Effects of Microplastic Combined with Cr(Ⅲ) on Apoptosis and Energy Pathway of Coral Endosymbiont

Chengyong Li (cyli@gdou.edu.cn)
Shenzhen Institutes of Guangdong Ocean University https://orcid.org/0000-0003-0018-165X
Baohua Xiao
Shenzhen Institutes of Guangdong Ocean University
Dongdong Li
Shenzhen Institutes of Guangdong Ocean University
Baolin Liao
Shenzhen Institutes of Guangdong Ocean University
Huina Zheng
Shenzhen Institutes of Guangdong Ocean University
Xiaodong Yang
Shenzhen Institutes of Guangdong Ocean University
Yongqi Xie
Shenzhen Institutes of Guangdong Ocean University
Ziqiang Xie
Shenzhen Institutes of Guangdong Ocean University

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Abstract

The combined effect of polyethylene (PE) microplastic and chromium (Cr(III)) was investigated on scleractinian coral *Acropora pruinosa* (*A. pruinosa*). The endpoints analyzed in this study included the density of endosymbiont, chlorophyll a + c content, activity of enzymes involved in apoptosis (caspase-1; caspase-3), glycolysis (lactate dehydrogenase, LDH), pentose phosphate pathway (glucose-6-phosphate dehydrogenase, G6PDH) and electron transfer coenzyme (nicotinamide adenine dinucleotide, NAD^+/NADH). During the 7-day exposure to PE and Cr(III) stress, the density of endosymbiont and the chlorophyll content were decreased gradually. Caspase-1 and-3 activities were increased in the high concentration Cr(III) exposure group. Furthermore, the LDH and G6PDH activities were decreased significantly, and the ratio of NAD^+/NADH was decreased significantly. In summary, the results showed that PE and Cr(III) stress had inhibited the energy metabolism enzymes of *A. pruinosa*, and further led to the apoptosis of endosymbiont in coral. In addition, the combination of stressors exposure revealed as the concentration of Cr(III) keep at 10 μg/L, the toxic effects of heavy metals on endosymbiont was temporarily relieved with elevated PE In contrary, coral polyps were kept under the concentration of PE at 5 mg/L and increasing Cr(III), the metabolic activities of corals were seriously disturbed, which was increased the burden of energy consumption. In the short term, the deleterious effects of Cr(III) is more obvious due to it led to the apoptosis of endosymbiont and its irreversible damage. It is the first time to give insights into the combined effect of microplastic and Cr(III) stress on the apoptosis and energy pathway of coral endosymbiont. The results suggest that microplastic combined with Cr(III)contamination is an important factor affecting apoptosis and energy metabolism of coral *A. pruinosa*.

Introduction

Coral reef is one of the most important ecosystems in global owning to its abundant biodiversity and high primary productivity (Mooney et al., 2009). In recent years, the dual effects of natural and human factors have made coral reef ecosystems increasingly degraded, and many coral species are on the verge of extinction (Li et al., 2017; Hugdes et al., 2013). According to the latest marine environmental quality bulletin, the coral reef systems in China are in sub-health state. In the past five years, it has shown a more significant degradation trend, and the coral coverage has dropped to 16.8% by 2015 (State Oceanic Administaration 2015). Currently, the effects of toxic and harmful substances on coral reef ecosystem have attracted extensive attention because coral reef ecosystem is facing the joint toxicological effects of microplastics, heavy metals, persistent pollutants, antibiotics and pathogens (Wardrop et al., 2016; Tanaka et al., 2013). Significantly, heavy metals have a serious interference effect on the growth of marine organisms, and trace concentrations of heavy metals can lead to poisoning of organisms (Gissi F et al., 2019; Bielmyer et al., 2010; Nyström M et al., 2010).

Although heavy metal pollution has received widespread concern, there is still about 1.79×10^7 tons of multitudinous heavy metals imported into the ocean every year (Su et al., 2013). Heavy metals in seawater can be absorbed by corals resulting in toxicity. For example, Cu, Zn and Ni were shown to

exhibit toxicological injury to coral growth and its calcification (Dam et al., 2011; Rodriguez et al., 2016). In detail, the effects of heavy metals on the growth of corals are mainly manifested in the serious inhibition of their metabolism. It not only leaded to the damage of DNA, but also caused the genetic material mutation of organisms, and finally it resulted in slow growth and abnormal symptoms (Jones et al., 2004; Zhou et al., 2017). Heavy metals can decrease the survival rate of biological larvae, and it affected the diversity of marine organisms, which is a major threat to the marine ecosystem (Hudspith et al., 2013; Horwitz et al., 2014). Thompson's and Reichelt's results showed that as the concentration of heavy metals was increased, the growth of symbiotic algae in corals was inhibited. Meanwhile, the concentrations of Cu(II), Zn(II) and Cd(II) affected the survival rate of gametophyte fertilization, and it restrained the growth process of corals (Thompson et al., 1971; Reichelt-Brushett et al., 1999). In addition, the previous report showed that the increase of heavy metals would exert a certain inhibition effect on the development of coral larvae into corals, it leaded to the deepening of synergistic toxicity (Reichelt-Brushett et al., 1999). Endosymbiont was dissociated in the tissue of corals, which significantly reduced the growth rate and calcification rate of corals, thus it cause serious damage to the reconstruction process of coral reefs (Sabdono et al., 2009).

The degradation rate of plastics in the natural environment is very low. In the environment, the weathering processes will immediately begin to reduce the stability of polymers, resulting in increased surface roughness, shape change, fragmentation and chemical composition of plastics (Lebreton et al., 2017; Wright et al., 2013; Cooper and corcorcoran, 2010). These changes may enhance the adsorption of harmful substances from the surrounding environment. (Carbery et al., 2018). The adsorption process of heavy metals by microplastic particles occurs through a complex mechanism. It is found that the surface properties of plastic particles are changed and the active binding sites of various metal ions are formed due to the precipitation of inorganic minerals and organic matter on the surface of plastic particles (Rochman, C.M et al., 2014.). Several studies have described heavy metals are adsorbed on the surface of microplastics by electrostatic or complexation, and microplastics as a carrier can transport heavy metals to remote locations, which increased the ecotoxicity of heavy metals (Holmes et al., 2014; Wen et al., 2018). Lu's research showed that polystyrene microplastic enhanced the toxicity of Cd to zebrafish in a certain extent, as well as it resulted in oxidative damage and inflammation in zebrafish by combination exposure of polystyrene microplastic and Cd (Lu et al., 2018). Therefore, as microplastics pollution continues to increase, coral reef ecosystems exposure to microplastics and heavy metals may reach high levels in the future; It was potentially reducing coral resilience to environmental change, and also it was making ecosystems more vulnerable (Rodriguez et al., 2016; Lajeunesse et al., 2018). Since the contact of marine organisms with microplastics containing metal additives or metals adsorbed on their surfaces may have a negative impact (Gallo et al., 2018), it is of great significance to study the trace metal concentration of metals associated with microplastics in the coral (*A. pruinosa*).

