092×10^3	89.274%
Liver	2.092×10^1	78.539%
	2.092×10^2	78.695%
	2.092×10^3	81.126%
Muscle	2.092×10^1	85.467%
	2.092×10^2	79.503%
	2.092×10^3	82.031%
Gill	2.092×10^1	77.998%

	2.092×10^{2}	79.235%
	2.092×10^{3}	84.535%

174

175

176 **2.1.4 Standard curve**

177 The peak area of the elution curve of NP from the samples derived from
178 zebrafish tissues (Liver, muscle and gill) and aquaculture water have been measured,
179 the binary linear regression equation has been established by contrast the peak area of
180 each sample to the NP content of each sample (Table 2). The correlation coefficients
181 of these two factors were also been listed in table 2.

182 **Table 2 Regression equation and correlation coefficient of standard curve**

Samples	Equations	Correlation coefficient (R^2)
Water	y=370.65x+431635	0.9965
Liver	y=362.77x+356899	0.9931
Muscle	y=366.87x+400058	0.9922
Gill	y=361.34x+451265	0.9909

183

2.2 Optimum condition for SPME

Extraction processed by 75 μm PDMS/DVB, the optimal adsorption time is 20 min, resolution time is 5 min.

2.3 Exposure dose of NP

The mean NP contents of 10 measured water samples is 3.2133 μg/L, while the exposure doses of NP are 3.2133 μg/L, 32.133 μg/L and 321.33 μg/L, respectively.

2.4 The determination results and data analysis of NP in tissues

The NP contents in liver, muscle and gill of zebrafish at 7d, 15d and 30d were measured and calculated for single factor analysis of variance with Excel. The results showed that 1/100, 1× and 100× MMT exposure concentrations, organic solvent ethanol and test water had no effect on the experimental results. (In the following tables, N1, N2 and N3 respectively represents low, medium and high concentrations of NP. M1, M2, M3 respectively represents low, medium and high concentrations of MMT).

2.4.1 Variance analysis of NP contents in liver

Table 3, table 4 and table 5 are represented for the significance analysis of the mean difference of the NP contents in liver at 7d, 15d and 30d between the groups (N1, N2 and N3 respectively represent low, medium and high contents of NP. M1, M2, M3 respectively represents low, medium and high concentrations of MMT. In addition to the row of NP content, the cross of other rows and columns represents p value, /

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represents p≥0.05, * represents p<0.05, **represents p<0.01 and blank represents no comparison). The changes of NP contents of each experimental group at 7d, 15d and 30d were shown in figure 1 (* and / at the top of the figure: the first row represents analysis of difference significance between NP contents at 7d and 15d, the second row represents analysis at 7d and 30d and the third row represents analysis at 15d and 30d).

Table 3 Statistical analysis that NP contents in liver at 7d

	NP contents (μg/g)	**N2**	**N3**	**N1M1**	**N1M2**	**N1M3**	**N2M1**	**N2M2**	**N2M3**	**N3M1**	**N3M2**	**N3M3**
N1	0.1224±0.0096	*	*	**	**	**						
		*	*									
N2	0.0714±0.0066		/				/	**	**			
N3	0.0711±0.0014									**	**	**
N1M1	0.0699±0.0099				*	**	/			/		
N1	0.085±0.0129					*		*			/	

M2						
N1M3	0.099±0.0073			/		/
N2M1	0.0747±0.006		**	**	/	
N2M2	0.1001±0.008			/	**	
N2M3	0.1021±0.012	1				/
N3M1	0.0787±0.003	5			/	*
N3M2	0.0834±0.006	6				/

N3	0.0912±0.009												
M													
3	1												

211

212 **Table 4 Statistical analysis that NP contents in liver at 15d**

	NP contents	**N**	**N**	**N1**	**N1**	**N1**	**N2**	**N2**	**N2**	**N3**	**N3**	**N3**
				M	**M**	**M**	**M**	**M**	**M**	**M**	**M**	**M**
	(μg/g)	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
N1	0.0347±0.003	*	*	**	/	/						
	9	*	*									
N2	0.0422±0.002		*				**	**	\			
			*									
N3	0.0517±0.002									**	\	**
	2											
N1	0.0231±0.006				**	**	**			*		
M	8											
1												
N1	0.0358±0.002					*		\			\	
M	9											

