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不同官能团微塑料对斑马鱼胚胎菌群和代谢功能的胁迫效应

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摘要: 为探究官能团修饰对微塑料生物效应的影响规律和机制, 分析了3种聚苯乙烯微塑料(PS、PS—NH₂和PS—COOH)在斑马鱼(*Danio rerio*)胚胎内的累积特征, 并利用微生物组学和代谢组学相结合的分析技术, 解析了斑马鱼幼鱼体内代谢功能及微生物群落的响应规律。结果显示, 3种微塑料都可以在斑马鱼体内产生累积, 其含量范围为143~175 $\mu\text{g}\cdot\text{g}^{-1}$, 且不存在明显差异性。斑马鱼胚胎绒毛膜是微塑料早期暴露的主要累积场所, 但不能有效阻止小粒径微塑料的穿透。未修饰的PS明显影响了斑马鱼幼鱼体内氨基糖苷类的代谢能力, PS—NH₂主要对氨基酸的代谢过程产生了影响, 而PS—COOH则主要作用于三羧酸循环、氨基酸及糖酵解等代谢通路。3种微塑料均导致了斑马鱼幼鱼体内代谢功能的变化, 从而对斑马鱼产生了毒性效应, 而微塑料的官能团修饰则加强了这一影响程度。与对照组相比, PS—NH₂明显降低了斑马鱼幼鱼体内的微生物群落的多样性, 显著增加了变形菌门(Proteobacteria)的占比, 导致斑马鱼幼鱼体内的菌群失衡, 从而引起鱼体代谢功能的紊乱。微塑料的官能团修饰可能会明显改变其对生物体的胁迫效应, 导致难以预测的生态风险。

关键词: 微塑料; 官能团; 代谢物; 菌群; 累积

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Biological Effect of Microplastics with Different Functional Groups on the Bacterial Communities and Metabolic Functions of Zebrafish (*Danio rerio*) Embryos

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Abstract: To investigate the influences of functional groups on the biological effects caused by microplastics, the accumulation of three polystyrene microplastics (PS, PS—NH₂, and PS—COOH) in zebrafish (*Danio rerio*) embryos were analyzed, and then the responses of metabolic functions and microbial communities in zebrafish larvae were revealed using the combination of the microbiome and metabolome methods. The results showed that all microplastics could accumulate in zebrafish with concentrations ranging from 143 to 175 $\mu\text{g}\cdot\text{g}^{-1}$, and there were no significant differences in the accumulation potentials among different PS treatments. Exposure to plain PS significantly affected the metabolic capacity of aminoglycosides in zebrafish larvae, whereas the metabolic processes of amino acids were affected by PS—NH₂. In the PS—COOH treatment, the metabolic pathways of the tricarboxylic acid cycle, amino acids, and glycolysis in zebrafish were markedly altered. The metabolic functions of zebrafish larvae were changed by all PS microplastics, resulting in toxic effects on zebrafish, and the functional group modification of microplastics may have further enhanced these toxicities. Compared to that in the control, exposure to PS—NH₂ significantly reduced the diversity of microbial communities in zebrafish larvae and increased the proportion of Proteobacteria in the composition, leading to an imbalance of the bacterial community in zebrafish and thus disrupting the metabolic functions in the fish. Therefore, the functional modifications of microplastics may significantly alter the related stresses on aquatic organisms, leading to unpredictable ecological risks.

Key words: microplastic; functional groups; metabolites; bacterial community; accumulation

塑料制品由于性能优良而在日常生产生活中大量应用, 其全球年产量已超过4.6亿t, 并预计在2060年增长至12.31亿t^[1]。但由于管理不当和极低的回收利用效率, 约2/3的塑料产品直接进入环境成为塑料垃圾, 并在自然风化和降解等作用下形成微塑料^[2,3]。当前, 微塑料广泛分布在河流、湖泊和海洋等水体环境中, 并能通过其自身及载体效应传播致病菌和抗性基因, 甚至经食物链和代际传递等过程, 对水生生物体和生态系统安全造成严重危害^[4~7]。因此, 联合国环境规划署将微塑料列为全球性新污染物, 我国也在2022年将其列入《新污染物治理行动方案》。面

向微塑料污染防控的国家重大需求, 解析微塑料的生物效应及机制是当前迫切需要解决的科学问题, 也是实施微塑料污染防控的基础和前提。

微塑料对水生动物的影响研究已在全世界范围内广泛开展起来^[8~10]。由于尺寸和浮游生物相类似, 水环境中的微塑料极易被鱼、虾和贝等水生生物所摄取, 并产生生物累积现象^[11,12]。例如, Kashiwada^[13]发现聚苯乙烯(PS)微塑料可以穿透血脑屏障进入青

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鲮鱼(*Oryzias latipes*)的血液和脑组织中。Farrell等^[14]也证实微塑料可以被贻贝所摄取,并通过食物链传递累积在螃蟹的鳃、胃和血淋巴等组织中。而累积的微塑料则可进一步通过生长抑制、神经干扰、组织病理损伤及氧化应激等途径对生物体产生各种不良的生物效应和毒性作用^[15]。例如,聚氯乙烯微塑料暴露会抑制鲤鱼(*Cyprinus carpio*)的生长,导致其肝、肠和鳃的氧化损伤,并引起肝脏组织学病变^[16]。经PS微塑料处理后,罗非鱼(*Oreochromis mossambicus*)脑组织中的神经酶活性受到显著抑制,产生了明显的神经毒性^[17]。此外,肠道作为微塑料在生物体内的重要聚集器官,更易受到微塑料的影响,进而出现绒毛破裂、炎症以及阻塞等肠道疾病和症状^[18]。尽管已有研究关注微塑料对水生生物体肠道功能的影响,但其影响过程及机制还不明确。