At present, There is a knowledge gap on the physiological effects of Cr(III) and microplastics combined on corals. It is urgent to analyze the physiological changes of corals responding to microplastic and Cr(III) stress. In this study, *A. pruinosa*, an important species in the South China Sea, was used to study the response of microplastic and Cr(III) combination to coral stress in laboratory. Physiological

characteristics were detected including chlorophyll content, endosymbiont, apoptosis related protein enzyme, energy metabolism pathway enzyme activity and other indicators. The overall goals of this study were to determine sensitivity and physiological response of coral to PE and Cr(III) exposure and to elucidate the mechanism of PE and Cr(III) toxicity in *A. pruinosa*, which is of great significance for the protection of marine biological resources.

Materials And Methods

Materials and chemical reagents

Polyethylene (PE) is one of the most commonly used plastics globally, and commonly seen in packaging. PE was selected because it is a common plastic polymer used in microplastics sampling in marine and beaches (Ding et al., 2019; Jensen et al., 2019). Polyethylene microplastic was purchased from Yineng Platic Materials CO., Ltd. (Dongguan, China). Polyethylene suspension was prepared by ultraviolet disinfection of aerated seawater. The composition of polyethylene was confirmed by Raman spectroscopy (RS, SR-510 Pro, Ocean optics Asia, 785 nm laser, Raman shift 50-3500 cm^{-1}), and it showed in Fig S1. Analytically pure acetone (AR 99.9% purity), chromium chloride hydrate of analytical grade (2:5) (99.0% purity) were purchased from Macklin Chemical Co., Ltd. (Shanghai, China). In filtered sea water, 100 μg/L and 50 μg/L Cr(III) stock solutions were prepared. Assay kits for measurement of levels of NDA^{+}/NADH, G6DPH, LDH, Caspase-3 and Caspase-1 were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). BCA protein assay kit was offered by Beyotime Institute of Biotechnology (Jiangsu, China). The seawater used in the whole experiments was artificial.

The corals collection and treatment

Corals

The colonies of stony corals were collected from a coral reef in Nan'ao Bay, Guangdong Province, China, transferred and cultured in an in flow-through aquaria (ca. 200 L) filled with seawater in a facility located at the Shen Zhen Institute of Guangdong Ocean University. The branches in the colonies were split as nubbins (long 3-5 cm), they were attached to the ceramic matrix bases with two-component glue, and 300 nubbins were generated in total. In laboratory, the light source was Chihiros LED lighting system (21 W), and 12 hours of light was given every day from 06:00 to 18:00, with a light 70 ± 10 μmol quanta m^{-2} s^{-1} cycle for one year, and the sea water was regularly renewed to ensure the nutrition supply. All corals were not fed with any exogenous food during the experiment. The temperature of seawater for culture was controlled at 23-24 ℃, and the salinity was about 35.0 ± 0.2 ppt.

Stress experimental design

After the nubbins were transferred into the acrylic laboratorial aquariums (15 L), the growth status was recorded regularly, and the stress experiment was started when the nubbins condition was stable. In the experiment, except for stressors, the experimental environment was consistent with the conditions of adaptation period. 30 acrylic aquariums were used to store coral nubbins (3-5 cm in length), including 6 as the control group and 24 as the experimental group, and the growth of coral in this environment was monitored in real time. 240 nubbins were used for PE and Cr(III) stress experiments (8 nubbins per tank). In detail, PE-MP were added into filtered seawater to prepare seawater with the concentrations of 2 mg/L, 10 mg/L and 20 mg/L, respectively. Similarly, chromium chloride ($CrCl_3$) was added into filtered seawater to prepare Cr(III) seawater with final concentration of 2 μg/L, 10 μg/L and 20 μg/L, respectively. The detailed operation is shown in Fig.S2. Then, 96 nubbins were transferred to the seawater with elevated Cr(III), hereinafter referred to as Cr(III) group. Another 96 of the coral nubbins were transferred to the seawater with elevated PE, hereinafter referred to as microplastic group. The other 48 coral nubbins were served as control group, which were incubated only in filtered seawater. There were 3 biological replicates in each treatment group. In addition, The seawater in all tanks was replaced once every 24 h with freshly filtered seawater from the coral culture system to ensure a suitable aquaculture water environment, and new PE and Cr(III) were also added at the same time. After 24 h and 7 days of incubation, the 3 nubbins were randomly sampled from Cr(III), PE, Cr(III)-10&PE(2, 10, 20 mg/L), PE -5&Cr(III)(2, 10, 20 μg/L) and control groups.

Before the beginning of the experiment, the mass of $CrCl_3$ and PE were accurately weighed to prepare the actual Cr (III) and PE concentrations in each treatment group. During the experiment, the pH of seawater was measured daily by a pH meter calibrated with standard liquid, and the salinity and temperature of water were measured by a salinometer and a thermometer respectively. The related results are shown in Table S1.

Endosymbiont density measurement

The density of endosymbiont was measured according to Higuchi's work with slight modification (Higuchi et al., 2015). In short, the tissue homogenate was prepared with Waterpik water jet, and then it was stripped from the coral skeleton into about 10 mL filtered seawater (0.22 μm). The flushing liquid containing endosymbiont was poured into a cleaning measurement barrel, and finally it was recorded the total volume. The flushing liquid was centrifuged at 4000 rpm 4℃ for 3min, and the supernatant was collected. Re-suspended endosymbiont precipitate with filtered seawater, it repeated this operation until no impurities were detected under microscope. A total of 3 copies of homogenate (mixture of coral soft tissue and endosymbiont, 9 mL/share) were centrifuged. And then the endosymbiont deposition at the bottom of the centrifuge tube was collected and it was fixed in 1 mL 10% formaldehyde for 2-4 h. The preserved endosymbiont solution was mixed evenly, and the number of endosymbiont (n =10-12) was calculated with a neubauer hemocytometer (QIUJING, China). The quantity of endosymbiont contained in the total volume solution was obtained by conversion (A: cell/mL). The aluminum foil was wrapped on the surface of coral bone, and the weight of aluminum foil

was weighed. The area of aluminum foil was calculated according to the density and weight of the known aluminum foil (Johannes et al., 1970). That is, the surface area of coral bones (S, cm^2). According to the following formula: density (D, cells/cm^2): D = A/S, the density of endosymbiont was defined as the number of endosymbiont per unit coral nubbins surface area.

Chlorophyll measurement

The chlorophyll content was determined according to Jeffrey's method (Jeffrey et al., 1975). At 1 and 7 d, the coral nubbins with size of 4-5 polyps and diameter of 1 cm were quickly removed with forceps under water. The excess seawater was wiped off with absorbent paper, and then coral nubbins was transferred to a centrifuge tube containing 10 mL acetone, following it was extracted for 24 h at 4 ℃ under dark condition. Then the acetone extract was centrifuged at 4000 rpm for 10 min. The chlorophyll concentration was determined by thermo nanodrop 2000 visible light spectrophotometer. The calculation formula is as follows:

$Chl\text{-}a=11.85A_{664}-1.54A_{647}-0.08A_{630}$

$Chl\text{-}c=24.52A_{664}-1.67A_{647}-7.6A_{630}$

Where Chl-a/c is the chlorophyll content per unit area of coral surface (μg/cm^2), and A is the light absorption value under different wavelengths. The chlorophyll content per unit area of coral surface can be obtained, which combined with the above-mentioned method.