2						
N1M3	0.0401±0.0021		\			*
N2M1	0.0253±0.0066	*	**	*		
N2M2	0.0346±0.0053		*		\	
N2M3	0.0425±0.0033					**
N3M1	0.0338±0.0042				\	**
N3M2	0.0349±0.004					**
N3	0.0465±0.006					

M 7

3

213

214

215 **Table 5 Statistical analysis that NP contents in liver at 30d**

NP contents (μg/g)	N2	N3	N1M1	N1M3	N2M1	N2M2	N2M3	N3M1	N3M2	N3M3
N1 0.0331±0.0038	/	*	/	/						
N2 0.0318±0.0035		*			/	*	*			
N3 0.0408±0.0063								/	/	/
N1M1 0.0357±0.0045				/	/			/		
N1M3 0.0341±0.004				/		/			/	

2							
N1	0.0344±0.005						
M					/		*
3	5						
N2	0.0354±0.003						
M				/	/	/	
1	4						
N2	0.0389±0.006						
M					/	/	
2	2						
N2	0.0366±0.004						
M							*
3	0						
N3	0.0325±0.007						
M						/	*
1	7						
N3	0.0368±0.003						
M							*
2	6						
N3	0.0436±0.004						

M 6

3

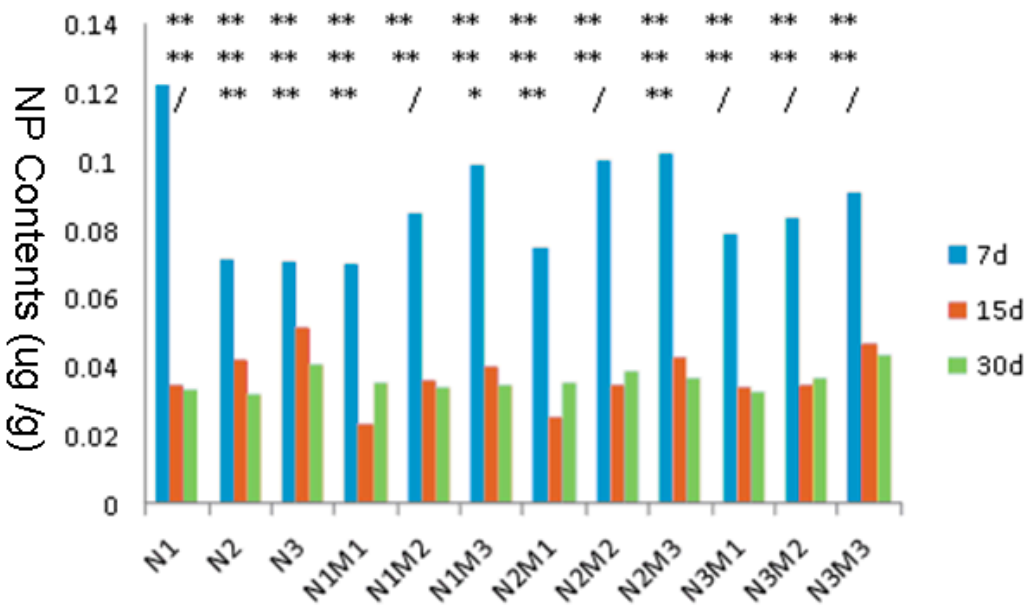

Figure 1. Statistical analysis of the content variation of NP in liver at 7d, 15d and 30d.

2.4.2 Variance analysis of NP contents in muscle

The NP contents in zebrafish muscle at 7d, 15d and 30d were listed in table 6, table 7 and table 8, respectively. Significance analysis of the means of all experimental groups was also demonstrated in these tables. The content variation of NP in every experimental group at 7d, 15d and 30d were illustrated in figure 2.

