1 **Effects of chronic dietary hexavalent chromium on bioaccumulation and** 2 **immune responses in the sea cucumber *Apostichopus japonicus***

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6 **ABSTRACT**

7 Sea cucumbers *Apostichopus japonicus* (3.54 ± 0.01 g of wet weight) were exposed
8 to five concentrations of dietary hexavalent chromium [0 (control), 100, 200, 400, and
9 800 mg Cr⁶⁺/kg dry weight] amended with K₂Cr₂O₇ for 30 days. The bioaccumulation
10 and immune responses [antioxidant enzymes: superoxide dismutase (SOD) and
11 catalase (CAT); hydrolytic enzymes: acid phosphatase (ACP) and alkaline
12 phosphatase (AKP)] of sea cucumbers were subsequently evaluated. This study found
13 that the order of Cr accumulation in the experimental tissues was respiratory tree >
14 intestine > body wall. Significantly lower SOD activities occurred in the 400 mg/kg
15 group compared to that in the control group. Higher dietary Cr⁶⁺ exposure (400 and
16 800 mg Cr⁶⁺ /kg dry weight) did not negatively alter the CAT activities, but
17 significantly inhibited CAT activities in 100 mg/kg group, compared to control group.
18 ACP activities in groups 200, 400 and 800 mg/kg were significantly lower than those
19 in control group, while no significant differences occurred in AKP activities among
20 groups. The present study provides important information into the bioaccumulation
21 and immune responses of the sea cucumber *A. japonicus* in response to chronic
22 dietary Cr⁶⁺ exposure.

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24 **Keywords:** Hexavalent chromium; *Apostichopus japonicus*; bioaccumulation;
25 antioxidant enzymes; hydrolase

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51 1. Introduction

52 Chronic contamination by heavy metals is one of the most important issues in
 53 aquatic environment because they invariably remain in the environment (Ashraf,
 54 2005). In this context, chromium (Cr) has been well documented that causing health
 55 concerns and environmental pollution around the world (Dhal et al., 2013). Cr has
 56 different valence states (II–VI), and its chemical properties and toxicity vary with
 57 these valence states. Hexavalent chromium (Cr^{6+}) is one of the most harmful
 58 pollutants listed by the United States' Environmental Protection Agency (US EPA)
 59 (Jiang et al., 2019; Tang et al., 2021), because it induces negatively impacts at cellular,
 60 molecular and ecosystem (Olibet et al., 1995). Nowadays, Cr^{6+} is widely applied by
 61 many industries, such as dyeing, fungicide, ceramic glass and refining (Zahoor and
 62 Rehman, 2009). Serious pollution of Cr^{6+} occurred in aquatic environment due to
 63 improper disposal of these industrial wastes (Ashraf and Hanfiah., 2017; Tang et al.,
 64 2021). Using Cr^{6+} as the filling materials in the junkyard further makes it more easily
 65 to penetrate groundwater (Domingues et al., 2010). The concentrations of Cr^{6+} in
 66 aquatic environment (12 mg/L), for example, are much higher than the national
 67 standards in India (100 $\mu\text{g/L}$) (Sharma et al., 2012; Awasthi et al., 2018).

68 Sea cucumber *Apostichopus japonicus* is deposit animal (Ru et al., 2019) and like
 69 to habit seabed rich in algae and sea mud because of the abundant organic matters,
 70 such as benthic diatoms, bacteria and organic debris of animals and/or plants (Jiang et
 71 al., 2013; Yokoyama, 2013). The habits of sea cucumbers lead them to be more
 72 vulnerable to the heavy metals in sediments (Jiang et al., 2015). Sun et al. (2007)
 73 documented that Cr^{6+} in water displayed acute toxicity on *A. japonicus*, and the LC_{50}
 74 of 24 h, 48 h and 72 h were 31.974, 7.499 and 3.808 mg/L, respectively. Significantly
 75 lower growth rate occurred when sea cucumbers *A. japonicus* were fed Cr^{6+} diet after

a short period (10 days) (Li et al., 2019). However, the bioaccumulation and immune of *A. japonicus*, which are the most important biological responses, have not been fully studied when they were exposed to chronic dietary Cr^{6+} exposure.

