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硕 士 学 位 论 文

Cu²⁺或 Zn²⁺及 Cu²⁺与 Zn²⁺交互胁迫对拟穴青蟹(*Scylla paramamosain*)生理生化的影响

**Physiological-Biochemistrial Effects of
Scylla paramamosain to Cu²⁺ or Zn²⁺ or Interaction of Cu²⁺
and Zn²⁺ Stress**

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中文摘要

运用酶学分析方法和组织学技术,研究了水体中不同浓度 Cu^{2+} 或 Zn^{2+} 及 Cu^{2+} 与 Zn^{2+} 交互作用胁迫对拟穴青蟹生理生化以及鳃和肝胰腺显微结构的影响,探讨 Cu^{2+} 或 Zn^{2+} 及 Cu^{2+} 与 Zn^{2+} 交互作用胁迫对拟穴青蟹生理生化的影响及免疫防御机制,以期为其健康养殖水质管理提供理论指导,并为甲壳动物环境免疫学研究积累基础资料。本试验的主要结果和结论如下:

1. Cu^{2+} 及 Cu^{2+} 与 Zn^{2+} 交互作用胁迫对拟穴青蟹血淋巴中 THC 的影响

在 $\text{Cu}^{2+} \leq 0.1 \text{ mg} \cdot \text{L}^{-1}$ 胁迫第 1 天可诱导拟穴青蟹血淋巴中 THC 的升高,随着胁迫时间的延长,THC 显著下降后回升至对照组水平,在 $\text{Cu}^{2+} \geq 2.0 \text{ mg} \cdot \text{L}^{-1}$ 及 Cu^{2+} 与 Zn^{2+} 交互作用胁迫下的 9d 试验期间,THC 均显著低于对照组($P < 0.05$)。结合本试验拟穴青蟹血清中 PO 活性变化,THC 与 PO 活性存在一定相关性,THC 较多时,PO 活性较高,THC 减少时,PO 活性较低。

2. Cu^{2+} 或 Zn^{2+} 及 Cu^{2+} 与 Zn^{2+} 交互作用胁迫显著影响拟穴青蟹的生理生化,且存在一定的时间-剂量效应。

$\text{Cu}^{2+} \leq 0.1 \text{ mg} \cdot \text{L}^{-1}$ 胁迫下,拟穴青蟹体内 CuZn-SOD、GSH-Px 活性在短时间内可被激活,随着胁迫时间延长,其活性回落,而 MDA 含量变化不显著($P > 0.05$)。 $\text{Cu}^{2+} \geq 1.0 \text{ mg} \cdot \text{L}^{-1}$ 胁迫下,CuZn-SOD 活性表现为持续抑制作用,其中肌肉中 CuZn-SOD 活性低于对照组虽被抑制却呈逐步上升趋势;而拟穴青蟹体内 GSH-Px 活性被持续激活。同时,较低 Cu^{2+} 浓度组 GSH-Px 活性与 MDA 含量存在负相关,而较高 Cu^{2+} 浓度组 GSH-Px 活性与 MDA 含量存在正相关,即较高 Cu^{2+} 浓度组拟穴青蟹体内 MDA 含量显著增加($P < 0.05$)。 Cu^{2+} 胁迫可以显著激活拟穴青蟹体内 ACP 活性($P < 0.05$),同时随时间的延长会对 ACP 活性产生抑制作用。较低 Cu^{2+} 浓度胁迫在激活拟穴青蟹体内 AKP 活性后下降至对照组水平,而较高 Cu^{2+} 胁迫则对 AKP 活性初期有抑制作用,后 AKP 活性有所回升。较低 Cu^{2+} 浓度胁迫下拟穴青蟹血清中抗菌力(U_a)呈上升趋势,而较高 Cu^{2+} 胁迫则对 U_a 有抑制作用,同时 Cu^{2+} 胁迫对 U_a 的抑制作用具有时间累积效应。在 Cu^{2+} 胁迫初期拟穴青蟹血清中溶菌酶(U_L)活性随着 Cu^{2+} 浓度的增大有逐步上升趋势,而随着 Cu^{2+} 胁迫进一步进行,较高 Cu^{2+} 浓度组 U_L 活性逐步下降。

$Zn^{2+} \leq 1.00 mg \cdot L^{-1}$ 胁迫下, 拟穴青蟹血清中 PO、CuZn-SOD、GSH-Px 以及体内 CuZn-SOD、ACP、AKP 活性被显著诱导后呈下降趋势, 而 U_a 呈上升趋势; $Zn^{2+} \geq 20.0 mg \cdot L^{-1}$ 胁迫下, 对拟穴青蟹体内 PO、CuZn-SOD、GSH-Px、ACP、AKP、 U_a 活性表现出明显的抑制作用, 后随胁迫时间延长略有回升。 U_L 活性随着 Zn^{2+} 浓度的增大呈下降趋势。