肠道菌群是在动物肠道中栖息的各种微生物的总称,其在生物体营养吸收、生长发育和免疫调节等过程中发挥着重要作用,对维持宿主的健康至关重要^[19,20]。尤其是肠道菌群可以通过参与调节宿主的代谢功能而影响其新陈代谢和免疫作用,且影响范围不仅局限于肠道环境,而是能够通过脑肠轴作用对生物体全身产生干扰^[21]。肠道菌群的组成和代谢功能不仅与宿主的生命阶段相关,也受到抗生素和重金属等环境污染物的影响,进而导致肠道菌群失调和代谢紊乱,对宿主产生一系列的毒性作用^[22-24]。然而,微塑料是否会影响生物体肠道菌群的组成和代谢功能等还不清晰,微塑料在自然及人工条件下产生的官能团修饰是否会对肠道菌群及代谢功能产生不一致的影响仍需深入探究。

斑马鱼是研究肠道菌群与宿主相互作用的理想模型,其幼鱼肠道在受精后2 d内即开始发育,并在4 d内发育完成开始有微生物定殖^[25]。探究环境污染条件下斑马鱼幼鱼肠道菌群变化与代谢功能响应是解析其对生物体早期生长发育和健康效应影响的重要方法。因此,本文选取了3种不同官能团修饰的PS微塑料为研究对象,以斑马鱼胚胎为受试生物,分析了不同种类微塑料在斑马鱼体内的累积水平,并利用不同组学方法解析了斑马鱼幼鱼肠道菌群的组成和代谢功能变化,揭示了不同官能团微塑料对斑马鱼幼鱼菌群失调和代谢紊乱的作用过程,以期掌握微塑料对水生生物健康效应的影响提供科学基础。

1 材料与方法

1.1 主要材料

PS是环境介质中检出频率较高的一种微塑料聚合物,也是目前研究毒性效应常用的微塑料种类。因

此,选取不同官能团修饰的PS微塑料为研究对象,分别为未修饰PS微塑料(PS)、氨基化PS微塑料(PS—NH₂)和羧基化PS微塑料(PS—COOH),直径均为100 nm,均购自天津塞尔群科技有限公司(天津,中国)。同时,选取相同直径和官能团的绿色荧光标记PS微塑料用于生物累积实验,其最大激发波长和最大发射波长为488 nm和518 nm,且均购自天津塞尔群科技有限公司(天津,中国)。所有微塑料在使用前均在乙醇中用超声波清洗3次,以去除表面的分散剂等化学物质。

1.2 受试生物

成年野生型斑马鱼(AB型)由南京大学模式动物研究所提供,并在实验室的斑马鱼循环水系统(上海海圣,中国)中驯化和饲养。水温保持为(27±1)℃,光/暗周期设置为14 h/10 h,pH在7.0~7.2之间,溶解氧>7 mg·L⁻¹,每天喂食2次活体丰年虾。

利用光线刺激触发斑马鱼的受精行为,收集鱼卵并先后用亚甲基蓝溶液和标准稀释水冲洗,并在暴露4 h后使用SZX16体视显微镜(Olympus,日本)筛选健康的胚胎用于后续暴露实验,实验过程遵循OECD 236的规定进行^[26]。

1.3 微塑料累积暴露实验

根据微塑料在不同水环境中检出的最高环境浓度(4.65 mg·L⁻¹)并考虑到其不断积累的特征^[27,28],本实验中微塑料的暴露浓度设置为10 mg·L⁻¹。在此浓度下,PS微塑料不会对斑马鱼胚胎的生长发育产生显著影响,这一结果得到赵佳等^[29]及本实验室前期研究的证实^[30]。为了便于观察和计数,将绿色荧光标记的PS微塑料用于累积暴露实验,标记该塑料的荧光材料包埋在微塑料内部,在暴露和处理过程中不会发生泄漏。为保证微塑料的分散性,暴露溶液现配现用。暴露组设置为空白对照组(control)、未修饰PS处理组(PS)、氨基化PS处理组(PS—NH₂)和羧基化PS处理组(PS—COOH)共4个处理组。

将发育良好的斑马鱼胚胎分别放入直径18 cm的玻璃培养皿中,并加入相应的暴露溶液80 mL,每组包含150粒受精卵,设置4个平行组别。所有胚胎均在置顶LED光照培养箱中培育,光/暗周期、水温、pH和溶解氧等水质参数和斑马鱼驯化阶段相同。暴露期间每天更换90%的暴露溶液以确保微塑料浓度的相对稳定。暴露24 h后,收集各组斑马鱼胚胎并在标准稀释水冲洗3次,随后借助荧光显微镜和高分辨扫描电镜(SEM)观察不同官能团微塑料在斑马鱼胚胎中的赋存情况。继续暴露至第5 d,统计微塑料在斑马鱼幼鱼中的生物累积情况。

1.4 微塑料毒性暴露实验

结合微塑料的累积实验,利用非荧光标记的PS微塑料开展斑马鱼胚胎毒性暴露实验。暴露组别仍设置为对照组(control)、PS处理组、PS—NH₂处理组和PS—COOH处理组这4个组别,暴露浓度为10 mg·L⁻¹。其余暴露条件设置与累积实验相同,暴露周期设置为5 d,随后采集斑马鱼幼鱼用于随后的微生物多样性和代谢组学分析。

1.5 微塑料累积测试

累积暴露实验结束后,每个处理平行随机选取30条斑马鱼幼鱼,用稀释水冲洗3次,以除去其表面粘附的微塑料颗粒。随后,转移幼鱼至冰上使其停止生命活动,并转移至2 mL离心管称重并制备全鱼组织匀浆。基于文献[31],采用10% KOH在60℃下消解12 h。获取消解液后,采用荧光分光光度计(Hitachi F7000,日本)测定累积微塑料的荧光强度。