Here, sea cucumbers were chronic exposed to five concentrations of dietary Cr^{6+} [0 (control), 100, 200, 400, and 800 mg Cr^{6+} /kg dry weight] for 30 days to investigate: 1) How the dietary Cr^{6+} accumulates in different tissues (body wall, intestine, respiratory tree) of *A. japonicus*; 2) whether dietary Cr^{6+} affects the immune responses of *A. japonicus*.

2. Materials and methods

2.1 Experimental diets

Five formulated diets containing different levels of Cr^{6+} (0, 100, 200, 400, 800 mg Cr^{6+} /kg dry weight) amended with $\text{K}_2\text{Cr}_2\text{O}_7$ (Wuxi Hongxing Chemical Industry Factory, Wuxi, China) were used in the present study (Table 1). The actual Cr concentrations were 19.87, 139.40, 243.70, 359.00, 930.60 mg Cr /kg dry weight, respectively. Dry ingredients were mingled (Xindou Food Machinery Factory, Jiangmen, China) with freshwater at a ratio of 600 g/kg (Wang et al., 2015). They then were shaped into pellets (1.88 mm of test diameter) using a pellet mill (Pinzheng Equipment Co. Ltd., Changzhou, China). All diets were subsequently dried at 40 °C in a hot air oven (Jinghong Test Equipment Co. Ltd., Shanghai, China) for 24 h and then stored at -20 °C for the following experiment.

2.2 Sea cucumber and experimental design

Eight hundred juvenile sea cucumbers (~3 g) were transported to Dalian Ocean University (121°56' N, 38° 87' E) from Dalian Yinharma Aquatic Products Co., Ltd (121°54' N, 39°38' E) on 26 October 2020. They were maintained in a 250-L tank (length × width × height: 76 × 55 × 60 cm) with aeration and fed control diet for two

101 weeks to acclimatized the lab environment. Sea cucumbers were fasted for 24 h
102 before the experiment began on 6 November 2020.

103 Formulated diets with different concentrations of Cr^{6+} were the experimental
104 factors. Each group has three replicates and the experiment duration lasted for 30 days
105 (from 6 November 2020 to 5 December 2020). Twenty-five sea cucumbers ($3.54 \pm$
106 0.01 g) were randomly selected and placed into each of 15 plastic tanks (length $\times$
107 width $\times$ height: $50 \times 35 \times 29$ cm) with aeration according to the experimental design.
108 Sea cucumbers were fed the corresponding diets at 3% of body weight once a day at
109 17:00 during the experiment (Wang et al., 2015; Li et al., 2018). These diets were not
110 dissolved within 24 hours. One-half of seawater was exchanged every day. Water
111 temperature, salinity, and pH were measured weekly by a portable water quality meter
112 (HACH, HQ40D, USA). They were 14.73 ± 0.26 °C, 31.2 ± 0.13 ‰ and 7.92 ± 0.01 ,
113 respectively. Five sea cucumbers from each tank were randomly chosen before they
114 were starved for 24 h. The coelomic fluid, body wall, intestine, and respiratory tree of
115 sea cucumbers were obtained and kept at -80 °C for the further analyses of enzyme
116 activity of coelomic fluid and Cr concentrations in tissues (body wall, intestine and
117 respiratory tree) ($N = 3$).

118 *2.3 Growth performance*

119 The final body weight (FBW), body weight gain (BWG), specific growth rate
120 (SGR), and survival rate (SR) were assessed at the end of the experiment on 5
121 December 2020 ($N = 3$). Growth parameters were calculated according to Wang et al
122 (2016): $\text{BWG (\%)} = 100 \times (\text{final mean body weight} - \text{initial mean body weight}) /$
123 $\text{initial mean body weight}$; $\text{SGR (\%/day)} = (\ln \text{final mean body weight} - \ln \text{initial}$
124 $\text{mean body weight}) / \text{feeding days}$; $\text{SR (\%)} = 100 \times (\text{final sea cucumber number}) /$
125 $(\text{initial sea cucumber number})$.

126 2.4 Chromium concentration in sea cucumber tissues

127 The Cr⁶⁺ concentration in experimental tissues was analyzed on days 10, 20 and 30
128 according to Kim and Kang (2016) as follows. The 65% HNO₃ (v/v) was added to
129 digest these tissues at 120 °C and the digested solution was further diluted with 2%
130 HNO₃ (v/v) to make ~25 mL liquid following a filtration using a membrane filter (0.2
131 µm). The Cr concentration in these tissues was determined using atomic absorption
132 spectrometer (Perkin Elmer Instruments Shanghai Co. Ltd. Shanghai, China) and
133 expressed as mg/kg wet weight.

