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亚慢性铜暴露对背角无齿蚌鳃抗氧化系统的影响

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摘 要:为探究淡水环境中铜(Copper, Cu)污染对背角无齿蚌(*Anodonta woodiana*)鳃抗氧化系统的影响,检测了 Cu^{2+} (0.137、0.548、2.192 $\text{mg} \cdot \text{L}^{-1}$)暴露于背角无齿蚌7、14、21、28 d后,其鳃中超氧化物歧化酶(Superoxide dismutase, SOD)、过氧化氢酶(Catalase, CAT)、谷胱甘肽过氧化物酶(Glutathione peroxidase, GPx)和谷胱甘肽转硫酶(Glutathione-S-transferase, GST)的活性,还原型谷胱甘肽(Reduced glutathione, GSH)和丙二醛(Malondialdehyde, MDA)的含量及总抗氧化能力(Total antioxidant capacity, T-AOC)的变化。结果显示:随着 Cu^{2+} 浓度的增加和暴露时间的延长,MDA含量逐渐上升,表现出明显的“剂量-效应”和“时间-效应”关系;抗氧化酶SOD、GPx和GST活性均被显著诱导($P < 0.05$),CAT活性被显著抑制($P < 0.05$),抗氧化剂GSH含量显著降低($P < 0.05$); Cu^{2+} 暴露14、21、28 d,T-AOC与对照组相比均显著升高($P < 0.05$)。总体而言,背角无齿蚌鳃抗氧化系统在 Cu^{2+} 暴露下被激活,但是鳃组织器官仍然受到了氧化损伤。GPx、GST和GSH对 Cu^{2+} 暴露响应最为灵敏。

关键词:背角无齿蚌;鳃;抗氧化系统;铜

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Effect of sub-chronic copper exposure on the antioxidant system in the gills of *Anodonta woodiana*

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Abstract: To explore the effect of freshwater copper (Cu) pollution on the antioxidant system in the gills of the mussel *Anodonta woodiana*, the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST), and the contents of reduced glutathione (GSH), and malondialdehyde (MDA), and total antioxidant capacity (T-AOC) in mussel gills exposed to Cu^{2+} (0.137, 0.548, 2.192 $\text{mg} \cdot \text{L}^{-1}$) for 7, 14, 21 and 28 d, were tested. The results showed that: MDA content increased with increased Cu^{2+} concentration and extended exposure time, showing “dose-effect” and “time-effect” relationships. With Cu^{2+} exposure, the activity of antioxidant enzymes SOD, GPx, and GST were significantly induced ($P < 0.05$), while the activity of CAT was significantly inhibited ($P < 0.05$), in comparison to the control group. In addition, the content of the antioxidant GSH decreased significantly ($P < 0.05$). T-AOC was significantly higher ($P < 0.05$) than the control group after exposure to Cu^{2+} for 14, 21 and 28 d. Overall, the antioxidant system in gills of *A. woodiana* was activated under Cu^{2+} exposure, but the gills still suffered oxidative damage. In addition, the responses of GPx, GST, and GSH to Cu^{2+} exposure were the most sensitive among the indicators detected in this study, which could provide a scientific basis for using *A. woodiana* to monitor and assess freshwater copper pollution.

Keywords: *Anodonta woodiana*; gills; antioxidant system; copper

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铜(Copper, Cu)是机体必需的微量元素之一^[1],是超氧化物歧化酶(Superoxide dismutase, SOD)、细胞色素c氧化酶(Cytochrome c oxidase)、铜氧化酶(Ceruloplasmin)等许多抗氧化酶的辅因子,且具有促进机体造血、加快生长繁殖和增强机体免疫力的功能^[2]。然而,过多的Cu可诱发机体产生大量活性氧(Reactive oxygen species, ROS),引起生物膜损伤、蛋白质变性、酶失活及DNA突变^[3-4]。