1.6 代谢组学分析

毒理学暴露周期结束后,每组随机选取5个平行样本,每个样本随机挑选120条幼鱼经冷冻处理后置于2 mL离心管中,同时加入500 μL提取液(甲醇:水=4:1,含有2%的L-2氯苯丙氨酸),在-10℃匀浆3 min,而后加入200 μL氯仿,继续研磨3 min。超声萃取10 min后,萃取液用离心机高速离心20 min(4℃, 12 000 r·min⁻¹)。取上清液真空抽干,加入80 μL甲氧胺吡啶溶液,于37℃反应90 min;再加入80 μL三氟乙酰胺(含1%三甲基氯硅烷)和20 μL正己烷,在70℃下反应60 min。反应液在室温下静置30 min,而后取1 μL利用Agilent公司的8890B-5977B气相色谱质谱联用仪(GC-MS, Agilent, 美国)进行代谢组学分析。其色谱条件为:HP-5MS UI毛细管柱,初始温度60℃,平衡30 s后以8℃·min⁻¹的速度升至310℃并维持6 min。进样口温度为260℃,载气为高纯氮气,载气流速为1 mL·min⁻¹。质谱条件为:电子轰击离子源,离子源温度230℃,四极杆温度150℃,电子能量70 eV,扫描方式为全扫描模式(SCAN),质量扫描范围 m/z 为50~500 u,扫描频率为3.2 scan·s⁻¹,进而获得总离子流色谱图。

1.7 微生物组成分析

毒性实验结束后,每组随机挑选120条幼鱼,3个平行样品用于微生物组成和多样性分析。样品总DNA采用E. Z. N. A.® soil DNA kit(Omega Bio-tek, 美国)进行提取,随后使用1%的琼脂糖凝胶电泳对DNA的提取质量进行检测,并使用NanoDrop2000(Thermo, 美国)进行浓度和纯度测定。随后使用338F(5'-ACTCCTACGGGAGGCAGCAG-3')和806R(5'-GGACTACHVGGGTWTCTAAT-3')对16S rRNA基因

的V3-V4可变区进行PCR扩增。扩增程序为95℃预变性3 min,27个循环(95℃变性30 s,55℃退火30 s,72℃延伸30 s),然后于72℃稳定延伸10 min,4℃保存。每个样本进行3个重复扩增。而后将同一样本的PCR产物混合后使用2%琼脂糖凝胶进行回收和纯化,并对回收产物进行定量检测。使用NEXTflex™ Rapid DNA-Seq Kit(Bioo Scientific, 美国)进行建库并利用Illumina公司的MiSeq PE300/NovaSeq PE250平台进行测序。

1.8 数据分析

斑马鱼胚胎的代谢组学数据在质控样品基础上借助ChromaTOF软件进行处理,采用偏最小二乘判别分析方法(PLS-DA)分析不同处理组之间的代谢差异及组内样本变异度大小,依据正交偏最小二乘法判别分析(OPLS-DA)模型的VIP值和 t 检验 P 值筛选显著差异代谢物,并根据差异代谢物在MetaboAnalyst 4.0和iPath 3平台进行代谢通路及途径分析。微生物组成和多样性数据利用Omicsmart实时交互平台(<http://www.omicsmart.com>)进行分析,其中利用操作性分类单元(operational taxonomic unit, OTU)聚类分析物种的丰富程度,利用Mothur(version 1.33.3)进行alpha多样性分析(Shannon和Simpson等)。不同处理之间的多重比较采用单因素方差分析中的Turkey统计检验法, $P<0.05$ 为统计显著性。

2 结果与讨论

2.1 微塑料的生物累积

暴露24 h后,不同官能团PS微塑料在斑马鱼胚胎中的累积情况如图1所示,包括斑马鱼胚胎上荧光微塑料的累积情况和同时时间段经去膜化处理后的胚胎。由图1可知,PS微塑料处理后,其在斑马鱼胚胎绒毛膜上的生物累积明显增强,但3种PS微塑料处理组之间没有显著差异性。这表明胚胎绒毛膜是PS微塑料的重要累积场所,对PS微塑料进入生物体具有一定的保护效果。同时,去膜化处理后的斑马鱼胚胎卵黄囊中也发现了微塑料的累积,这表明100 nm的PS微塑料可能会穿透绒毛膜进入到斑马鱼胚胎内部,这一结果也得到Pitt等的证实^[32],其发现斑马鱼胚胎绒毛膜可以有效拦截大尺寸微塑料,但对小粒径的微塑料阻拦效果不佳。

为了进一步证实斑马鱼胚胎绒毛膜对小粒径PS微塑料的拦截效果,随后对斑马鱼胚胎绒毛膜的结构进行了SEM扫描,结果见图1。斑马鱼胚胎绒毛膜外侧平滑,存在均匀分布的绒毛膜孔通道,直径约为660 nm。经100 nm微塑料暴露后,在胚胎绒毛膜的外侧和内侧同时发现了微塑料的存在,证实

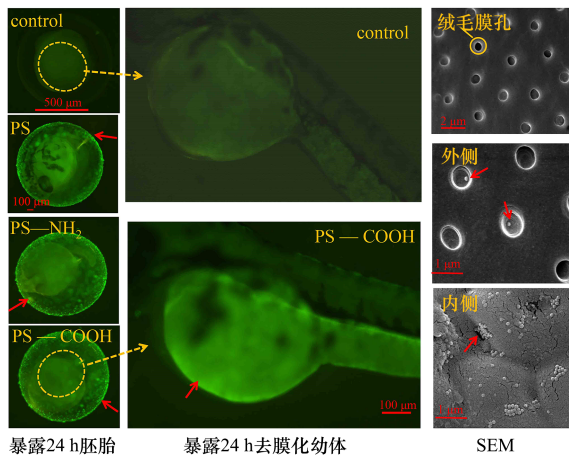


图1 不同荧光微塑料在斑马鱼胚胎中的累积情况
及斑马鱼胚胎绒毛膜SEM扫描图片

Fig. 1 Accumulation of different fluorescent microplastics in zebrafish embryos and SEM images of zebrafish embryonic chorionic membrane