Table 6 Statistical analysis that NP contents in muscle at 7d

NP contents	N	N	N1	N1	N1	N2	N2	N2	N3	N3	N3

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	(μg/g)	2	3	M 1	M 2	M 3	M 1	M 2	M 3	M 1	M 2	M 3
N1	0.0894±0.006 2	/	* *	**	**	*						
N2	0.0976±0.011 8		* *				/	/	/			
N3	0.0696±0.007 2									**	**	**
N1 M 1	0.0620±0.002 8				/	**	**			**		
N1 M 2	0.0649±0.002 0					**		**			**	
N1 M 3	0.0776±0.007 0								**			*
N2 M 1	0.0909±0.002 2							**	*	*		

N2 M 2	0.1054±0.009 1					/	/			
N2 M 3	0.1039±0.009 7							/		
N3 M 1	0.1062±0.012 5						/	/		
N3 M 2	0.0888±0.011 3							/		
N3 M 3	0.0971±0.014									

225

226 **Table 7 Statistical analysis that NP contents in muscle at 15d**

NP contents (μg/g)	N 2	N 3	N1 M 1	N1 M 2	N1 M 3	N2 M 1	N2 M 2	N2 M 3	N3 M 1	N3 M 2	N3 M 3

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N1	0.0390±0.0022	*	* *	/	/	*						
N2	0.0430±0.0033		* *				/	/	/			
N3	0.0348±0.0015									/	/	/
N1M1	0.0363±0.0046				/	/	/			/		
N1M2	0.0332±0.0071					/		/			/	
N1M3	0.0349±0.0036								/			/
N2M1	0.0384±0.0048							/	/	/		
N2M	0.0388±0.0034								/		/	

2												
N2M3	0.0383±0.007											/
N3M1	0.0365±0.006									/	/	
N3M2	0.0426±0.008											/
N3M3	0.038±0.0085											

227

228 **Table 8 Statistical analysis that NP contents in muscle at 30d**

NP contents (μg/g)		N2	N3	N1M1	N1M2	N1M3	N2M1	N2M2	N2M3	N3M1	N3M2	N3M3
N1	0.0410±0.006	/	/	*	/	*						

	5									
N2	0.0399±0.0057	/			/	/	/			
N3	0.0333±0.0114							/	/	/
N1M1	0.0263±0.0121		/	/	/			/		
N1M2	0.0357±0.0073			/		/			/	
N1M3	0.0328±0.0048						/			/
N2M1	0.0333±0.0101					/	/	/		
N2M2	0.0448±0.0117						/		/	

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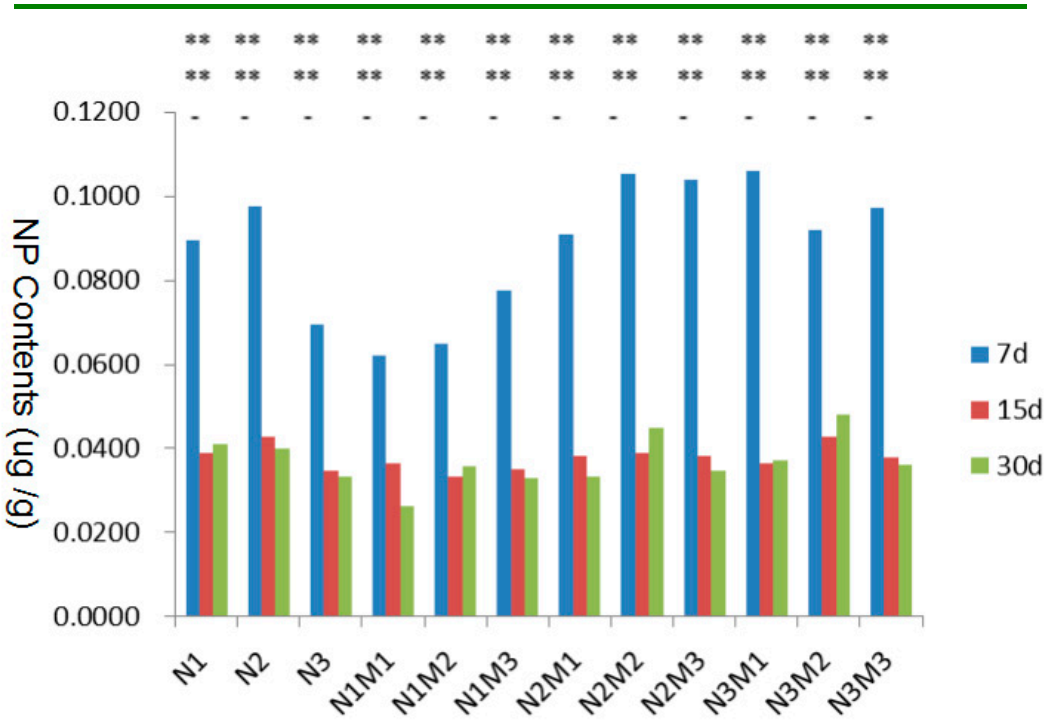
N2
0.0348±0.007
M /
5
3

N3
0.0373±0.006
M / /
3
1

N3
0.0480±0.013
M /
0
2

N3
M 0.0361±0.003
3



229

Figure 2. Statistical analysis of the content variation of NP in muscle at 7d, 15d and 30d.