Biochemical evaluation of coral tissue homogenates

To get the homogenates, the endosymbiont deposition were homogenized in 5 mL of filtered seawater by using an Automatic Sample Rapid Grinding Instrument (JingXin, Shanghai, China), and it was centrifuged at 5500 rpm for 15 min. The precipitation was washed thrice with millipore filtered seawater. The supernatant was transferred to a new tube for analyzing the contents of biochemical parameters. The activities of Caspase-1, Caspase-3, LDH, G6DPH and NAD^+/NDAH were measured by commercial kits. Caspase is the general term of cysteinyl aspartate specific protein, as the first signal protein identified in mammalian cells, it mediates the apoptosis of certain types of cells (Zhou et al., 2017; Hengartner et al., 2000). Nicotinamide adenine dinucleotide (NAD+), abbreviated as coenzyme I, is an essential coenzyme in the redox process. The NAD+ involves in many physiological activities such as cell metabolism, energy synthesis, DNA repair and so on (Hosseini et al., 2014; Marangoni et al., 2017). NADH (reduced coenzyme I) is the reductive state of nicotinamide adenine dinucleotide (Ying et al., 2008; Babot et al., 2014). As a carrier and electron donor of biological hydrogen, NADH transfers energy to ATP synthesis through oxidative phosphorylation process in mitochondrial inner membrane. NADH plays an important role in cell growth, cell differentiation and maintenance (anner et al., 2000; Sauve et al.,

2006). After the total enzyme activities were obtained, the concentration of total protein in the supernatant was quantified using BCA method (Zhou et al., 2018).

Statistical analysis

All experiments were repeated at least three times to ensure the accuracy and reproducibility of the results. All data are presented as the mean ± standard error of the mean. One-way ANOVA test of non-parametric equivalent was applied for statistical analysis on the significance of difference by SPSS 20, And they were followed by the Student-Newman-Keuls post-test. For all test, $p<0.05$ was considered statistically significant. The commercial statistical Origin 2019 was used to finish the column diagrams. The letter a expressed as the significant difference among the control, Cr(III) and microplastic treatment groups respectively ($p<0.05$). Letter of b represented the differences of Cr(III)-10 *vs* PE-2, 10, 20 respectively ($p<0.05$), and c represented the differences of PE-5 *vs* Cr(III)-2, 10, 20, respectively ($p<0.05$).

Results

Effects of Cr(III) and PE on density of endosymbiont in corals

Fig.1A shows the density of endosymbiont in corals was decreased under the stress of Cr(III) (10 μg/L) and PE (2 mg/L, 10 mg/L, 20 mg/L). On the day 7, the corals exposed to Cr(III) (10 μg/L) alone showed a significantly lower the density of endosymbiont (2.68×10^6 cell/cm^2, $P < 0.05$). Similarly, the density of endosymbiont in corals was also decreased with the increase of PE concentration, and it was reached the lowest value (2.90×10^6 cell/cm^2, 3.01×10^6 cell/cm^2, 3.46×10^6 cell/cm^2, $P < 0.05$) in the PE group (PE 2 mg/L, 10 mg/L, 20 mg/L) on the day 7. In Fig.1B, the effect of PE exposure alone as well as the joint effect of PE and Cr(III) (2 μg/L, 10 μg/L, 20 μg/L) on the density of endosymbiont in corals were observed after 7 days of exposure. In detail, the density of endosymbiont was decreased after exposure to PE (5 mg/L) combined with Cr(III) (2 μg/L, 10 μg/L, 20μg/L). Especially, the density of endosymbiont in corals was decreased gradually in the high concentration group (Cr(III) 20 μg/L) and it was reached the lowest level on day 7. However, in the low concentration of PE, the effect of increasing the concentration of Cr(III) on the density of endosymbiont was decreased (Fig.1B). In the short term, the effect of Cr(III) on the density of endosymbiont was more obvious.

Effects of Cr(III) and PE on the content of chlorophyll a+c in coral

As shown in Fig.2A, significant effects were showed in chlorophyll a+c after 7 days of exposure to isolated stressors (Cr(III) 10 μg/L). On day 7, the content of chlorophyll a+c in endosymbiont of coral was

the lowest (1.46 μg/cm^2, $P < 0.05$). In addition, after exposure to Cr(III) combined with PE (2 mg/L, 10 mg/L, 20 mg/L), the content of chlorophyll a+c in endosymbiont was decreased gradually with the extension of time. The PE (2 mg/L) in low concentration group had the lowest value (1.54 μg/cm^2, $P < 0.05$) on day 7. In Fig.2B, The adverse effects due to the combination of stressors were observed in chlorophyll a+c content after 7 days of exposure. The chlorophyll a+c content was decreased with the exposure to Cr(III) stress time in PE and Cr(III) groups (2 μg/L, 10 μg/L, 20 μg/L). Especially, it was decreased gradually and reached the lowest level (3.62μg/cm^2, 3.21μg/cm^2, 2.28 μg/cm^2, $P < 0.05$) on day 7 in all concentration groups. Short term combination of stressors exposure may lead to the collapse of coral endosymbiont symbiosis and the expulsion of endosymbiont. The toxic effects of Cr(III) may be the main factors leading to the decrease of chlorophyll content.

Effects of Cr(III) stress on the caspase-3 activation level

The effects were observed in Caspase-3 activity for the treatments tested after 7 days of exposure, the activity of Caspase-3 (1.88 U/g prot, 2.29 U/g prot, $P < 0.05$) was increased with the increase of stress time (Fig.3A). Also, the activity of Caspase-3 was increased gradually with the increasing PE (2 mg/L, 10 mg/L and 20 mg/L respectively) concentration. Moreover the corals exposure to 10 μg/L Cr(III) combined with PE was presented a lower caspase-3 activity than those exposure to Cr(III) alone ($P < 0.05$). Meanwhile, the activity of Caspase-3 in low concentration PE group was higher ($P < 0.05$) than that in high concentration PE group. In Fig.3B, Corals exposed to PE (5 mg/L) showed that the activity of Caspase-3 was increased with the stress time. In addition, exposure to 5 mg/L PE combined with Cr(III) (2 μg/L, 10 μg/L, 20 μg/L) showed that the activity of Caspase-3 was increased with the increase of Cr(III) concentration. On day 7, there was significant difference in activity of Caspase-3 (2.02 U/g prot, 2.51 U/g prot, 2.95 U/g prot, $P < 0.05$) between Cr(III) exposure group and control group, aslo the activity of Caspase-3 in all concentration Cr(III) group was higher than that in PE group.