134 2.5 Biochemical analysis

135 The enzymes activities were measured at the end of the experiment following the
136 kit introductions (Nanjing Jiancheng Bio-engineering Institute, Nanjing, China). The
137 superoxide dismutase (SOD) was measured according to Marklund and Marklund
138 (1974). The activities of catalase (CAT) were analyzed following Góth (1991). The
139 levels of malondialdehyde (MDA) were quantified according to Scown et al. (2010).
140 The activities of acid phosphatase (ACP) and alkaline phosphatase (AKP) were
141 determined by colorimetric method (Malamy and Horecker, 1966).

142 2.6 Statistical analyses

143 The differences of SOD activities were analyzed using Kruskal-Wallis H. One-way
144 ANOVA was performed to analyze the differences of all remaining data followed by
145 the LSD multiple comparison. All data were expressed as mean ± S.E and analyzed
146 using SPSS software version 19.0 (SPSS, Chicago, IL, USA). A value of $P < 0.05$
147 was considered significant.

148 3. Results

149 3.1 Growth performance

150 Significantly lower BWG and SGR occurred in 800 mg/kg group than those in
151 control group after 30-day Cr⁶⁺ exposure ($P < 0.05$, Table 2). However, there was no
152 significant difference in SR among groups ($P > 0.05$, Table 2).

153 3.2 Chromium concentration in tissues

154 Cr accumulation was found in body wall (Fig. 1A), intestine (Fig. 1B) and
155 respiratory tree (Fig. 1C) of sea cucumbers during the experiment. The order of Cr
156 accumulation in tissues was respiratory tree > intestine > body wall after 30 days of
157 dietary Cr⁶⁺ exposure. The Cr burden in respiratory tree was ~6 times greater than that
158 in the body wall on day 30.

159 The Cr accumulation in body wall increased with concentration increasing. The 800
160 mg/kg group showed significantly higher Cr burden in body wall on days 10 ($0.74 \pm$
161 0.03 mg/kg), 20 (0.85 ± 0.11 mg/kg) and 30 (1.58 ± 0.02 mg/kg), compared to that in
162 other groups ($P < 0.05$, Fig. 1A).

163 The Cr burden in intestine was significantly greater in groups 400 mg/kg ($3.81 \pm$
164 0.77 mg/kg for day 10, 3.93 ± 0.72 mg/kg for day 20 and 3.37 ± 0.41 mg/kg for day
165 30) and 800 mg/kg (5.69 ± 0.98 mg/kg for day 10, 5.57 ± 1.87 mg/kg for day 20 and
166 6.55 ± 0.64 mg/kg for day 30) than that in control group on days 10, 20 and 30 ($P <$
167 0.05 , Fig. 1B).

168 Significantly higher Cr burden of respiratory tree was found in groups 200 mg/kg
169 (2.30 ± 0.01 mg/kg for day 10, 3.04 ± 0.98 mg/kg for day 20 and 5.09 ± 0.01 mg/kg
170 for day 30), 400 mg/kg (4.09 ± 0.03 mg/kg for day 10, 3.99 ± 0.21 mg/kg for day 20
171 and 7.45 ± 0.34 mg/kg for day 30) and 800 mg/kg (2.56 ± 0.01 mg/kg for day 10, 5.65
172 ± 0.63 mg/kg for day 20 and 10.24 ± 1.95 mg/kg for day 30) than that in control
173 group during the experiment ($P < 0.05$, Fig. 1C).