近年来,巢湖、太湖、鄱阳湖Cu污染均已达中度以上水平,其污染来源主要是采矿废水、化肥施用等^[5-7]。研究表明,Cu可通过呼吸、进食和体表渗透等途径被水生动物吸收^[1],对水生动物的毒性较哺乳动物更大^[8-9]。贝类被认为是监测水体重金属污染的理想指示生物^[10-11],其体内的抗氧化酶及小分子抗氧化剂可作为生物标志物用来监测和评价水体Cu等重金属的污染程度^[12-15]。

背角无齿蚌(*Anodonta woodiana*)是在我国分布广泛的淡水蚌,对Cu具有较强的富集能力^[16],曾作为指示生物用于太湖五里湖重金属污染监测评估^[17]。然而,有关Cu对背角无齿蚌毒性的研究较少,且鳃是重金属蓄积的重要靶器官^[15]。因此,本研究通过检测亚慢性Cu²⁺暴露后背角无齿蚌鳃中抗氧化指标,包括超氧化物歧化酶、过氧化氢酶(Catalase, CAT)、谷胱甘肽过氧化物酶(Glutathione peroxidase, GPx)和谷胱甘肽转硫酶(Glutathione S-transferase, GST)活性,还原型谷胱甘肽(Reduced glutathione, GSH)和丙二醛(Malondialdehyde, MDA)的含量及总抗氧化能力(Total antioxidant capacity, T-AOC)水平,筛选可指示Cu污染的合适的生物标志物,为背角无齿蚌用于淡水Cu污染监测和评估提供科学依据。

1 材料与方法

1.1 实验材料

实验所用背角无齿蚌(以下简称“河蚌”)购自湖南省郴州市苏仙区栖凤渡镇水产市场,将其置于盛有曝气48 h以上自来水(pH 6.8, 溶氧6.0~6.3 mg·L⁻¹, 水温20±2℃)的塑料箱(长×宽×高为60 cm×42 cm×35 cm)中暂养3周,驯养期间每2 d换1次水,每日每只河蚌喂3.5×10⁴个小球藻,并维持室温20℃左右。

1.2 实验方法

1.2.1 主要仪器及试剂

仪器:高速冷冻离心机(Eppendorf, 5804R型),电动匀浆仪(FLUKO, F6/10型),电热恒温水浴锅(Ep-

pendorf, HHS型),多功能酶标仪(SpectraMax M5型)。

试剂:氯化铜(CuCl₂·2H₂O, 分析纯)、氯化钠(NaCl, 分析纯),蛋白定量测定试剂盒, SOD、CAT、GPx、GST测定试剂盒, GSH和MDA含量测定试剂盒, T-AOC测定试剂盒, 均购于南京建成生物工程所。

1.2.2 实验设计

称取5.884 g CuCl₂·2H₂O于小烧杯中,加入200 mL双蒸水充分溶解配制10 955 mg·L⁻¹的Cu²⁺母液。根据Cu²⁺对河蚌96 h的LC₅₀(109.55 mg·L⁻¹)设置3个Cu²⁺(0.137、0.548、2.192 mg·L⁻¹)暴露组和1个空白对照组,实验共进行28 d。选取个体大小相近(壳长6.4±2.7 cm、壳宽4.0±1.7 cm、壳顶高2.2±2.0 cm)的河蚌分别置于4个塑料箱(长×宽×高为51 cm×35 cm×30 cm)中,每箱随机投放20只,对照组加入20 L曝气48 h以上的自来水,暴露组加入20 L不同浓度的Cu²⁺溶液。暴露期间与驯养期间饲养条件保持一致。定时检查河蚌的死亡情况(本实验期间河蚌死亡率为87.5%),并及时挑出死亡个体。每组设4个平行。