Effects of Cr(III) stress on the caspase-1 activation level

Fig.4A shows that the caspase-1 activity was significantly changed (3.95 U/g prot, $P < 0.05$) after 7 days of exposure to Cr(III) stressor. An increased caspase-1 activity was observed in corals exposed to different combinations of increasing PE concentrations (2 mg/L, 10 mg/L, 20 mg/L) and Cr (10 μg/L) addition. On day 7, the caspase-1 activity in low concentration PE (2 mg/L) exposure group has reached highest levels (3.66 U/g prot, $P < 0.05$). As shown in Fig.4B, the activity of Caspase-1 was increased with the exposure to PE stress time. Under the stress of PE (5 mg/L) combined with Cr(III) (2 μg/L, 10 μg/L, 20 μg/L), the activity of Caspase-1 gradually was increased, and the activity of caspase-1in high concentration Cr(III) group was significantly increased (6.54 U/g prot, $P < 0.05$). In addition, the activity of caspase-1in Cr(III) group was higher than that in PE group, the up regulation of Caspase-1 was more obvious with the increase of Cr(III) concentration.

Activity of glycolysis enzymes

In Fig.5A, the content of LDH in the control group showed not changes significant with the extension of time ($P > 0.05$). On the day 7, the LDH activity was decreased significantly after Cr(III) (10 μg/L) exposure alone, and the LDH activity reached the lowest level (0.013 U/mg prot, $P < 0.05$). Corals exposed to Cr(III) (10 μg/L) combined with PE (2 mg/L, 10 mg/L and 20 mg/L) showed lower LDH activity. The LDH activity was significantly decreased (0.023 U/mg prot, 0.021 U/mg prot, 0.0249 U/mg prot, $P < 0.05$) in all PE groups. In Fig.5B, the LDH activity was decreased ($P < 0.05$) significantly under joint stress of PE (5 mg/L) combined with Cr(III) (2 μg/L, 10 μg/L, 20 μg/L). In the same period of time, LDH activity in low concentration Cr(III) group was higher than that in high concentration Cr(III) group. The LDH activity (0.018 U/mg prot, $P < 0.05$) of Cr(III) high concentration group was significantly lower than those control group on the day 7. During the acute 1 day exposure, the LDH activity of *A. pruinosa* was inhibited by joint stress of PE combined with Cr(III). This inhibition was significantly enhanced upon 7 days exposue days exposure, indicating that the interaction of PE and Cr(III) was dynamic and with persistence within the experimental period.

Activity of the phosphate pentoses pathway

Fig. 6A show the effect of Cr(III) (10 μg/L) addition alone as well as the effect of PE (2 mg/L, 10 mg/L, 20 mg/L) combined with Cr(III). The G6PDH activity in corals was decreased significantly after Cr(III) stress. A lower G6PDH activity (0.110 mU/mg prot, $P < 0.05$) was observed on day 7. Lower G6PDH activity ($P < 0.05$) was detected in corals exposed to 2 mg/L, 10 mg/L and 20 mg/L PE. G6PDH activity was decreased with the increase of PE concentration. Furthermore, the G6PDH activity was decreased with prolongation of time, proving that the G6PDH activity was inhibited. In Fig. 6B, under the stress of 5 mg/L PE, the G6PDH activity was decreased. On the day 7, the G6PDH activity reached the lowest level. In addition, with the increase of Cr(III) concentration (2 μg/L, 10 μg/L, 20 μg/L), the G6PDH activity was decreased. Also, G6PDH activity in each treatment group was decreased with the exposure to Cr(III) stress time, and the G6PDH activity in Cr(III) group (2 μg/L,10 μg/L, 20μg/L) was decreased (0.0216 mU/mg prot, 0.0214 mU/mg prot, 0.0136 mU/mg prot, $P < 0.05$).

The of ratio NAD^+/NADH

In Fig.7A, the NAD^+/NADH ratio was reduced in the stony coral *A. pruinosa* after Cr(III) stress alone and combined stress of Cr(III) and PE. The ratio of NAD^+/NADH was reduced ($P < 0.05$) significantly after exposed to Cr(III) (10 μg/L). Also, after exposed to Cr(III) combined with PE (2 mg/L, 10 mg/L, 20 mg/L) showed a lower the ratio of NAD^+/NADH. Interestingly, the NAD^+/NADH ratio under joint stress of Cr(III) combined with PE was higher than that under Cr(III) stress alone. In Fig.7B, the coral was exposed to PE and Cr(III) (2 μg/L, 10 μ g/L, 20 μg/L), the ratio of NAD^+/NADH was reduced (Fig. 7B). In high concentration Cr(III) (20 μg/L) group, the ratio of NAD^+/NADH was significantly lower ($P < 0.05$).

Moreover, the ratio of NAD^+/NADH was reached the lowest level on day 7. In conclusion, the ratio of NAD^+/NADH in corals were more sensitive to Cr(III) exposure.

Discussion

Effect of microplastic and Cr(III) on symbiotic relationship of endosymbiont

The endosymbiont in corals provide most of nutrients for growth and reproduction of host through photosynthesis (Pierson et al., 2017), and the chlorophyll content can affect the growth of endosymbiont. Substantial heavy metals accumulation in both endosymbiont and tissue fractions of branching corals has been previously expounded (Bielmyer et al., 2010; Mitchelmore et al., 2007). Reichelt-Brushett found that under heavy metal pollution, endosymbiont may accumulate certain heavy metals, and the heavy metals seriously inhibited photosynthesis and lead to *Goniastrea aspera* decline and death (Reichelt-Brushett et al; 1999). In the short term, the density of endosymbiont in corals was decreased under the stress of PE and Cr(III), the balance between the endosymbiont and the host was disturbed to a certain extent. In addition, the chlorophyll content of coral endosymbiont in the control group was the maximum value, and it was decreased with the increase of PE and Cr(III) concentration. We assumed that the cause for the collapse after PE stress may be similar to the response after heat stress, which attributed to the changes in the internal environment of the coral, such as the increase of ROS level (Mendrik et al., 2020), the activation of apoptosis (Zhou et al., 2017). The joint stress of microplastic and Cr(III) can inhibit the photosynthesis of coral endosymbiont. Moreover, the chlorophyll content of endosymbiont under Cr(III) stress alone was significantly lower than that under PE alone, it suggested that endosymbiont in coral was more sensitive to Cr(III) stress. The inhibition effect of heavy metal on photosynthesis may be ascribed to the toxic effect of Cr(III), which seriously interfered with the electron transfer between PS I and PS II (Aro et al., 2005; Bhattacharya et al., 2010), resulting in the decrease of energy conversion and the decline of photosynthesis.