174 3.3 Antioxidant enzyme activities

175 Significantly lower SOD activity occurred in 400 mg/kg group (129.47 ± 1.35
176 U/mL) than that in control group (156.94 ± 8.21 U/mL) ($P \leq 0.05$, Fig. 2A). The
177 CAT activities in control group (13.91 ± 0.45 U/mL) were significantly higher than
178 those in groups 100 mg/kg (7.05 ± 2.04 U/mL) and 200 mg/kg (10.06 ± 0.74 U/mL)
179 (both $P \leq 0.05$, Fig. 2B). Significantly better MDA levels were found in 800 mg/kg
180 group (0.67 ± 0.14 n mol/mL) than those in control group (0.36 ± 0.05 n mol/mL) (P
181 ≤ 0.05 , Fig. 2C)

182 *3.4 Hydrolytic enzymes activities*

183 The ACP activities in control group (3.15 ± 0.02 U/100 mL) were significantly
184 higher than those in groups 200 mg/kg (2.72 ± 0.06 U/100 mL), 400 mg/kg ($2.28 \pm$
185 0.07 U/100 mL) and 800 mg/kg (2.19 ± 0.02 U/100 mL) ($P < 0.05$, Fig. 3A). There
186 were significantly higher ACP activities in 200 mg/kg group than those in groups 400
187 mg/kg and 800 mg/kg ($P < 0.05$, Fig. 3A). The AKP activities were not significant
188 difference in all groups ($P \leq 0.05$, Fig. 3B).

189 **Discussion**

190 Heavy metals are persistent contamination with highly toxic, causing various
191 negative impacts on animals in aquatic ecosystems (Yin et al., 2020). The emission of
192 Cr^{6+} in wastewater, for example, is 24, 844 kg in China in 2017 (Ning, 2020). It is
193 necessary to investigate the effects of chronic dietary Cr^{6+} on bioaccumulation,
194 immunity responses in sea cucumbers *A. japonicus*, especially considering its high
195 commercial and ecological value (Ru et al., 2019).

196 *4.1 Chromium concentration in tissues*

197 The bioaccumulation patterns of animals contribute to evaluating environmental
198 metal pollution (Kim et al., 2004). Metal accumulation in aquatic animals (e.g fishes)
199 either directly uptake from the water using their gills or indirectly absorb from diets

by intestine (Oost et al., 2003). In this context, dietary exposure is considered as the main cause of metal accumulation in fishes (Hall et al., 1997; Farag et al., 1999) and the intestine is therefore the primary target of metal accumulation (Bernhoft, 2012). For example, Li et al. (2018) observed that the profile of mercury accumulation in tissues was intestine > respiratory tree > body wall. However, the present study showed that the order of Cr accumulation in the experimental tissues was respiratory tree > intestine > body wall, suggesting respiratory tree accumulated the highest concentration of Cr. Respiratory tree of sea cucumbers is similar to gill of fish (Wang et al., 2015), which plays important role in detoxification, gaseous exchange and ion regulation (Goss et al., 1998). Accumulation of Cr in the gills probably is caused by the switch of dietary Cr to the basolateral cell membranes of the gill cells through the bloodstream (Szebedinszky et al., 2001). Wang et al. (2015) documented that the order of tissues Pb accumulation was as follows: body wall > intestine > respiratory tree when *A. japonicus* fed dietary Pb with different levels (100, 500 and 1000 mg Pb/kg dry weight) for 30 days. Li et al. (2019) found that the profile of Cr accumulation in tissues was body wall > intestine after *A. japonicus* was exposed to dietary chromium (58.27 mg Hg/kg dry weight) for 10 days. Together with these previous results, the present study further suggests that the Cr accumulation in tissues is probably related to the exposure time and ability of metal accumulation in each tissue.

4.2 Immunity responses

Cr^{6+} enters a cell with the help of sulphur anion transport system (Holland and Avery, 2011) and becomes more stable form (e.g. Cr^{3+}) by cellular reductants (Krumshabel and Nawaz, 2004). Various chemicals, including hydroxyl radicals ($\text{OH}^\cdot$), superoxide ($\text{O}_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), increasing the levels of lipid

225 peroxidation in this process (Vlahogianni et al., 2007). In other words, Cr^{6+} exposure
 226 probably directly facilitates the production of reactive oxygen species (ROS) via the
 227 Fenton-like cycling mechanism (Sinha et al., 2006) and/or indirectly encourages
 228 oxidative stress (OS) by interacting with mitochondria (Pourahmad et al., 2003). ROS
 229 attacks cellular constituents (Li et al., 2010; Sturve et al., 2008), alters the defense
 230 processes and the antioxidant enzymes activities in cells (Jindal and Handa, 2019).
 231 The present study found that significantly higher MDA levels occurred in 800 mg/kg
 232 group than those in control group. High MDA levels destroy physiologic integrity by
 233 decreasing the resistance and fluidity in cytomembrane (Chandran et al., 2005). These
 234 results are consistent with Lushchak et al. (2009), who found that MDA levels
 235 increase with the increase of exposed Cr^{6+} concentration (Kubrak et al., 2010) in
 236 goldfish, indicating that sea cucumbers were suffering from oxidative stress in
 237 relation to ROS generation (Ahmad et al., 2006).