了微塑料可以穿过绒毛膜孔。结果表明,绒毛膜是斑马鱼胚胎发育前期(从受精至咽囊期)实施自我保护的天然屏障,可以有效控制大颗粒物质的流入或外排^[33],但尺寸远远小于绒毛膜孔直径的微塑料则可以穿透绒毛膜屏障,累积在胚胎内部的卵黄囊中。卵黄是给斑马鱼胚胎早期发育提供营养和能量的关键部位,累积在卵黄囊中的微塑料可能会随着胚胎的发育而逐步转移到胃肠道、肝脏、心脏和大脑等关键部位^[32],进而威胁斑马鱼正常的生长发育过程。

暴露 120 h 后,不同 PS 微塑料在斑马鱼幼鱼体内的累积情况如图 2 所示,其含量范围介于 $(143 \pm 36) \sim (175 \pm 24) \mu\text{g} \cdot \text{g}^{-1}$ 之间,相应的生物累积系数(BCF)为 14.8~17.7,且 3 种微塑料之间不存在显著差异性。Liu 等在先前的研究中也发现,PS 微塑料在青鳉鱼体内的 BCF 在 16.8~139.9 之间,微塑料的尺寸越小,其生物累积能力越强^[34]。考虑到 3 种 PS 微塑料在斑马鱼幼鱼体内的 BCF 均远远小于 1 000,均不具有生物

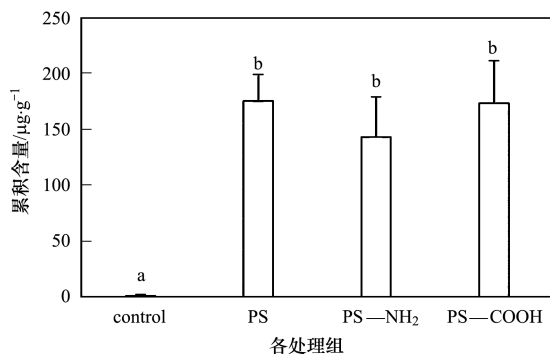


图2 不同 PS 微塑料在斑马鱼幼鱼中的累积含量

Fig. 2 Accumulations of different PS microplastics in zebrafish embryos

不同小写字母表示各组之间存在显著性差异($P < 0.05$)

累积性。

2.2 代谢差异物

利用有监督的 PLS-DA 分析不同官能团 PS 微塑料处理组及对照组斑马鱼幼鱼体内的代谢图谱,得分见图 3。结果显示,不同官能团 PS 微塑料暴露处理的斑马鱼幼鱼代谢图谱在图 3 中聚集良好且与对照组相比完全分开,且模型解释率($R^2 \geq 0.9$)和预测率($Q^2 \geq 0.8$)数值较高,表明 3 种不同官能团 PS 微塑料暴露均对斑马鱼幼鱼体内的代谢功能具有不同的调节作用且诱导产生的代谢谱也存在明显差异性。

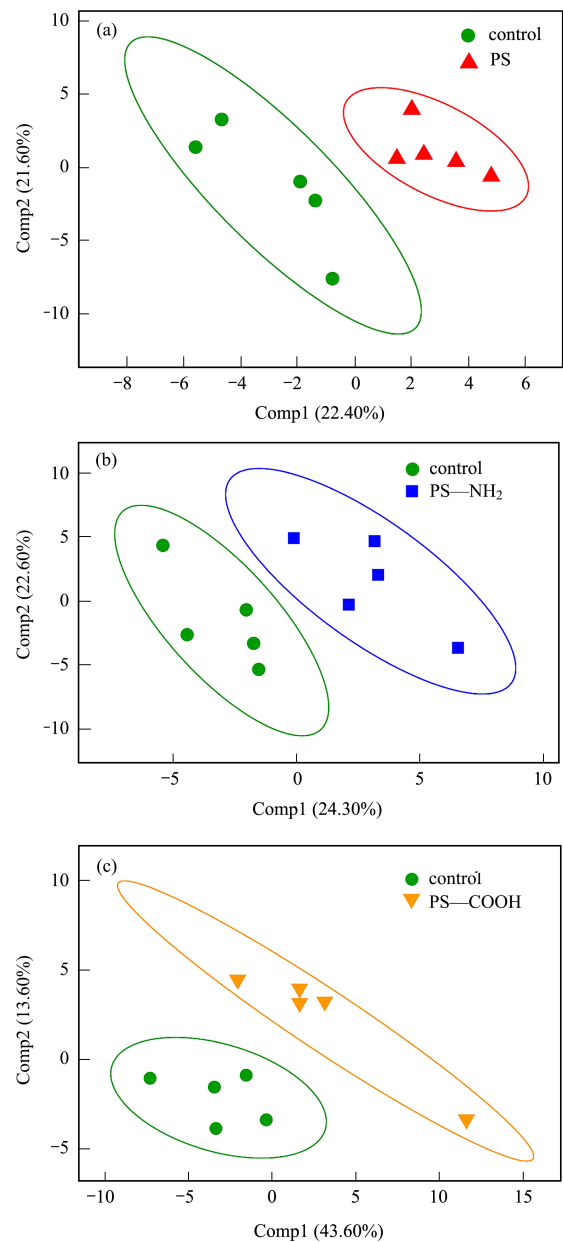


图3 不同微塑料处理组与对照组相比较的 PLS-DA 得分

Fig. 3 PLS-DA plots of different microplastic treatments compared to the control treatment

为了更好地反映不同官能团 PS 微塑料处理组与对照组之间差异情况,采用有监督的 OPLS-DA 进一

步对代谢物数据进行分析,选择变量重要性(VIP)值 >1 且 P 值 <0.05 的代谢物作为处理组与对照组之间的差异代谢物.进一步通过NIST谱库检索,并将代谢物数据与KEGG和HMDB等数据库进行在线对比确认,得到差异代谢物在数据库中的注释信息,不同微塑料处理组诱导产生的差异代谢物见图4.