1.2.3 样品制备

Cu²⁺暴露7、14、21、28 d后,每组随机取4只河蚌置于冰上,迅速剖取其鳃组织约0.1 g(称质量前用滤纸吸净组织表面水分),置于离心管中,用液氮速冻后转移至-80℃低温冰箱待测。按质量:体积为1:4的比例加入预冷的生理盐水,在冰上用电动匀浆器(FLUKO, F6/10型)制备匀浆,然后4℃下2800 r·min⁻¹离心10 min,取其上清液暂置于冰上。

1.2.4 测定方法

用考马斯亮蓝法测定组织蛋白含量,羟胺法测定组织SOD活力,可见光法测定CAT活力,比色法测定GPx和GST活力, TBA法测定MDA含量,微板法测定GSH含量, FRAP法测定T-AOC。

1.3 数据处理

采用SPSS 18.0软件对实验数据进行单因素方差分析(One-way ANOVA),运用Duncan法比较各暴露组和对照组间的差异,组间不同字母表示差异显著($P<0.05$)。实验结果均以“平均值±标准差”表示。

2 结果与分析

2.1 Cu²⁺暴露对河蚌鳃SOD活性的影响

如图1所示,随着暴露时间的延长,各暴露组河蚌SOD活性均表现为“诱导-恢复-诱导”的变化趋势。其中,低、中浓度组SOD活性在Cu²⁺暴露14 d以及高浓度组SOD活性在Cu²⁺暴露21 d时恢复到对照

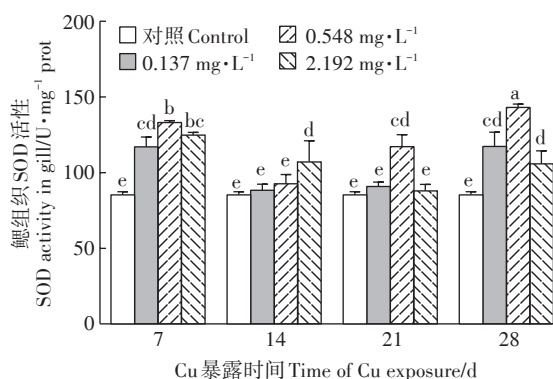
组水平。在 Cu^{2+} 暴露 28 d 时,随着 Cu^{2+} 浓度的增加, SOD 活性先升高后降低,且均显著高于对照组 ($P < 0.05$)。

2.2 Cu^{2+} 暴露对河蚌鳃 CAT 活性的影响

由图 2 可知,经 Cu^{2+} 暴露后,除高浓度组 Cu^{2+} 暴露 28 d CAT 活性较对照组无显著差异外,其余各组与对照组相比均被显著抑制 ($P < 0.05$)。从 Cu^{2+} 暴露 7 d 到 14 d,中浓度组 CAT 活性下降,从 Cu^{2+} 暴露 21 d 到 28 d,中浓度组 CAT 活性上升。

2.3 Cu^{2+} 暴露对河蚌鳃 GPx 活性的影响

由图 3 可知,经 Cu^{2+} 暴露后,河蚌鳃 GPx 活性较对照组被显著诱导 ($P < 0.05$)。低、高浓度组 GPx 活性随着暴露时间的延长呈现先升后降的变化趋势,且各暴露组 GPx 活性均在 Cu^{2+} 暴露 28 d 时达到最低,但仍显著高于对照组 ($P < 0.05$)。 Cu^{2+} 暴露 7、21、28 d 时,中、高浓度组 GPx 活性较低浓度组均显著降低 ($P < 0.05$)。



不同字母表示组间存在显著性差异 ($P < 0.05$)。下同
Different letters in figure indicate significant differences among groups ($P < 0.05$). The same below