3. CA 组、CB 组的拟穴青蟹在 9d 试验期间, 其体内 THC、PO、GSH-Px、ACP、 U_a 以及 U_L 等指标分别低于 C100 组、C200 组, 且 CB 组小于 CA 组, 表明在外加固定浓度 Zn^{2+} 情况下, Cu^{2+} 与 Zn^{2+} 交互作用比单一 Cu^{2+} 胁迫对上述指标更具抑制作用, 对拟穴青蟹更具有毒性作用, Cu^{2+} 与 Zn^{2+} 表现出协同作用。 Cu^{2+} 与 Zn^{2+} 交互胁迫对拟穴青蟹体内 CuZn-SOD 及 AKP 等酶活性产生激活效应。 Cu^{2+} 与 Zn^{2+} 交互作用胁迫对拟穴青蟹体内 MDA 含量变化差异不显著 ($P > 0.05$)。

4. 组织学观察表明, Cu^{2+} 及 Cu^{2+} 与 Zn^{2+} 交互作用胁迫下拟穴青蟹鳃及肝胰腺组织细胞结构发生了氧化损伤, 作用于鳃上皮细胞及肝胰腺肝小管引起细胞结构受损, 影响了拟穴青蟹正常的生理生化功能, 且这种损伤随着水体中添加的重金属浓度的升高而加剧。

综上所述, Cu^{2+} 或 Zn^{2+} 及 Cu^{2+} 与 Zn^{2+} 交互作用胁迫对拟穴青蟹体内主要生理生化影响显著, 而且损伤其鳃、肝胰腺等器官的正常结构和功能。

关键词: 拟穴青蟹; Cu^{2+} 胁迫; Zn^{2+} 胁迫; 生理生化; 显微结构

Abstract

The effects of Cu^{2+} or Zn^{2+} or interaction of Cu^{2+} and Zn^{2+} stress on the physiological-biochemical parameters (THC, PO, SOD, GSH-Px, MDA, ACP, AKP, U_a , U_L) and histological structure of gill and hepatopancreas of mud crab *Scylla paramamosain* were determined by enzyme analysis and histology technique during 9d experimental time. The aim was to clarify the physiological-biochemical effects and mechanisms of immune defence of *Scylla paramamosain* under Cu^{2+} or Zn^{2+} or interaction of Cu^{2+} and Zn^{2+} stress. The research has provided theoretical guidances to water quality management for mud crab healthy aquaculture and accumulated basic data for the environmental immunology of crustaceans. The main results and conclusions were as follows:

1. Total haemocyte counts (THCs) in haemolymph of *S. paramamosain* were affected by Cu^{2+} or interaction of Cu^{2+} and Zn^{2+} stress.

The THCs of mud crabs *S. paramamosain* were increased below $0.1\text{mg}\cdot\text{L}^{-1}$ Cu^{2+} stress on the first day. With the stress time prolonged, the THCs were decreased markedly which had partially returned finally. Above $2.0\text{mg}\cdot\text{L}^{-1}$ Cu^{2+} stress or the interaction of Cu^{2+} and Zn^{2+} stress during the whole experimental period, the THCs were significantly lower than the control group ($P<0.05$). And referring to changes of phenoloxidase activities in the serum, it showed that PO activities were significantly correlated to the THC, the PO activities were higher when THC increased, and vice versa.

2. Physiological-biochemical parameters in mud crabs were significantly affected by Cu^{2+} or Zn^{2+} stress, which showed dose-time response.

The activities of CuZn-SOD and GSH-Px in mud crabs were activated in short time and decreased with the stress time prolonged below the $0.1\text{mg}\cdot\text{L}^{-1}$ Cu^{2+} stress, Meanwhile there were no significant changes on MDA level ($P>0.05$). Above the $1.0\text{mg}\cdot\text{L}^{-1}$ Cu^{2+} stress, the CuZn-SOD activities in mud crabs were continuously inhibited; on contrary, the GSH-Px activities were induced. It showed that MDA levels in mud crabs were significantly increased at higher Cu^{2+} concentration, which

showed a positive correlation to GSH-Px activities. The ACP activities in mud crabs were significantly activated and decreased with stress time prolonging. The AKP activities in mud crabs were activated initially and dropped to control group level later under lower concentration of Cu^{2+} stress. And under higher concentration of Cu^{2+} stress, the AKP activities were inhibited initially and recovered partially later. There were increasing trend of antibacterial activities in lower concentration of Cu^{2+} stress. And in higher concentration of Cu^{2+} stress the antibacterial activities had an inhibitory effect which in association with the time dosage. With the increase of Cu^{2+} stress, Lysozyme activities induced initially and decreased in higher concentration of Cu^{2+} stress.