238 The antioxidant defense system (e.g. SOD and CAT) can neutralize the ROS and
 239 consequently protect cells from adverse variation (Ritola et al., 2002; Kubrak et al.,
 240 2010). The SOD is considered as the first line against oxygen toxicity in the cells (Li
 241 et al., 2010) because it catalyzes the decomposition of superoxide anions into H_2O
 242 and H_2O_2 (Shao et al., 2012). The present study found that significantly lower SOD
 243 activities occurred in 400 mg/kg group than those in the control group. Significantly
 244 decline in SOD activity has also been observed after fishes *Labeo rohita* were
 245 exposed to dietary Cr^{6+} (Kumari et al., 2014). Long-term exposure of Cr^{6+} increases
 246 the substrate levels (superoxide radicals) of SOD, whereas SOD activity decreased
 247 with the increase in its substrate levels in this study, indicating that antioxidant
 248 defense probably has been impaired in sea cucumbers. In terms of CAT, as an
 249 important enzymatic scavenger (Chen et al., 2015), removing the H_2O_2 by catalyzing

its decomposition. The present study found that significantly lower CAT activities were found in groups 100 and 200 mg/kg than those in control group. Similar findings also showed that CAT activity decreased significantly in kidney and liver tissues of fishes *Cyprinus carpio* (Kumar et al., 2013) and goldfish (Lushchak et al., 2009). Earlier studies suggested that the overproduction of superoxide anion caused by Cr^{6+} inhibited the CAT activities (Pandey et al., 2001). The glucose-6-phosphate dehydrogenase activity, which is essential for the conservation of CAT activity, probably also affects the CAT activities (Scott et al., 1991). Further, we found that CAT activities in groups 400 and 800 mg/kg increased significantly compared to those in the 100 mg/kg group, while they were not significantly different from those in control group. The interaction between CAT and Cr^{6+} changes the structure of substrate (H_2O_2), leading the substrate to more easily reach the enzyme (Zhang et al., 2016). These results suggest higher dietary Cr^{6+} exposure (at least 400 and 800 mg/kg) did not negatively alter the CAT activity, although significantly inhibited CAT activity occurred in lower dietary Cr^{6+} exposure (e.g 100 mg/kg).

It has been well documented that dietary Cr^{6+} exposure influenced immune responses in various animals (Glaser et al., 1985; Arunkumar et al., 2000), including the macrophage functions (Tian and Lawrence 1996), distribution of leukocytes (Arunkumar et al., 2000) and the proliferation of lymphocytes (Wang et al., 1996). The present study further found that ACP activities decreased significantly in groups 200, 400 and 800 mg/kg than those in control group, while no significant differences in AKP activities were found among groups. Jiang et al. (2010) consistently found that significantly decline of ACP occurred in the mud crabs *Scylla paramamosain* after dietary Cr^{6+} exposure. ACP is one of the phosphatases attaching the phosphate groups from other molecules (Cajaraville et al., 2000). It functions when the lysosome

fuses with the nuclear endosome (Rajalakshmi and Mohandas, 2005). AKP plays an important role in catalyzing the hydrolysis of phosphate monoesters (Zang et al., 2012). They are both involved in the degradation of lipids and carbohydrates (Ottaviani, 1984; Xue and Renault, 2000). This study found Cr^{6+} displayed an inhibitory effect on ACP activities but not on AKP activities, which probably explains why the nonspecific immune responses were suppressed in fishes *Salmo trutta* L. and *Cyprinus carpio* L. when they were experienced Cr^{6+} exposure (O'Neill, 1981). Collectively, the present study found that high dietary Cr^{6+} exposure (200, 400 and 800 mg Cr^{6+} /kg dry weight) probably impairs the immune function of sea cucumbers *A. japonicus*.

285 Declaration of competing interest

286 The authors declare no competing interests.

287 Ethical Approval

288 All applicable international, national, and/or institutional guidelines for the care and
289 use of animals were followed by the authors.