图1 Cu^{2+} 对背角无齿蚌鳃组织 SOD 活性的影响

Figure 1 Effect of Cu^{2+} on SOD activities in gills of *A. woodiana*

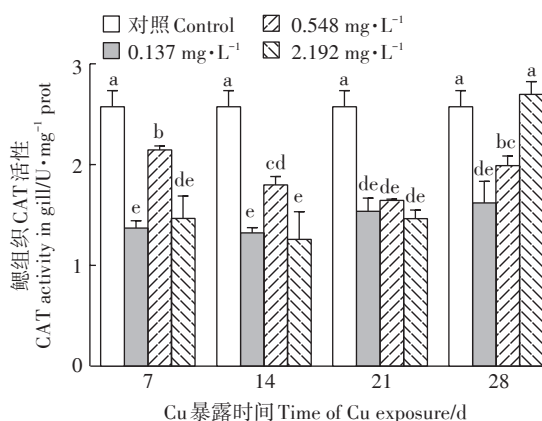


图2 Cu^{2+} 对背角无齿蚌鳃组织 CAT 活性的影响

Figure 2 Effect of Cu^{2+} on CAT activities in gills of *A. woodiana*

2.4 Cu^{2+} 暴露对河蚌鳃 GST 活性的影响

如图 4 所示,经 Cu^{2+} 暴露后,河蚌鳃 GST 活性较对照组被显著诱导 ($P < 0.05$)。随着暴露时间的延长,低浓度组 GST 活性无显著变化,中浓度组 GST 活性显著降低,高浓度组 GST 活性先升高后降低,且中、高浓度组 GST 活性均在 Cu^{2+} 暴露 28 d 时达到最低,但仍显著高于对照组 ($P < 0.05$)。 Cu^{2+} 暴露 21、28 d 时,中、高浓度组 GPx 活性较低浓度组均显著下降 ($P < 0.05$)。

2.5 Cu^{2+} 暴露对河蚌鳃 GSH 含量的影响

由图 5 可知,经 Cu^{2+} 暴露后,河蚌鳃 GSH 含量较对照组显著降低 ($P < 0.05$)。低、中浓度组 GSH 含量随着暴露时间的延长均先升高后降低,且均在 Cu^{2+} 暴露 28 d 时达到最低。 Cu^{2+} 暴露 7、21、28 d 时,高浓度组 GSH 含量均显著高于低浓度组 ($P < 0.05$)。

2.6 Cu^{2+} 暴露对河蚌鳃 T-AOC 的影响

如图 6 所示, Cu^{2+} 暴露 14、21、28 d 时,河蚌鳃 T-AOC 较对照组均显著升高 ($P < 0.05$),且各暴露组 T-AOC 随着暴露时间的延长均有所上升。 Cu^{2+} 暴露 21、28 d 时,随着 Cu^{2+} 浓度的增加, T-AOC 均先升后降,但