The activities of PO、CuZn-SOD、GSH-Px、ACP、AKP in mud crabs were significantly activated in a short time ($P<0.05$) and decreased with the stress time prolonged below the $1.00\text{mg}\cdot\text{L}^{-1}$ Zn^{2+} stress, but the antibacterial activities increased. Above the $20.0\text{mg}\cdot\text{L}^{-1}$ Zn^{2+} stress, these parameters were inhibited and recovered partially later. The lysozyme activities decreased with the Cu^{2+} concentration increasing.

3. The biochemical parameters (THC、PO、GSH-Px、ACP、 U_a 、 U_L) of the mud crabs in CB group and CA group were lower than those in C100 group and C200 group, and the biochemical parameters of the mud crabs in CB group were less than those in CA group. It showed that the interaction of Cu^{2+} and Zn^{2+} stress represented inhibitor to these and more toxicity to mud crabs than single metal ion stress, which indicted a synergistic effect by the stress of Cu^{2+} and Zn^{2+} . Meanwhile the activities of CuZn-SOD and AKP in mud crabs had been activated, and there were no significantly changes in MDA levels ($P>0.05$).

4. The histological structure changes of gills and hepatopancreas in mud crabs under Cu^{2+} stress or interaction of Cu^{2+} and Zn^{2+} stress were studied by microscopy. The results showed that there were oxidative injuries to the structure of epithelium of gill and hepatopancreatic tubules in mud crabs under Cu^{2+} stress or interaction of Cu^{2+} and Zn^{2+} stress, the physiological functions of mud crabs were depressed and the structures of gill and hepatopancreas were damaged. It was more obviously injured in

structures of gill and hepatopancreas in mud crab with the Cu^{2+} stress increasing.

In conclusion, the physiological-biochemical parameters of *S. paramamosain* had been affected significantly, and the normal structures and functions of gill and hepatopancreas had been injured by Cu^{2+} stress or Zn^{2+} stress or interaction of Cu^{2+} and Zn^{2+} stress.

Keywords: *Scylla paramamosain*; copper(Cu^{2+}) stress; zinc (Zn^{2+}) stress; physiological-biochemical parameters; microstructure

第一章 绪 论

第一节 海洋重金属污染研究现状

伴随着工农业的发展和人口不断增长,人们生产和生活过程中产生的废弃物也越来越多,这些废弃物中的绝大部分最终直接或间接进入海洋,使得海洋(特别是近岸和近海海域)中的物质组成和能量分布的平衡关系受到影响或者破坏,产生严重的污染问题。资料表明^[1],我国近岸和近海海域的主要污染物 85%以上来自于陆源排污,每年有上百亿吨的工业和生活污水携带大量的有害物质排入海,造成近岸海域的水质发生恶化。如沿海工业大量排放的重金属污染物导致了诸如长江口^[2]、珠江口^[3]、九龙江口^[4]等河口地区重金属污染严重。2006 年度的《中国渔业生态环境状况公报》显示:2006 年,全国局部地区的渔业水域污染仍比较严重,主要污染物为氮、磷、石油类和铜;强调指出海洋渔业水域沉积物中,主要受到铜、镉、砷和铅的污染,其中铜和镉污染以东海区和南海区部分区域相对较重;砷和铅污染以南海区部分区域相对较重。

重金属是一类具有潜在危害的污染物,具有持久性、生物富集和放大作用的特点,其对生物的影响已经成为生态毒理学研究的重要内容。甲壳动物在水生生态系统之中分布广泛,是水生生物食物链中具有重要作用的物种,对水环境中发生的物理、化学和生物性的各种变化反应十分灵敏,在毒理学和生态风险评价中具有重要的实用价值。由于海洋环境中的重金属污染会对水生生物产生毒害,并且通过食物链及生物的富集作用产生蓄积,人类如果食用了这些含重金属超标的鱼虾贝类等生物会造成不同程度的中毒现象^[5,6],因此重金属污染对环境的影响越来越多地受到国内外科学家和国际环境组织的关注,陆续开展了各项科学研究以及全球海域重金属污染的环境检测、评价项目^[7-9]。目前,除了采用化学方法对水体进行监测外,也常用生物学方法来测定和评价化学物质对水生甲壳动物的影响。在重金属离子对水生甲壳动物的毒理学研究中,许多学者已经把其免疫相关因子的变化作为水生甲壳动物对环境重金属胁迫的评价指标^[10-12]。

1.1 重金属污染的来源

海洋中的重金属来源分为人为来源和自然来源。各种地质、地球化学作用,如构造活动、陆地和海底岩石风化、侵蚀及水动力作用等,都可造成金属元素在

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