2.4.3 Variance analysis of NP contents in gill

The NP contents in zebrafish gill at 7d, 15d and 30d and the significance analysis of the means of all experimental groups were demonstrated in table 6, table 7 and table 8, respectively. The content variation of NP in every experimental group at 7d, 15d and 30d were illustrated in figure 3.

Table 9 Statistical analysis that NP contents in gill at 7d

	NP contents (μg/g)	N2	N3	N1M1	N1M2	N1M3	N2M1	N2M2	N2M3	N3M1	N3M2	N3M3
N1	0.0988±0.007	/	*		**	**	**					
N2	0.1090±0.0195		/				*	**	/			
N3	0.1281±0.0122									*	/	/
N1	0.0687±0.004				/	/	*			**		

M	6												
1													
N1													
M	0.0759±0.010 4							/		/		/	
2													
N1													
M	0.0759±0.008 9									**		*	
3													
N2													
M	0.0833±0.010 3									/	/	/	
1													
N2													
M	0.0824±0.004 2									**		/	
2													
N2													
M	0.0932±0.006 1												/
3													
N3													
M	0.0885±0.007 5											/	/
1													

N3	0.0817±0.011											
M												/
	1											
2												
N3	0.0859±0.006											
M												
	2											
3												

239

240 **Table 10 Statistical analysis that NP contents in gill at 15d**

	NP contents (μg/g)	**N2**	**N3**	**N1M1**	**N1M2**	**N1M3**	**N2M1**	**N2M2**	**N2M3**	**N3M1**	**N3M2**	**N3M3**
N1	0.0425±0.007											
	2	/	/	*	/	*						
N2	0.0363±0.006											
	8		/				/	/	/			
N3	0.0398±0.002											
	9									/	/	/
N1	0.0328±0.006											
M	5				/	/	/			/		

1

N1 0.0410±0.007

M 9

2 / / /

N1 0.0316±0.009

M 5

3 / /

N2 0.0361±0.005

M 8

1 / / /

N2 0.0425±0.004

M 9

2 / /

N2 0.0381±0.007

M 2

3 /

N3 0.0334±0.004

M 8

1 / /

N3 0.0378±0.005 /

M 2	1											
N3 M 3	0.0392±0.004 4											

241

242 **Table 11 Statistical analysis that NP contents in gill at 30d**

NP contents (μg/g)		N 2	N 3	N1 M 1	N1 M 2	N1 M 3	N2 M 1	N2 M 2	N2 M 3	N3 M 1	N3 M 2	N3 M 3
N1	0.0414±0.004 8	/	/	*	*	/						
N2	0.0398±0.009 8		/				/	/	/			
N3	0.0430±0.003 6									*	/	/
N1 M 1	0.0309±0.008 6				/	*	/			/		

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N1M2	0.0302±0.0099	/	/			/	
N1M3	0.0400±0.0052			/			/
N2M1	0.0308±0.0054		/	*	*		
N2M2	0.0398±0.0103			/		/	
N2M3	0.0374±0.0035						/
N3M1	0.0378±0.0034					/	/
N3M	0.0385±0.0057						/