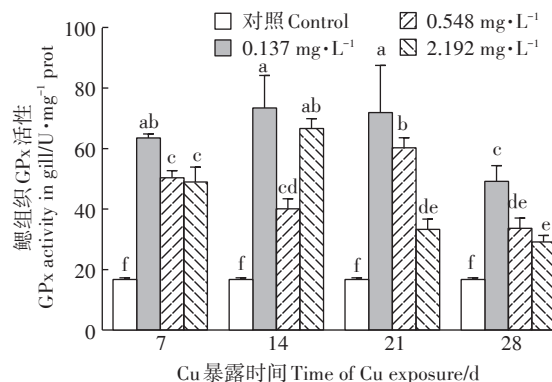


图3 Cu^{2+} 对背角无齿蚌鳃组织 GPx 活性的影响

Figure 3 Effect of Cu^{2+} on GPx activities in gills of *A. woodiana*

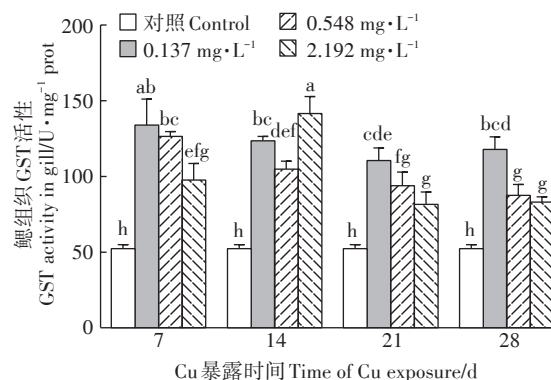


图4 Cu^{2+} 对背角无齿蚌鳃组织 GST 活性的影响

Figure 4 Effect of Cu^{2+} on GST activities in gills of *A. woodiana*

均显著高于对照组($P<0.05$)。

2.7 Cu^{2+} 暴露对河蚌鳃MDA含量的影响

由图7可知, Cu^{2+} 暴露14、21、28 d时, 河蚌鳃MDA含量较对照组均显著升高($P<0.05$)。各暴露组MDA含量均随着暴露时间的延长而上升。 Cu^{2+} 暴露14、21、28 d时, 高浓度组MDA含量较低、中浓度组均显著升高($P<0.05$)。