The effect of PE and Cr(III) on the apoptosis of endosymbiont

The combination of stressors led to a higher expression on the apoptotic protease related to corals endosymbiont (Tang et al., 2020; Su et al., 2019). Deleterious effects due to the combination of PE and Cr(III) stressors were observed on Caspase-1 activity after short exposure. The strengthen in this Caspase-1 activity may an important source of apoptotic production for *A. pruinosa*, considering that Caspase-1 is important for the activation-induced cell death in organisms with low metabolism. In addition, the programmed cell death (PCD) mediated by caspase-1 can effectively improve the body's ability to resist endogenous and exogenous stimuli, and it protect the host (Mariathasan et al., 2004). The stress source PE and Cr(III) were combined, the activation level of Caspase-1 was increased. It indicated that the host

cell apoptosis or the separation of endosymbiont, which is consistent with the decrease of chlorophyll. Specifically, the concentration of Cr(III) was increased, and the activation level of Caspase-1 was significantly higher than that in other treatment groups. Caspase-3 is the key executive enzyme as well as the final effector of apoptosis (Xu et al., 2017). Therefore, the activation level of Caspase-3 was investigated to understand the apoptosis status of corals after short-term Cr(III) and PE stress. The activation level of Caspase-3 was increased significantly, which indicated that Cr(III) and PE induced the apoptosis of endosymbiont. Apoptosis was also observed in the same corals under heat (Tchernov et al., 2011), the mechanisms underlying the response of coral may be heavy metal stress induce the apoptosis of corals through TNF signaling pathway and caspase-3. Moreover, apoptosis will eventually lead to the collapse of symbiotic balance between host and endosymbiont, and it induces the expulsion of endosymbiont, resulting in coral bleaching (Tang et al., 2020; Su et al., 2019). In conclusion, our results provide preliminary evidence that the PE combined with Cr(III) may induce apoptosis of coral endosymbiont, but the underlying mechanism still needs to be explored in the future.

Effects of PE and Cr(III) on energy metabolism of coral endosymbiont

Glycolysis is considered to be the main energy production pathway of low metabolism invertebrates, which can produce ATP and NADH under anaerobic conditions (Nelson et al., 2008; Carvalho et al., 2008). After exposure to PE and Cr(III), LDH activity was inhibited in coral *A. pruinosa*. The results suggested that the corals exposed to 20 μg/L Cr(III) and 5 mg/L PE had lower enzyme activity than corals exposed to 10 μg/L Cr(III) alone. In addition, LDH activity also was decreased in corals exposed to different concentrations of Cr(III) and PE. Interestingly, under the stress of 10 μg/L Cr(III) and 2, 10 and 20 mg/L PE, the LDH activity of corals was higher than those corals exposed to the same concentration of Cr(III) alone. The fact was that in these cases the combination of stressors led to inhibition effects on enzymatic activity (Carvalho et al., 2008). The exposure of *A. pruinosa* to PE can reduce the effects of the Cr(III) in LDH activity. In this context, the decrease of the LDH activity in the present study may be related to detoxification pathways activation due to higher PE concentrations (Tang et al., 2020; Liao et al., 2021). In the short term, the toxicity of Cr(III) to corals was significantly higher than that of PE. High concentration of PE can slightly alleviate the LDH damage of corals induced by Cr(III). When coral was exposed to the same heavy metal Cr (III) and different microplastic concentrations, the higher of microplastic concentration, the toxicological effect on coral energy metabolism enzyme LDH was weakened, which may be the adsorption of heavy metals by microplastic (Mao et al., 2020; Zou et al., 2020; Wu et al., 2018). At the same concentration of PE (5 mg/L), the attenuation effect of Cr(III) on G6PDH activity was observed. Although Cr(III) and other metals are essential nutrients for the metabolism of organisms, especially invertebrates (Lee et al., 2005), excessive heavy metals can seriously interfere with the energy metabolism of host symbionts (Tang et al., 2020; Fonseca et al., 2019). In fact, a short exposure time may have yielded same results. Fonseca found Cu(II) was assimilated by adsorption or ingestion by corals, which seriously interfered with glycolysis and pentose phosphate metabolism

(Fonseca et al., 2019). Exposure to PE combined with Cr(III) could inhibit the activity of G6PDH, the inhibition of G6PDH activity may reduce the NADH synthesis for antioxidant enzymes (Carvalho et al., 2008), leading an oxidative stress. Similarly, under the stress of 5 mg/L PE, this attenuating effect on G6PDH activity is observed, and exposure to combined stressors inhibited the activity of this enzyme.

Effects of PE and Cr(III) on NAD^+/NADH of endosymbiont

The NAD^+/NADH ratio was significantly reduced in coral *A. pruinosa* after Cr(III) stress alone as well as combined stress of Cr(III) and PE. The joint stress of Cr(III) and PE inhibited the ratio of NAD^+/NADH in endosymbiont of corals. In addition, stress of PE combined with Cr(III) reduced the ratio of NAD^+/NADH in endosymbiont and may further inhibit the expression of SIRT1 protein (Fonseca et al., 2019). we suspect that On the one hand, NAD^+ dysfunction blocked the signal transduction and it induced the antagonistic effect of coral tissue enzymes. On the other hand, it activated the senescence related pathway of coral cells, which induced the apoptosis of endosymbiont and led to the dysfunction of ATP conversion function. NADH is a crucial factor in SIRT1 signaling pathway, and produced in citric acid cycle during glycolysis and cell respiration, which involves in metabolism of substance and energy in cells (Fonseca et al., 2019; Babot et al., 2014). It accelerated the collapse of coral endosymbiont symbiosis system due to the damage effects from the two aspects. Therefore, the decrease of NAD^+/NADH ratio may be the core mechanism of endosymbiont corals collapse. Noteworthily, Cr(III) can promote MP-induced apoptosis of endosymbiont in corals by inhibiting the NAD^+-SIRT1 pathway. And it increased the production of lysosome in host cells to accelerate the process of histiocytic lesion. The NAD^+/NADH ratio of *A. pruinosa* was lowered as a result of exposure to PE and Cr(III), which may have been caused by ROS production and/or damage to glycolysis pathway. Based on this result, it is apparent that exposure to PE and Cr(III) will cause breakdown of symbiosis between *A. pruinosa* and its algal endosymbionts. However, the mechanism of its action still needs to be investigated.

Conclusion

This study for the first time investigated the isolation and joint effects of PE and Cr(III) exposure on the energy metabolism enzymes and apoptosis of endosymbiont in the coral *A. pruinosa*. On summary, the results showed that the stress sources of PE and Cr(III) resulted in the transient inhibition of chlorophyll and endosymbiont of *A. pruinosa*. Caspase-1 and caspase-3 were significantly increased under these conditions, which leaded to the apoptosis of endosymbiont. In addition, the activities of LDH and G6PDH and the ratio of NAD^+/NADH were decreased significantly after 7 days of exposure to PE and Cr(III), indicating that the combination of PE and Cr(III) had a significant adverse effect on the energy metabolism of corals. Therefore, it can be inferred that the increase of marine microplastic combined with the Cr(III) stress will lead to the disorder of energy metabolism of corals, and accelerate the apoptosis of endosymbiont in corals.