2

N3
0.0406±0.007
M
2
3

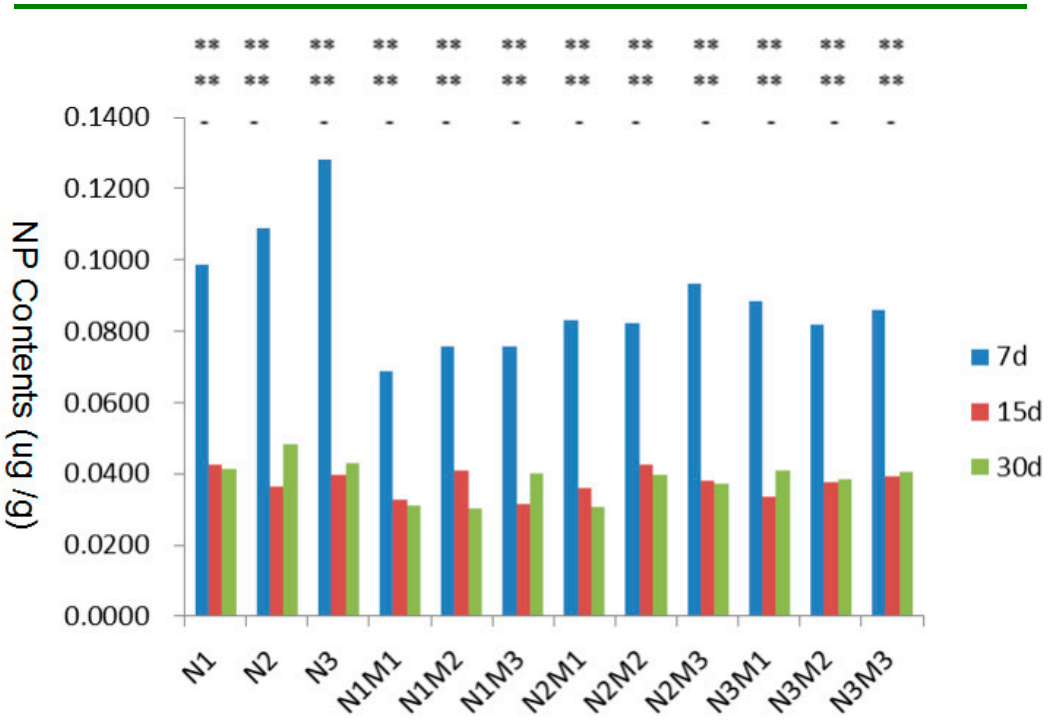

Figure 3. Statistical analysis of the content variation of NP in gill at 7d, 15d and 30d.

2.5 Enzymatic activity determinations

The average enzymatic activity of SOD and GST of zebrafish within aquaculture water group, MMT group and organic solvent group in each time spots were detected and data were analyzed. The results suggested that the MMT and the organic solvent have no effect on the enzyme activity of SOD and GST (Figure 4). In figure 4, we set the liver NP as the horizontal axis, the relative enzymatic activity (The average enzymatic activity of experimental groups/The average enzymatic activity of organic

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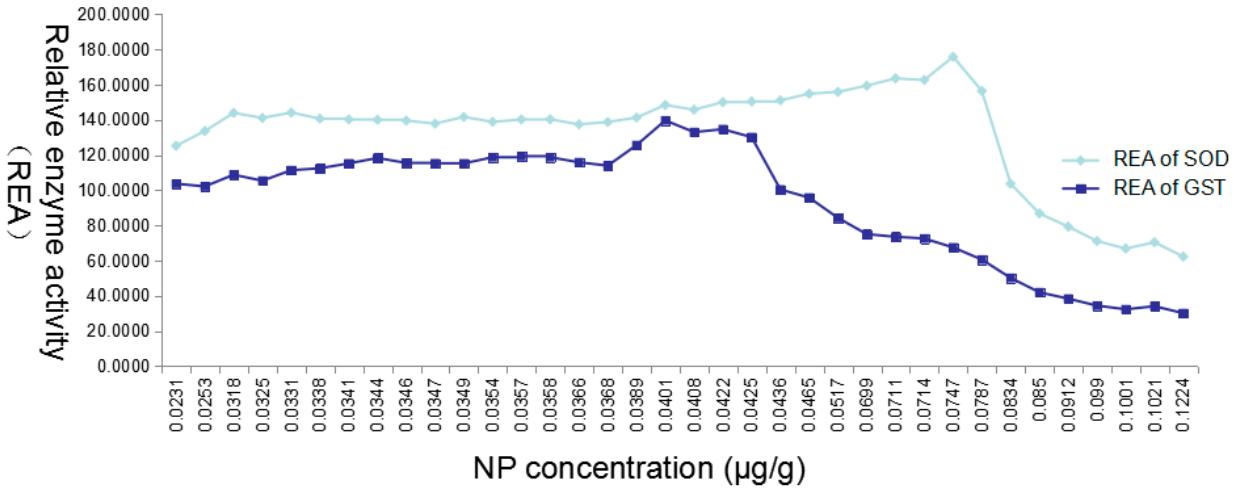
252 solvent group) as the vertical axis.