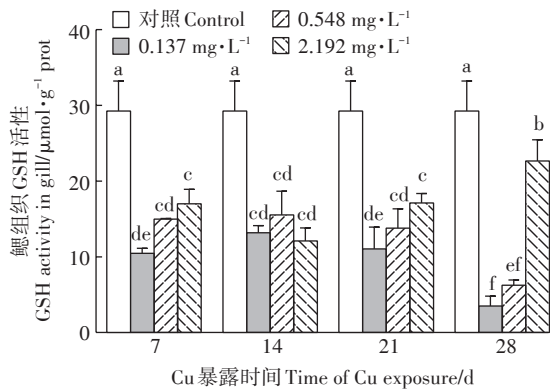


图5 Cu^{2+} 对背角无齿蚌鳃组织GSH含量的影响

Figure 5 Effect of Cu^{2+} on GSH content in gills of *A. woodiana*

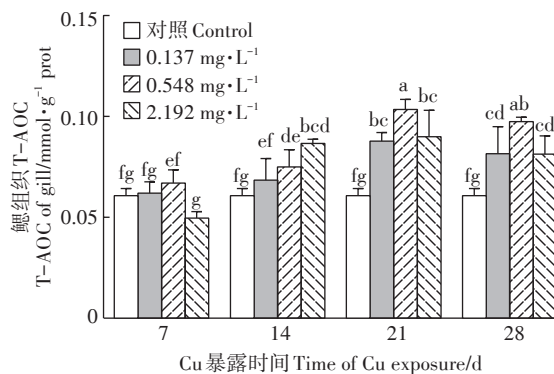


图6 Cu^{2+} 对背角无齿蚌鳃组织T-AOC的影响

Figure 6 Effect of Cu^{2+} on T-AOC in gills of *A. woodiana*

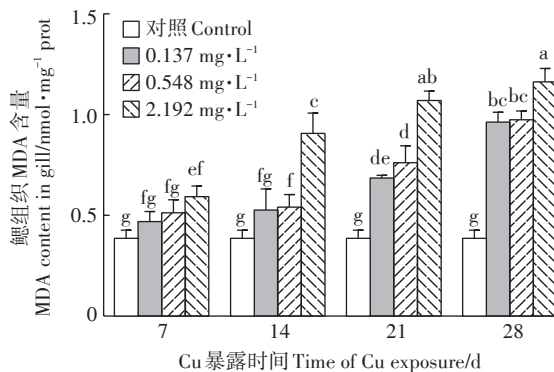


图7 Cu^{2+} 对背角无齿蚌鳃组织MDA含量的影响

Figure 7 Effect of Cu^{2+} on MDA content in gills of *A. woodiana*

3 讨论

SOD是生物体内重要的抗氧化酶,可清除生物体内超氧阴离子(Superoxide anion, $\text{O}_2^{\cdot-}$)^[18]。CAT是一种主要存在于生物线粒体和过氧化物酶体中富含巯基的氧化还原酶,可直接快速催化分解过氧化氢(Hydrogen peroxide, H_2O_2)转化为水和氧气^[19]。两者共同组成机体抗氧化系统的第一道防线^[20]。有研究报道,厚壳贻贝(*Mytilus coruscus*)和河蚌(*Corbicula fluminea*)经 Cu^{2+} 暴露后,其鳃SOD活性均被显著诱导^[21-22]。在本研究中,背角无齿蚌鳃SOD活性在 Cu^{2+} 暴露下也被诱导,可能是因为进入鳃中的 Cu^{2+} 诱发产生的 $\text{O}_2^{\cdot-}$ 促使SOD基因的表达上调,SOD蛋白的合成增加,酶活水平上升^[23-24]。 Cu^{2+} 暴露28 d时,各暴露组SOD活性均显著高于对照组,但高浓度组SOD活性较中浓度组显著降低,这与对葡萄牙牡蛎(*Crassostrea angulata*)研究所得到的结果类似^[25]。可能由于在长时间高浓度 Cu^{2+} 暴露下,背角无齿蚌鳃中产生了大量金属硫蛋白(Metallothionein, MT),MT可直接快速与 Cu^{2+} 结合来解毒重金属,使 $\text{O}_2^{\cdot-}$ 生成量减少,以 $\text{O}_2^{\cdot-}$ 为底物的SOD活性下降^[26],随着暴露时间的延长,各暴露组SOD活性均呈现“诱导-恢复-诱导”的变化趋势,表明 Cu^{2+} 暴露期间,背角无齿蚌鳃中 $\text{O}_2^{\cdot-}$ 生成量处于波动状态,受机体抗氧化系统动态调节。经 Cu^{2+} 暴露后,背角无齿蚌鳃CAT活性受到抑制,这与对厚壳贻贝的研究结果一致^[21]。可能由于背角无齿蚌鳃中积蓄的 Cu^{2+} 在参与机体内氧化还原反应(例如,芬顿反应)的过程中转化为 Cu^+ ,而 Cu^+ 又可与 H_2O_2 反应生成羟自由基,使得机体 H_2O_2 的含量降低,由于底物浓度的降低,引起CAT活性下降^[27-28]。随着暴露时间的延长,中浓度组CAT活性先降后升,但均显著低于对照组,这可能与中浓度组SOD活性变化有关。从 Cu^{2+} 暴露7 d到14 d,中浓度组SOD活性下降, H_2O_2 生成量的减少不足以为CAT提供底物,使CAT活性降低;从 Cu^{2+} 暴露21 d到28 d,中浓度组SOD活性上升, H_2O_2 生成量增加,CAT活性上升^[26]。

GPx在 H_2O_2 存在的情况下,可促进 H_2O_2 与GSH反应生成水及氧化型谷胱甘肽(Oxidized glutathione, GSSG)^[29]。GST是机体内具有清除有机过氧化物和解毒双重功能的酶,可催化某些外源有害物质的亲电子基团与GSH的巯基偶联,增强外源毒物疏水性,使其易于被分解排出体外,从而发挥解毒作用以保护机体细胞免受毒害^[29-30]。GSH是机体内的非蛋白巯基化