相较于对照组,PS微塑料处理组共筛选出18个显著差异代谢物;而PS—NH₂微塑料则导致产生了33个显著差异代谢物,包括上调了29个代谢物,降低了芥子酸、油酸酰胺、黄嘌呤核苷和蔗糖-6-磷酸等4个代谢物的水平;暴露于PS—COOH微塑料后,19种代谢物的含量显著增加,而绿原酸和氨丁三醇的含量则有所下降.这一结果表明,3种PS微塑料均会改变斑马鱼幼鱼体内的代谢物含量,且PS—COOH和PS—NH₂导致了更多的差异代谢物上调,而未修饰的PS则导致了大量代谢物含量下调,官能团修饰过程可能会使PS微塑料对斑马鱼代谢功能的抑制作用转向刺激效应.

在以上差异代谢物中,脂肪酸、氨基酸、碳水化合物和核酸等是主要的代谢物种类.脂肪酸作为生物体的骨架成分和能量供应者,在生物体信号传导和器官分化等细胞进程中有着重要作用.不同PS微塑料暴露对斑马鱼体内脂肪酸含量的影响表明,其可通过调节脂肪酸的生成和代谢过程而改变斑马鱼生命早期的生长和发育过程.此外,PS微塑料对斑马鱼的氧化损伤也会造成其体内脂肪酸含量的变化,这一影响过程可能会进一步导致生物膜的破坏和细胞功能的衰退^[35].此外,外源性的污染物可能会对斑马鱼肝脏功能产生影响,使得其能量代谢和氨基酸代谢等过程发生紊乱,导致生物体内氨基酸含量的明显增加^[36].在本研究中,PS—COOH和PS—NH₂微塑料暴露均导致了斑马鱼体内L-半胱氨酸、苏氨酸等氨基酸的大量生成,也证实了官能团修饰的PS微塑料会对斑马鱼肝脏产生损伤.此外,斑马鱼体内碳水化合物相关代谢物含量的变化表明,PS微塑料暴露会导致斑马鱼需要消耗更多的能量来维持正常的生理活动,进而导致生长发育迟缓等现象.

2.3 代谢通路

为进一步解析微塑料对斑马鱼代谢功能的影响,将不同微塑料处理组的差异代谢物导入MetaboAnalyst 5.0进行KEGG富集分析和拓扑学分析,并筛选出其潜在的代谢途径,结果如图5所示.未修饰的PS暴露显著提高了新霉素、亚油酸以及糖类等相关的合成和代谢功能,而PS—NH₂处理则导致了斑马鱼体内与氨酰tRNA、色氨酸、苯丙氨酸和组氨酸等相关的代谢通路受到显著影响.在

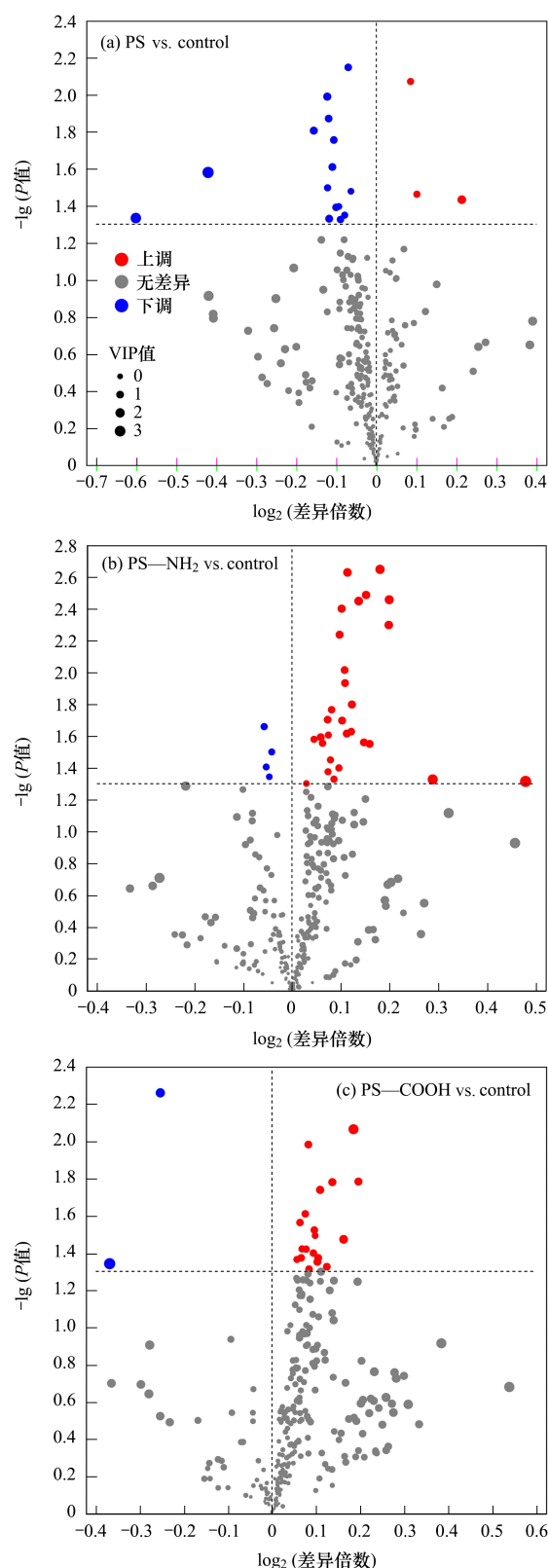
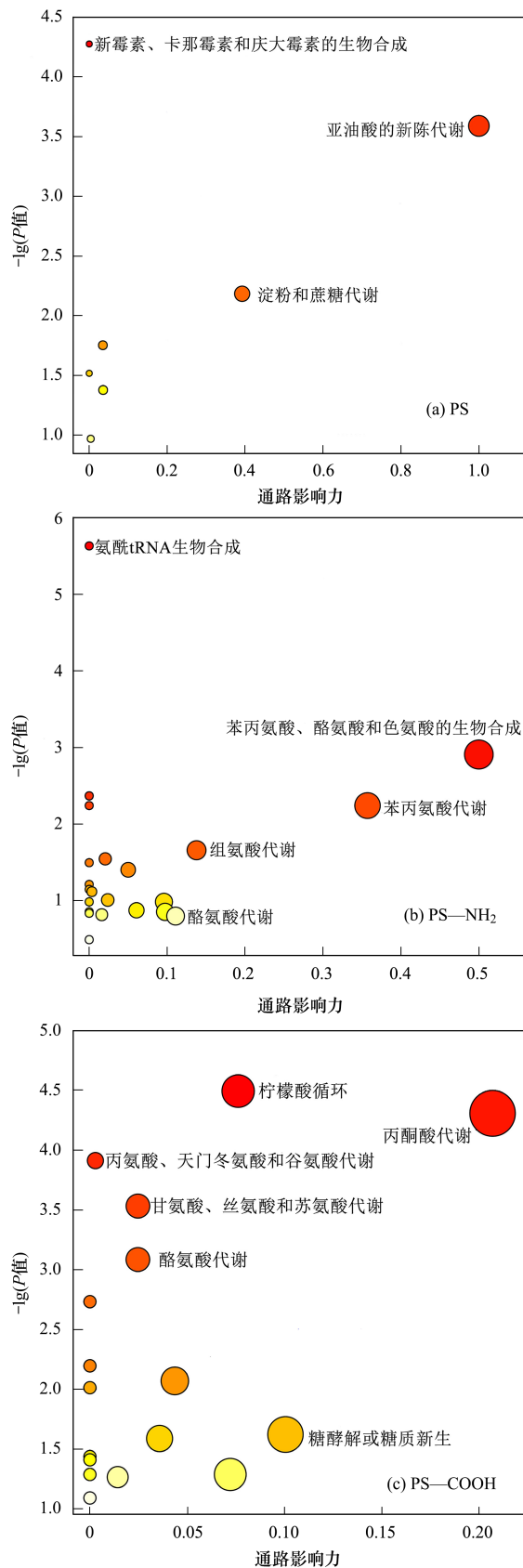