Declarations

Supporting Information

Treatment schedule of the study. Relevant seawater chemical parameters during exposure experiment.

Credit authorship contribution statement

Baohua Xiao: Conceptualization, Funding acquisition.

Dongdong Li: Conceptualization, Writing-Original Draft, Writing-Review & Editing.

Chengyong Li: Supervision, Project administration, Funding acquisition.

Baolin Liao: Investigation, Formal analysis.

Huina Zheng: Data Curation.

Xiaodong Yang: Methodology.

Yongqi Xie: Software, Supervision, Validation.

Ziqiang Xie: Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

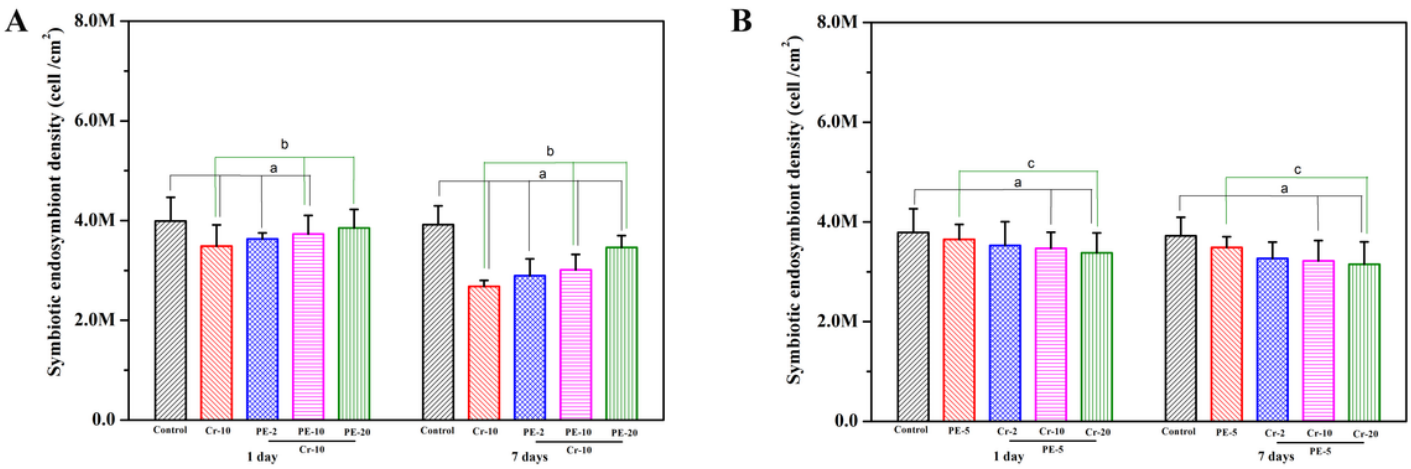

Figure 1

The density of endosymbiont in the coral *A. pruinosa* exposed to microplastic and Cr(III) for 1 day and 7 days. (A) Single exposure to heavy metal Cr(III) (10 μg/L) and Cr(III) combined with different concentrations of PE (2 mg/L, 10 mg/L, 20 mg/L). (B) Single exposure to PE (5 mg/L) and PE combined with different concentrations of heavy metal Cr(III) (2 μg/L, 10 μg/L, 20 μg/L). Data are expressed as mean ± standard error (n=3). The differences were considered significant at $p < 0.05$.

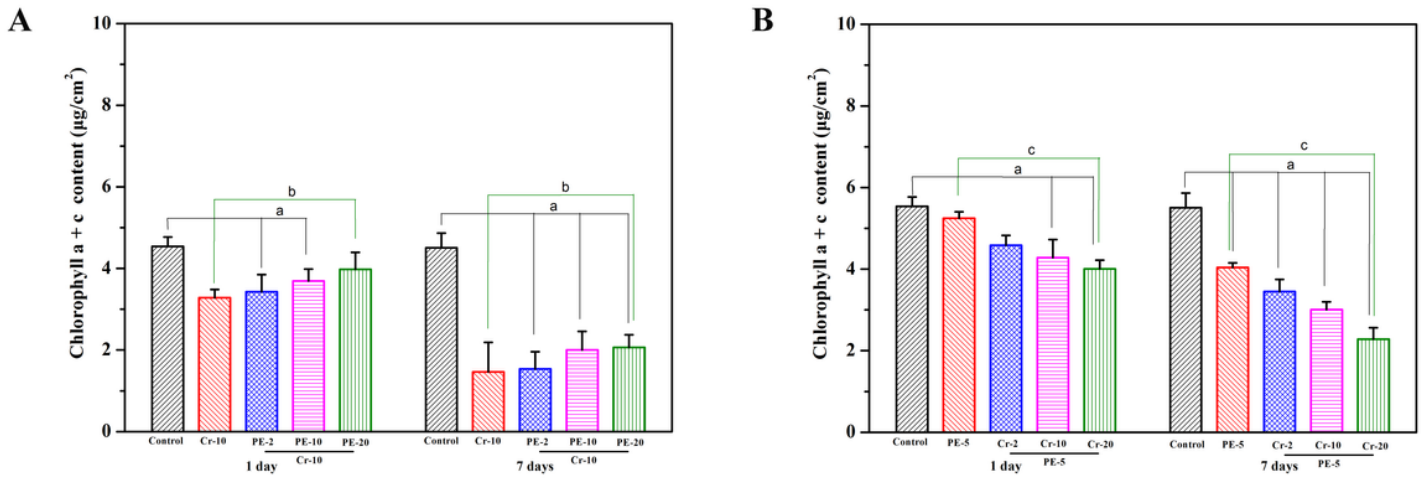

Figure 2

The chlorophyll a+c content in the coral *A. pruinosa* exposed to microplastic and Cr(III) for 1 day and 7 days. (A) Single exposure to heavy metal Cr(III) (10 μg/L) and Cr(III) combined with different concentrations of PE (2 mg/L, 10 mg/L, 20 mg/L). (B) Single exposure to PE (5 mg/L) and PE combined with different concentrations of heavy metal Cr(III) (2 μg/L, 10 μg/L, 20 μg/L). Data are expressed as mean ± standard error (n=3). The differences were considered significant at $p < 0.05$.