合物,具有清除自由基、解毒、维持红细胞膜完整性等多种重要生理功能^[31]。研究发现,河蚬及淡水贝 *Anodonta anatina* 经 Cu^{2+} 暴露后,其鳃 GPx 活性均被诱导^[32-33];菲律宾蛤仔 (*Ruditapes philippinarum*) 和翡翠贻贝 (*Perna viridis*) 经 Cu^{2+} 暴露后,其鳃 GST 活性均被诱导^[34-35]。在本研究中,经 Cu^{2+} 暴露后,背角无齿蚌鳃 GPx 活性被显著诱导,推测这可能是机体对 CAT 活性受到抑制作出代偿性调节的表现^[36]。背角无齿蚌鳃 GST 活性在 Cu^{2+} 暴露下也被显著诱导,可能由于在 Cu^{2+} 刺激下, GST 基因表达上调, GST 蛋白合成量增加,酶活水平升高^[37],加快催化了 Cu^{2+} 与 GSH 的巯基相结合,从而达到解毒目的^[30-31]。然而,经 Cu^{2+} 暴露后,背角无齿蚌鳃 GSH 含量显著降低,这与对背角无齿蚌进行急性镉暴露实验所得出的结果一致^[38]。可能是因为 GSH 一方面在 GPx 的催化下还原 H_2O_2 ,并在此过程中最终转化为 GSSG^[29],另一方面在 GST 催化下通过自身分子中的巯基或肽键等结构绑定结合重金属,从而降低重金属毒性^[30-31]。GSH 作为 GPx 和 GST 两种酶的共同底物,在 GPx 和 GST 两者活性均被显著诱导的情况下被大量消耗。 Cu^{2+} 暴露 7、21、28 d,各暴露组 GPx 和 GST 活性均显著高于对照组, GSH 含量显著低于对照组,高浓度组 GPx 和 GST 活性较低浓度组显著降低而高浓度组 GSH 含量较低浓度组显著上升,可能是因为当 Cu^{2+} 浓度升高到超出机体调节能力范围时,机体内 ROS 的产生与消除间的动态平衡被打破, ROS 不断增加,机体细胞毒性反应加重,酶合成受阻, GPx 和 GST 两者活性下降, GSH 消耗减少, GSH 含量回升^[27, 31]。

T-AOC 是衡量机体抗氧化酶系统与非酶促系统整体功能状态的综合性指标,表现为机体内各种抗氧化大分子、小分子和酶的总体水平^[39-40]。本研究结果显示, Cu^{2+} 暴露 14、21、28 d,背角无齿蚌鳃 T-AOC 较对照组均显著升高,且随着暴露时间的延长,各暴露组 T-AOC 均有所上升,表明随 Cu^{2+} 暴露时间延长,背角无齿蚌鳃中产生较为剧烈的氧化应激反应, T-AOC 适应性增高^[41]。在对美丽蚌 (*Lampsilis siliquoides*) 和三疣梭子蟹 (*Portunus trituberculatus*) 的研究中也得到了类似的结果^[42-43]。

MDA 是脂质过氧化的重要产物之一,其含量可直接反映机体脂质过氧化的程度,间接反映出细胞损伤的程度^[44]。研究表明, ROS 可攻击生物膜中的多不饱和脂肪酸,产生脂质过氧化物,从而对细胞造成损伤^[2]。本研究发现, Cu^{2+} 暴露 14、21、28 d,背角无齿

蚌鳃 MDA 含量较对照组均显著升高,且随 Cu^{2+} 浓度增加和暴露时间延长, MDA 含量逐渐上升,表现出明显的“剂量-效应”和“时间-效应”关系,类似的结果在对紫贻贝 (*Mytilus galloprovincialis*) 和沟纹蛤仔 (*Ruditapes decussatus*) 的研究中也被发现^[40, 45]。可能由于在长时间高浓度 Cu^{2+} 暴露下,背角无齿蚌鳃中 ROS 累积超过一定限度,尽管机体充分调动抗氧化系统来应对 Cu^{2+} 引起的氧化胁迫,但仍不足以保护鳃免受氧化损伤,脂质过氧化程度加深。

4 结论

(1) 亚慢性 Cu^{2+} 暴露对背角无齿蚌鳃 SOD、GPx、GST 活性均有诱导作用,对 CAT 活性有抑制作用。背角无齿蚌鳃抗氧化系统在 Cu^{2+} 暴露下被激活,但仍受到了氧化损伤。

(2) 背角无齿蚌鳃中 GPx、GST 和 GSH 对 Cu^{2+} 暴露响应最为灵敏,可以作为生物标志物用于水体 Cu 污染的监测和评估。

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