图4 不同PS微塑料处理组中斑马鱼幼鱼体内的差异代谢物火山图
Fig. 4 Volcano plots of different metabolites in zebrafish treated with different PS microplastics

PS—COOH暴露组中,斑马鱼幼鱼的三羧酸循环、丙酮酸、苏氨酸和酪氨酸的代谢功能以及糖酵解等途径受到明显影响.相较于未修饰的PS,两种官能团修饰的微塑料导致了斑马鱼体内更多代谢途径的

变化.



气泡面积大小与横坐标通路影响力成正比,气泡颜色深浅与纵坐标通路的富集显著性相关

图5 不同PS微塑料处理组中斑马鱼幼体内代谢通路的气泡图

Fig. 5 Bubble charts of metabolic pathway in zebrafish treated with different PS microplastics

将上述差异代谢物及代谢通路信息导入 iPath3, 并结合文献[37], 整合得到3种微塑料作用下斑马鱼体内受影响的主要代谢途径(图6). 结果显示, 3种微塑料主要通过改变斑马鱼体内三羧酸循环, 糖脂、核苷酸和氨基酸代谢等途径影响斑马鱼的代谢功能. 在先前研究中, PS微塑料暴露 21 d后显著改变了斑马鱼在转录水平上的生理和生化功能, 引起了斑马鱼肝脏糖脂代谢的紊乱^[38]. 此外, 微塑料暴露也扰乱了斑马鱼体内脂类、信号分子、脂肪醇代谢和 TCA 循环等功能^[39]. 以上研究结果与本研究 PS微塑料对斑马鱼体内糖脂代谢、核酸代谢和能量代谢等多个代谢功能的影响存在一定的差异性, 这可能是和实验所用斑马鱼所处的生命阶段有关, 斑马鱼在其生命早期对外源污染物的响应更为敏感. 此外, 斑马鱼品系的不同, 也会在其生理响应方面存在差异性^[40].

结合代谢差异物和代谢途径分析发现, PS可以通过下调腺嘌呤和胸腺嘌呤等途径而导致斑马鱼核苷酸代谢过程的紊乱. PS-NH₂暴露则可通过上调苯丙氨酸、酪氨酸和色氨酸而参与到多巴胺等神经递质的合成, 进而对斑马鱼幼鱼的神经系统发育产生重要影响; 也能通过上调果糖-6-磷酸的含量而影响糖酵解过程. 海藻糖和半乳糖醇是斑马鱼体内糖酵解代谢过程中的关键物质, PS-COOH对其上调作用表明, 斑马鱼体内的葡萄糖稳态受到了PS-COOH的破坏. 三羧酸循环是生物体内糖类、脂质、氨基酸三大物质代谢转化以及维持能量代谢的重要过程, 丙酮酸则是其中的关键物质. 在本研究中, PS-COOH暴露导致斑马鱼体内丙酮酸含量的显著上调, 显著影响了斑马鱼体内的三羧酸循环过程, 进而诱导斑马鱼产生自然防御性反应. 已有研究显示, 微塑料暴露可以显著改变水生生物的神经发育和行为学响应, 影响糖类关键生源物质的代谢过程^[41, 42], 进而导致生物体糖脂、氨基酸和三羧酸循环等过程受到明显干扰.

2.4 菌群多样性及组成

基于 OTU 对不同处理组别的微生物进行 alpha 多样性分析, 得到微生物在门和属水平下的 Shannon 指数和 Simpson 指数, 结果如图 7 所示. 相比于对照组, PS-NH₂显著降低了斑马鱼体内微生物在门和属分类水平上的 Shannon 指数, 并明显提升了其 Simpson 指数, 而 PS 和 PS-COOH 则对微生物的多样性指数没有显著影响. 这表明, 经过 PS-NH₂暴露处理的斑马鱼体内微生物群落的多样性有较明显的下降, 而其余两组的微生物多样性差异较小.