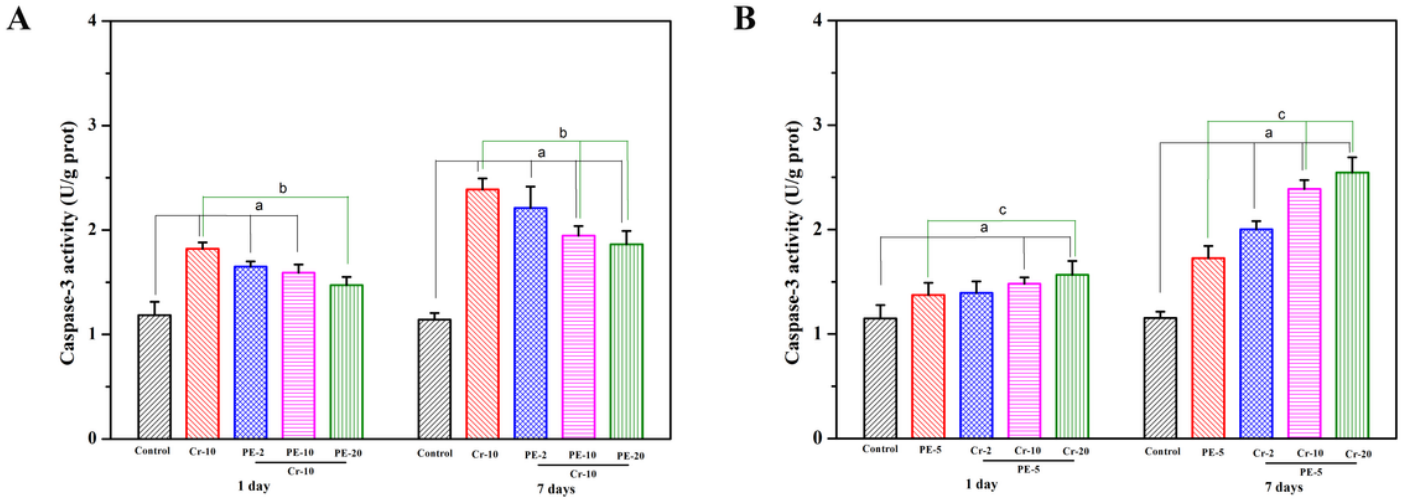

Figure 3

Cysteine protease protein (Caspase-3) activity in the coral *A. pruinosa* exposed to microplastic and Cr(III) for 1 day and 7 days. (A) Single exposure to heavy metal Cr(III) (10 μg/L) and Cr(III) combined with different concentrations of PE (2 mg/L, 10 mg/L, 20 mg/L). (B) Single exposure to PE (5 mg/L) and PE combined with different concentrations of heavy metal Cr(III) (2 μg/L, 10 μg/L, 20 μg/L). Data are expressed as mean ± standard error (n=3). The differences were considered significant at $p < 0.05$.

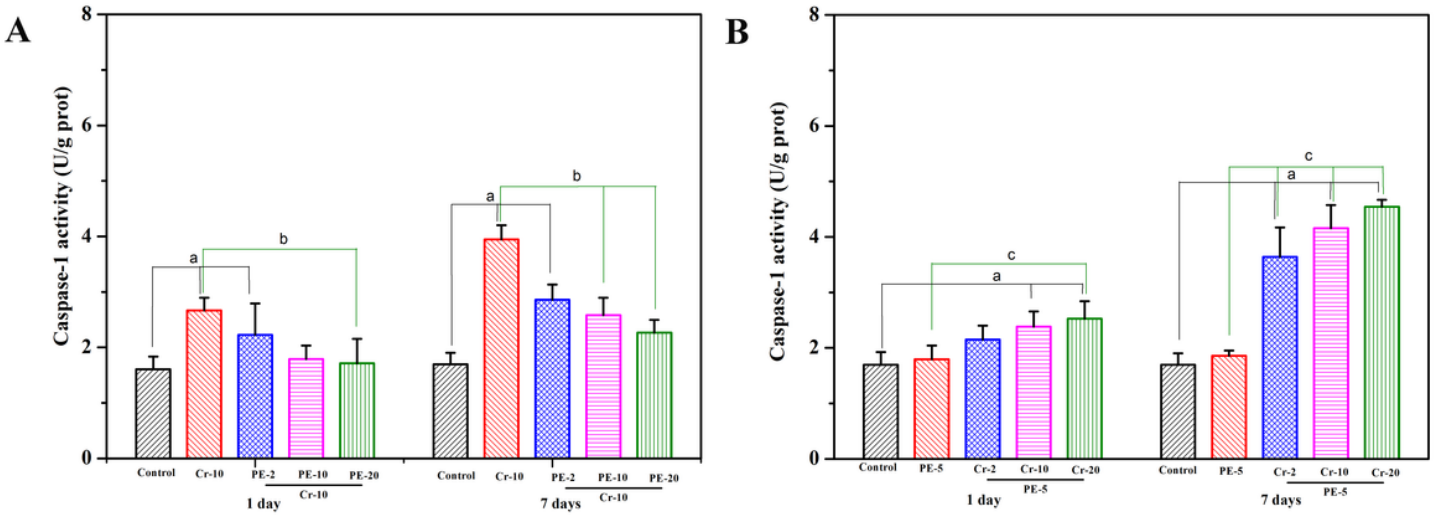

Figure 4

Cysteine protease protein (Caspase-1) activity in the coral *A. pruinosa* exposed to microplastic and Cr(III) for 1 day and 7 days. (A) Single exposure to heavy metal Cr(III) (10 μg/L) and Cr(III) combined with different concentrations of PE (2 mg/L, 10 mg/L, 20 mg/L). (B) Single exposure to PE (5 mg/L) and PE combined with different concentrations of heavy metal Cr(III) (2 μg/L, 10 μg/L, 20 μg/L). Data are expressed as mean ± standard error (n=3). The differences were considered significant at $p < 0.05$.

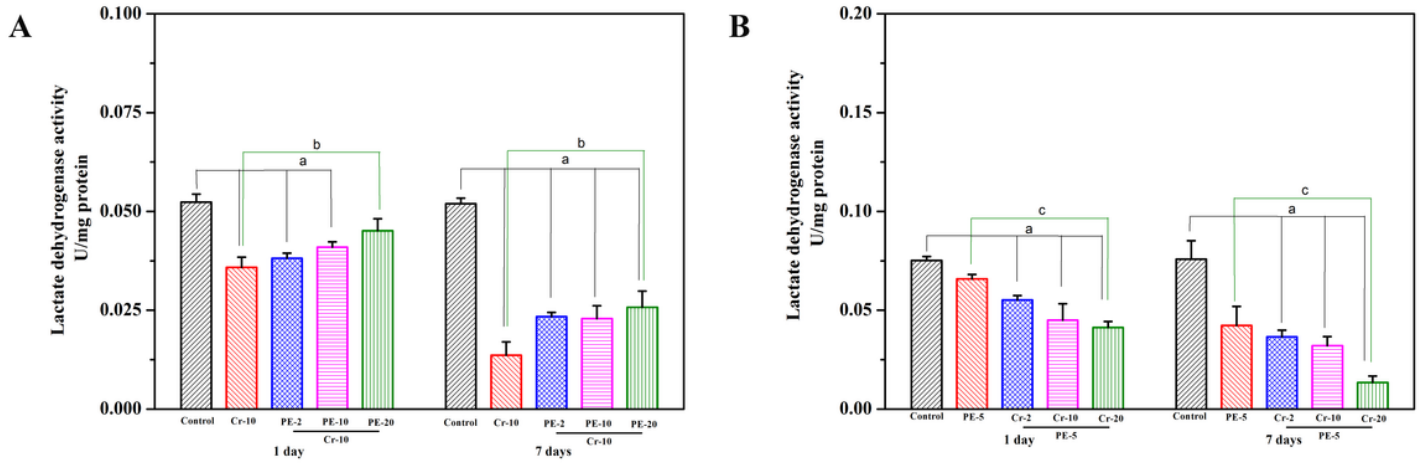

Figure 5

Lactate dehydrogenase (LDH) activity in the coral *A. pruinosa* exposed to microplastic and Cr(III) for 1 day and 7 days. (A) Single exposure to heavy metal Cr(III) (10 μg/L) and Cr(III) combined with different concentrations of PE (2 mg/L, 10 mg/L, 20 mg/L). (B) Single exposure to PE (5 mg/L) and PE combined with different concentrations of heavy metal Cr(III) (2 μg/L, 10 μg/L, 20 μg/L). Data are expressed as mean ± standard error (n=3). The differences were considered significant at $p < 0.05$.