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Table 1 Ingredients composition and nutrient levels of experimental diets (g/kg)

Ingredients	0 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
Fish meal	60	60	60	60	60
Soybean meal	100	100	100	100	100
<i>Scagassum</i>	300	300	300	300	300
<i>Laminaria japonica</i>	200	200	200	200	200
Sea mud	200	200	200	200	200
Yeast	40	40	40	40	40
Flour	50	50	50	50	50
Vitamin mixture ^a	10	10	10	10	10
Mineral mixture ^b	10	10	10	10	10
Guar gum	20	20	20	20	20
Microcrystalline cellulose	10	9.72	9.43	8.87	7.74
K ₂ Cr ₂ O ₇ ^c	0	0.28	0.57	1.13	2.26
Total	1000	1000	1000	1000	1000
Analyzed nutrients (% in dry basis)					
Moisture	5.64	5.29	6.62	5.18	6.68
Ash	38.33	38.10	38.37	38.94	38.40
Crude protein	15.15	14.87	15.17	14.19	14.73
Crude lipid	6.07	5.58	5.42	6.17	5.26

^a Vitamin mixture (per kilogram of premix): vitamin A, 1,000,000 IU; vitamin D₃, 300,000 IU; vitamin E, 4,000 IU; vitamin K₃, 1,000 mg; vitamin B₁, 2,000 mg; vitamin B₂, 1,500 mg; vitamin B₆, 1,000 mg; vitamin B₁₂, 5mg; nicotinic acid, 1,000 mg; vitamin C, 5,000 mg; Ca pantothenate, 5,000 mg; folic acid, 100 mg; inositol, 10,000 mg; carrierglucose and H₂O ≤ 10%.

^b Mineral mixture (0.025 mg/g of premix): NaCl, 107.79; MgSO₄·7H₂O, 380.02; NaHPO₄·2H₂O, 241.91; KH₂PO₄, 665.20; Ca(H₂PO₄)·2H₂O, 376.70; Fe citrate, 82.38; Ca lactate, 907.10; Al(OH)₃, 0.52; ZnSO₄·7H₂O, 9.90; CuSO₄, 0.28; MnSO₄·7H₂O, 2.22; Ca(IO₃)₂, 0.42; CoSO₄·H₂O, 2.77.

^c potassium dichromate (K₂Cr₂O₇)

539 **Table 2** Effects of dietary Cr⁶⁺ on the growth performance of sea cucumbers

540 *Apostichopus japonicus*.

Index	0 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	800 mg/kg
IBW (g)	3.53±0.01	3.55±0.02	3.55±0.03	3.54±0.01	3.53±0.01
FBW (g)	6.25±0.14 ^a	5.69±0.62 ^{ab}	5.15±0.63 ^{ab}	4.5±0.64 ^b	4.48±0.23 ^b
BWG (%)	77.12±3.56 ^a	59.96±16.93 ^{ab}	45.51±18.63 ^{ab}	40.79±19.84 ^{ab}	26.64±6.08 ^b
SGR (%/day)	1.9±0.07 ^a	1.53±0.34 ^{ab}	1.19±0.45 ^{ab}	1.11±0.47 ^{ab}	0.78±0.16 ^b
SR (%)	100.00±0	94.67±5.33	100.00±0	97.33±2.67	100.00±0

541 Data are expressed as mean ± S.E. (N = 3). Different letters refer to $P < 0.05$. IBW:

542 initial body weight; FBW: final body weight; BWG: body weight gain; SGR: special

543 growth rate; SR: survival rate.

544 **Figure legends**

545

546 **Fig. 1.** The Cr content in the body wall (A), intestine (B) and respiratory tree (C) of
 547 sea cucumbers *Apostichopus japonicus* when they were exposed to Cr⁶⁺ at different
 548 periodsof the experiment (mean ± S.E.). Data at the same exposure times without
 549 common letters are considered as $P \leq 0.05$.

550

551 **Fig. 2.** The enzyme activities of SOD (A), CAT (B), MDA (C) of *Apostichopus*
 552 *japonicus* at the end of experiment (mean ± S.E.). Different letters refer to $P < 0.05$.

553

554 **Fig. 3.** The enzyme activities of ACP (A) and AKP (B) of *Apostichopus japonicus* at
 555 the end of experiment (mean ± S.E.). Different letters refer to $P < 0.05$.