253

254 Figure 4. The relative enzyme activity (REA) of SOD and GST under the
255 different NP concentrations.

256 **Discussion**

257 The test NP concentrations employed in our study were set as 3.2133 μg/L,
258 which was consistent with the environmental NP concentration, this concentration of
259 NP as well as the NP concentrations of 32.133 μg/L and 321.33 μg/L were used in the
260 experiments respectively. The results showed that the concentrations of NP in the liver,
261 muscle, and gill of zebrafish in all the experimental groups reached a peak at 7d, then
262 decreased on 15d and 30d ($P<0.01$). There were no differences in the concentrations
263 of NP in muscle and gill between 15d and 30d for each group; data from the liver
264 were relatively complicated, i.e., the differences of N2, N3, N1M1, N2M1, N2M3
265 ($P<0.01$) and N1M3 ($P<0.05$) versus the control were significant, while those of the
266 other groups were not. Overall, NP concentrations in the liver, muscle and gill of all
267 the experimental groups were high at first then decreased later, and the declined
268 concentration at late period could be explained by enhanced resistance or

decomposition by the zebrafish. In the absence of MMT, enrichment of NP in the liver at 7d was more significant at a lower dose of NP, namely N1 > N2 > N3 ($P<0.01$), while at 15d and 30d, a higher enrichment effect was observed at higher dose; accumulation of NP in the muscle at 7d and 15d was higher at N1 and N2 than at N3 ($P < 0.01$); while enrichment of NP in gills was not highly correlated to its concentration. In the presence of MMT, enrichment of NP in the liver was significantly decreased at 7d in all N1 groups ($P<0.01$), but such decreased accumulation at N1 was only observed in N1M1 at 15d. Accumulation of NP was enhanced in all N2 and N3 groups except for the N2M1 group ($P<0.01$), but such enhanced accumulation was observed only in the N2M2 group till 30d. In the muscle, enrichment of NP at 7d was reduced by MMT at N1 but increased by MMT at N2 and N3, and the altered enrichment was maintained till 30d only in the N1M3 group. Concentration of NP in gills was influenced by MMT, reduced in all the experimental groups, but the difference was significant in only a few groups. In summary, as long as there was significant difference, higher concentrations of MMT or NP always led to higher accumulation of NP when the other was constant.

It can be speculated from the above-mentioned results that MMT exhibits different enrichment effect on NP in zebrafish. MMT adsorbs NP and reduces the actual concentration of NP by flocculation in water, and this effect is directly reflected in the water-contacted gills, in which the short-term enrichment of NP is reduced by MMT; once NP is ingested by the zebrafish, MMT contributes to the short-term accumulation of NP in the liver at both medium and high doses and in the muscle at

high dose as well, but not to the accumulation of NP in the liver and muscle at low dose. Analysis of experimental data also showed that the effects of MMT on NP enrichment decrease gradually over time. Due to the limitation of this study that only three time points were designed for each experimental group, further research is required to identify the time points when maximum concentration of NP is achieved.

The enzyme activity of liver SOD and GST were affected by the organic NP content. While the concentration of NP is lower than 0.00747 μg/g, the activity of SOD would be induced. By contrast, the activity of SOD would be inhibited when the concentration of NP is higher than 0.00747 μg/g, the inductive and inhibiting effect would be increased with the increase of the concentration of NP; The concentration of NP had a similar effect on the enzyme activity of GST while the critical concentration is 0.0401 μg/g. The induced enzyme activity of SOD could reach 175.82 % compared with the control enzyme activity, while the GST could only reach 139.65 % of the control enzyme activity, which of these results suggested that the SOD is more sensitive to the toxic effect of internal NP, and the NP has a more effective regulatory mechanism on the enzyme activity of SOD.

Conclusion

According to our results, in the short term, MMT could possibly reduce the enrichment of nonyl phenol in gill, and the high concentration of nonyl phenol is benefit of enrich itself in liver and muscle, while the low concentration of nonyl phenol would be against its enrichment. The enzymatic activity of SOD and GST

would exert inductive effect when the concentration of nonyl phenol in liver was reduced, by contrast, the enzymatic activity of SOD and GST would exert inhibiting effect while high-concentration of nonyl phenol was gathered in liver.

Conflict of interest:

The authors declare that they have no conflict of interest.

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