为进一步解析微生物组成的差异, 采用 RDP

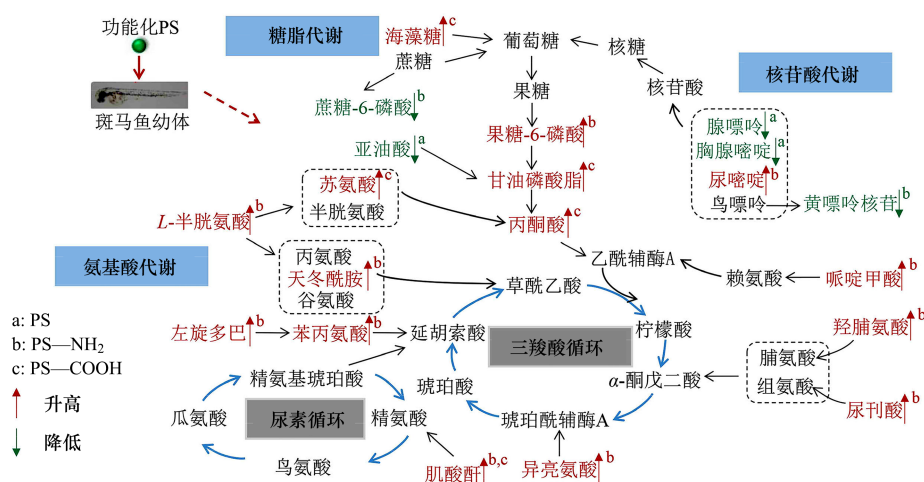


图6 不同PS微塑料处理组中斑马鱼差异代谢物可能参与的代谢通路

Fig. 6 Possible metabolic pathways involved in the differential metabolites of zebrafish in different PS microplastic treatments

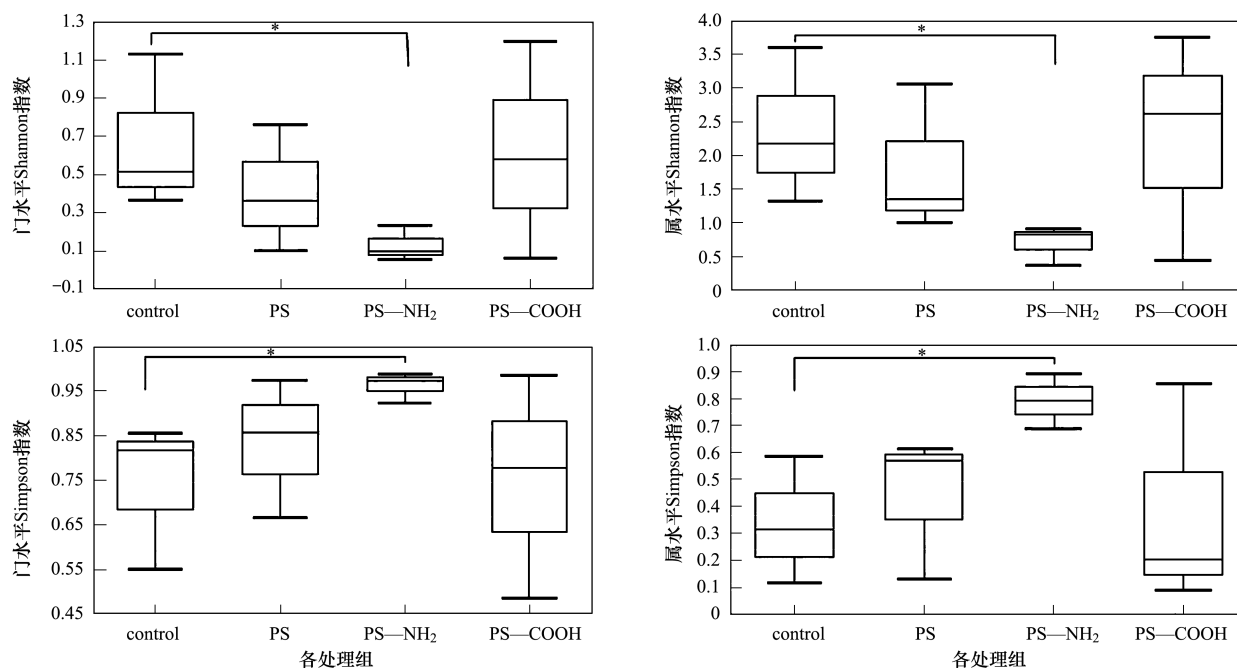
* 表示不同处理组之间存在显著性差异 ($P < 0.05$)

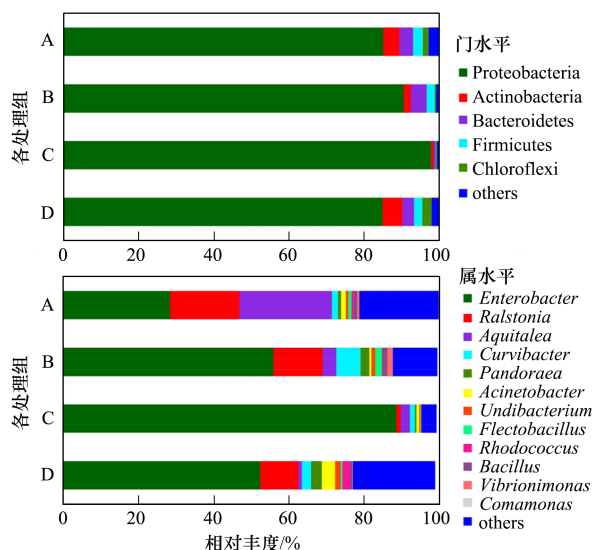
图7 不同处理组中斑马鱼菌群的门和属水平下的 Shannon 和 Simpson 指数

Fig. 7 Shannon and Simpson index of bacterial community in zebrafish in different treatments at the phylum level and genus level

classifier 贝叶斯算法对 OTU 进行分类学分析,并在门和属水平上统计不同处理组中斑马鱼体内微生物群落的物种组成,结果如图 8 所示. 在门水平上,不同微塑料处理组和对照组斑马鱼幼鱼体内的优势微生物群落组成相近,优势菌群主要包括变形菌门 (Proteobacteria)、放线菌门 (Actinobacteria)、拟杆菌门 (Bacteroidetes)、厚壁菌门 (Firmicutes) 和绿弯菌门 (Chloroflexi). 但不同菌门在各处理组中的占比不同,和对照组相比,PS-COOH 处理组的菌群占比十分相似;PS 处理组则略有不同,主要体现在绿弯菌门占比较小;而 PS-NH₂ 处理则导致了菌门占比的显著改变,其中变形菌门的占比明显增加,其余菌门明显

下降. 在属水平上,相比于对照组,各个微塑料处理组中的肠杆菌属 (*Enterobacter*) 比例均有增加,青枯菌属 (*Ralstonia*) 和 *Aquitalea* 则有所减少,尤其是 PS-NH₂ 处理组中,斑马鱼体内的菌属数目仅为对照组的 50%. 此外,微塑料的存在也增加了独有菌属的数目,其中约 30% 的菌属未在对照组中检出.