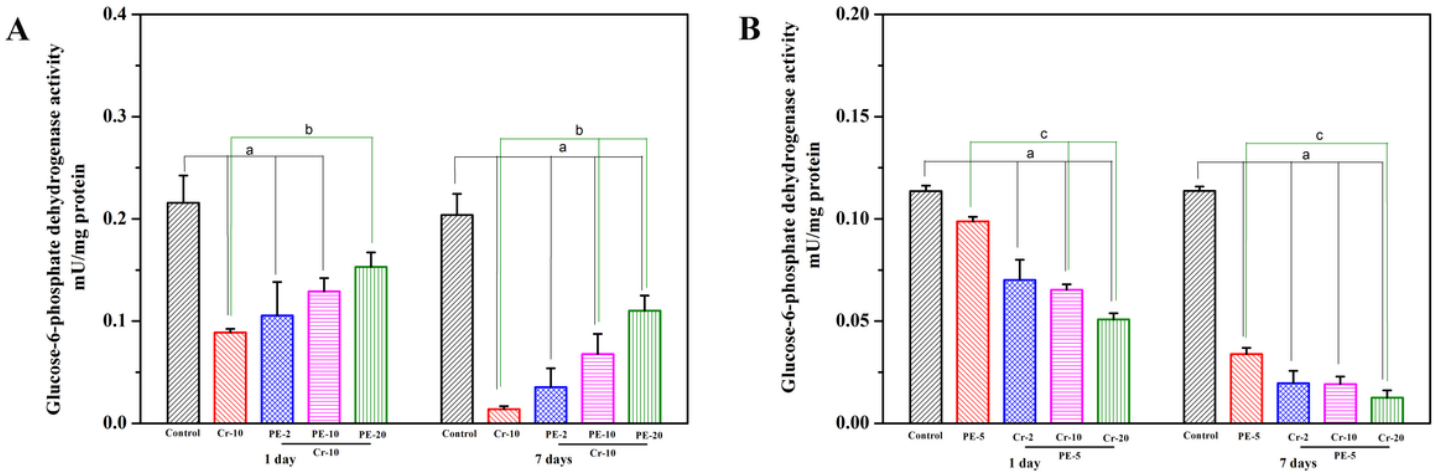

Figure 6

Glucose-6-phosphate dehydrogenase (G6PDH) activity in the coral *A. pruinosa* exposed to microplastics and Cr(III) for 1 day and 7 days. (A) Single exposure to heavy metal Cr(III) (10 μg/L) and Cr(III) combined with different concentrations of PE (2 mg/L, 10 mg/L, 20 mg/L). (B) Single exposure to PE (5 mg/L) and PE combined with different concentrations of heavy metal Cr(III) (2 μg/L, 10 μg/L, 20 μg/L). Data are expressed as mean ± standard error (n=3). The differences were considered significant at $p < 0.05$.

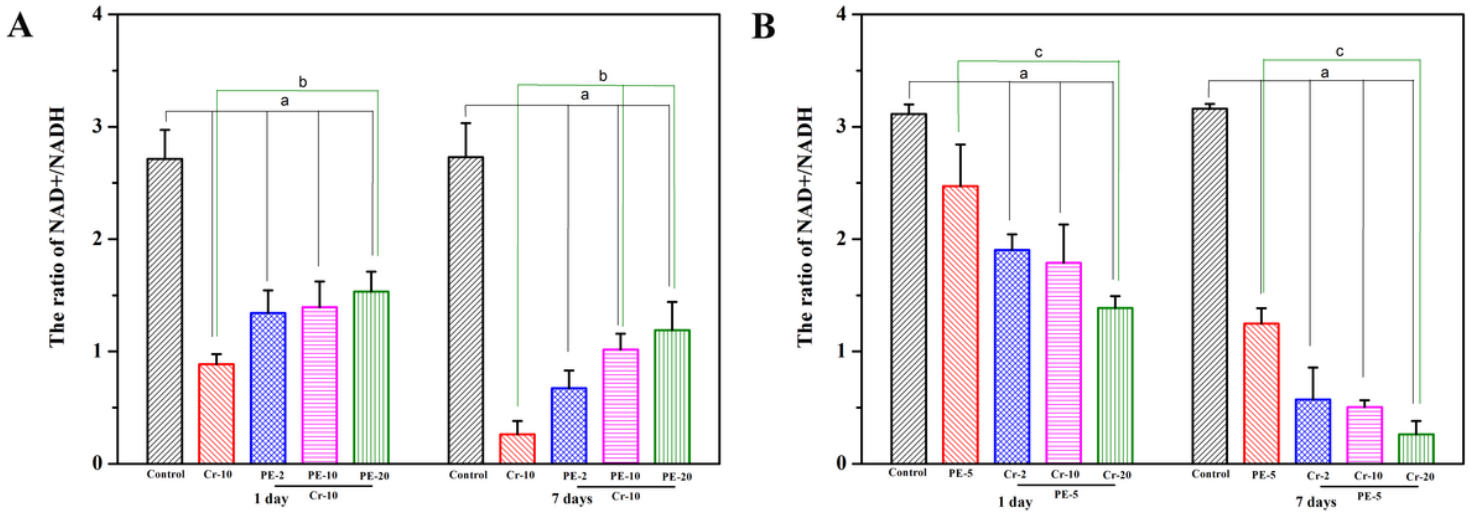

Figure 7

The ratio of NAD^+/NADH in the coral *A. pruinosa* exposed to microplastic and Cr(III) for 1 day and 7 days. (A) Single exposure to heavy metal Cr(III) (10 μg/L) and Cr(III) combined with different concentrations of PE (2 mg/L, 10 mg/L, 20 mg/L). (B) Single exposure to PE (5 mg/L) and PE combined with different concentrations of heavy metal Cr(III) (2 μg/L, 10 μg/L, 20 μg/L). Data are expressed as mean ± standard error (n=3). The differences were considered significant at $p < 0.05$.

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