近年来,生物体内的微生物群落被认为是宿主隐藏的代谢器官,不仅能够参与维生素和氨基酸的合成,也会影响能量的代谢和储存,参与机体的免疫调节、生长以及神经发育等多种生理功能. 生物体内菌群的失调往往会导致机体代谢功能的紊乱,影响正常的生理生化反应. 已有研究表明,宿主肠道内



A、B、C和D分别表示 control、PS、PS—NH₂和PS—COOH处理组
图8 不同处理组中细菌群落物种在门和属水平上的群落相对丰度

Fig. 8 Species composition and relative abundance distribution of bacterial community in different treatments at the phylum level and genus level

变形菌门的丰度增加往往反映出其肠道内的炎症水平^[43]。在本研究中,PS—NH₂微塑料导致斑马鱼幼鱼体内变形菌门细菌显著增加,表明PS—NH₂暴露可能会导致斑马鱼产生肠道炎症反应。此外,变形菌门在宿主肠道内还参与氨基酸的利用和分解过程^[43],因此变形菌门的明显变化可能会严重影响机体的氨基酸代谢能力,这一结果也再次验证了PS—NH₂暴露可以诱导斑马鱼体内氨基酸代谢过程的紊乱。此外,PS—NH₂暴露还导致了斑马鱼体内厚壁菌门和拟杆菌门丰度的明显下降。一般而言,厚壁菌门能够促进机体对营养物质的吸收进而提高机体的生长发育,拟杆菌门则在糖类发酵和代谢过程中有着不可替代的作用^[44]。PS—NH₂对两类菌群的明显抑制可能会影响斑马鱼正常的生长发育和糖酵解过程,这一结果也与代谢组学的结论相一致。相较于PS—NH₂,PS—COOH和PS微塑料对斑马鱼幼鱼菌群的影响较小,可能未通过菌群失衡而影响斑马鱼生命早期的生理活动。

总之,经官能团修饰后的PS微塑料PS—NH₂和PS—COOH诱导斑马鱼幼鱼体内产生了更多的差异代谢物,并对相关的代谢路径产生了严重干扰,特别是PS—NH₂微塑料导致斑马鱼体内的微生物群落多样性明显下降,菌群组成也更趋向于单一化。因此,微塑料的官能团修饰过程可能会加重其对斑马鱼胚胎的毒性效应,PS—NH₂因表面带有正电荷更易和表面负电荷的斑马鱼细胞相结合,导致更严重的生物胁迫效应。已有研究发现,微塑料表面电荷可以通过控制微塑料的稳定性影响其团聚行为和生物毒性效

应^[45-47]。在天然海水中,PS—NH₂和PS—COOH微塑料(100 nm)的水合粒径分别增长了1.6倍和2.1倍,PS—COOH相较于PS—NH₂微塑料更易形成聚集体,这导致PS—NH₂微塑料在海胆(*Paracentrotus lividus*)胚胎消化道内的累积程度更高,进而导致海胆幼体产生了严重的发育缺陷,但PS—COOH的影响却并不显著^[48]。Nolte等^[49]也发现微塑料携带的官能团会改变其表面电荷情况,影响其与藻类之间的吸附行为,进而导致微塑料对藻类产生不同的毒性效应。Yacobi等^[50]的进一步研究发现,脒基修饰的PS微塑料因表面带有正电荷,其在小鼠肺泡上皮细胞上的转运速度要远高于表面带有负电荷的PS微塑料。随后,Banerjee等^[51]也证实微塑料的官能团修饰作用会明显改变其在环境及生物体内的行为过程,进而影响其关联的生物效应。因此,带正电荷的PS—NH₂微塑料可能更易通过静电作用力而吸附在带负电的胚胎脂质双分子层上,进而通过不同传输途径进入生物体,产生比COOH阴离子修饰更强的生物毒性效应^[52,53]。

3 结论

(1)斑马鱼胚胎绒毛膜是微塑料暴露早期的主要累积场所,但其不能有效阻隔小粒径微塑料的穿透,卵黄囊是微塑料在胚胎内部的关键蓄积组织。3种微塑料在斑马鱼幼鱼体内的累积能力没有显著差异性,其累积系数均相对较小,在斑马鱼生命早期阶段不具有生物累积性。

(2)3种微塑料均使斑马鱼幼鱼体内的代谢物水平发生了显著变化,官能团修饰过程强化了这一差异性。其中,PS微塑料主要影响斑马鱼体内氨基糖苷类的生成和代谢,PS—NH₂则主要对氨基酸和蛋白质的合成代谢过程产生影响,而PS—COOH则主要作用于斑马鱼的三羧酸循环、氨基酸及糖酵解等代谢通路,且PS—NH₂微塑料对斑马鱼体内代谢功能的影响最为严重。

(3)斑马鱼幼鱼体内的菌群多样性和组成对3种微塑料产生了不同的响应,其中PS—NH₂微塑料的影响最为严重,不仅降低了菌群的多样性,还显著改变了不同菌群在门和属水平上的占比,明显增加了变形菌门的百分数,导致斑马鱼幼鱼体内的菌群失衡,进而可能导致鱼体代谢功能的紊乱。

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