食用蔬菜能吸收和积累微塑料

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摘要 微塑料(100 nm~5 mm)作为一种新型环境污染物,具有潜在的动植物和人体健康风险,其污染已成为高度关注的全球环境问题。当前已有不少关于微塑料在水生生物体内积累的报道,但对于陆地生态系统的研究则相对匮乏,高等植物对微塑料的吸收和积累更未见报道。本文基于室内培养实验报道了微塑料在生菜(*Lactuca sativa*)体内的吸收、传输及分布。通过激光扫描共聚焦荧光显微镜和扫描电子显微镜观察发现,聚苯乙烯微球(0.2 μm)可被生菜根部大量吸收和富集,并从根部迁移到地上部,积累和分布在可被直接食用的茎叶之中。研究结果为开展土壤-植物系统中微塑料积累机制及食物链传递与健康风险研究提供了新依据。

关键词 微塑料, 生菜, 聚苯乙烯微球, 吸收, 积累, 健康风险

塑料污染正在成为地球表层生态系统最严重的威胁之一^[1]。微塑料(100 nm~5 mm)是一种新型全球环境污染物,近年来受到国内外广泛关注。原生微塑料^[2]主要是指人为制造具有特定用途的粒径<5 mm的塑料颗粒,如工业磨料或塑料微珠等,它大量存在于日常生活中使用的一些洗涤用品以及个人清洁用品中。次生微塑料源自于外力作用下较大塑料碎片的碎裂,如塑料碎片、纤维等。微塑料粒径小、数量大、分布广且可作为污染物的载体,易被生物所摄食并且能在食物链中积累,从而可能会对生态系统和人体健康产生不利影响。目前,有关微塑料的研究多集中于海洋、海岸、河口、湖泊等水域生态系统^[3~5]。陆地环境是微塑料污染的重要源头和主要汇集地,如污水、污泥和有机肥的施用,塑料薄膜的使用以及大气沉降等均可能导致土壤微塑料的累积^[6~8]。有研究估算了每年排放到欧洲和北美农田的微塑料分别达到了110000和730000吨^[9]。

这一数字甚至超过了海洋表面水体的年均微塑料增加量^[10]。关于土壤微塑料污染问题已成为近年来全球关注的热点,亟需开展其含量、来源、迁移转化、生态环境效应及健康风险等方面的系统研究工作。

已有大量关于水生生物积累微塑料的报道,特别是在海洋生物中^[11~14],这些研究表明微塑料在水生食物链中已无处不在。有关微塑料在土壤生物中累积及其效应的研究则相对匮乏,尤其是高等植物的研究更是鲜见报道^[15~17]。高等植物作为人类和动物赖以生存不可或缺的重要组成部分之一,也是人类和动物的主要食物来源之一,它对保持生态系统平衡起到了至关重要的作用。值得关注的是,亚微米甚至纳米微塑料颗粒有可能被植物吸收、蓄积、生物放大、生物转化,但目前还缺乏证据。微塑料一旦被可食植物吸收,将有可能通过多种暴露途径导致在生物体和人体中的累积,而对生物体和人类健康产生潜在不利影响。研究微塑

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料在植物(尤其是可食用作物)的吸收、积累对于其生态环境与健康风险评估具有重要意义。

生菜(*Lactuca sativa*)是一种在全球范围被广为直接食用的绿叶蔬菜。聚苯乙烯则是在光学、电子和化妆品等不同领域广泛使用的塑料聚合物之一,同时也是在陆海环境中经常被检出的微塑料污染物类型^[1]。本研究在实验室培养试验条件下,利用荧光标记的不同尺寸的聚苯乙烯微球,通过激光共聚焦显微镜(confocal laser scanning microscopy, CLSM)和扫描电子显微镜(scanning electron microscope, SEM)观察,研究了其在生菜体内的吸收、输移、积累和分布。

1 材料与方法

1.1 塑料微球

本研究采用0.2和1.0 μm 的不同荧光标记的两种聚苯乙烯塑料微球(红色和绿色荧光),其均购自天津大鹅科技有限公司。微塑料颗粒呈球形,平均粒径分别为 $0.23\pm0.04\ \mu\text{m}$ 和 $0.98\pm0.09\ \mu\text{m}$ (图1(a), (b)),在水相中分散、保存,本实验储备液浓度为 $10\ \text{mg mL}^{-1}$ 。微球在被激发后可观察到高亮度荧光,且具有良好的稳定性,在溶液中未发现染料泄露(图S1(a), (b))。从红外光谱表征图可以明显看出聚苯乙烯的特征峰(图S2)。两种微球水动力学直径分别为 $0.27\pm0.04\ \mu\text{m}$ 和 $1.5\pm0.2\ \mu\text{m}$ 。其表面带负电荷,Zeta电位分别为 $-42.7\pm0.9\ \text{mV}$ 和 $-13.1\pm0.6\ \text{mV}$ 。

1.2 供试生菜及生长条件

生菜(*Lactuca sativa* L., Rosa)种子由中国农业科学院提供。挑选饱满大小一致的种子,先用0.5% NaClO溶液浸泡处理10 min,进行表面灭菌。随后用去离子水将种子洗涤3次,以洗去残留的NaClO溶液。将种子置于湿润的滤纸上,在20℃下,避光催芽2 d。种子萌发后在泥炭土中生长21 d。然后将生菜幼苗取出洗净,转移到Hoagland营养液中,在人工气候室 $25\pm2\ ^\circ\text{C}$, 12:12 h光照:黑暗和70%相对湿度条件,继续培养生长7 d。Hoagland营养液组成为: 2.5 mmol/L CaNO_3 和 KNO_3 , 10 $\mu\text{mol/L}$ FeEDTANa_2 , 0.1 mmol/L K_2HPO_4 , 75 $\mu\text{mol/L}$ KCl , 10 $\mu\text{mol/L}$ H_3BO_3 , 1 mmol/L MgSO_4 , 4 $\mu\text{mol/L}$ $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$, 0.5 $\mu\text{mol/L}$ $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$, 1 $\mu\text{mol/L}$ $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ 和0.2 $\mu\text{mol/L}$ $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$ 。

图1 两种尺寸聚苯乙烯微球TEM图片

Figure 1 Transmission electron microscopy (TEM) images of two sizes polystyrene microbeads

1.3 吸收富集实验

将红色和绿色荧光标记的聚苯乙烯微球储备液超声分散3 min后,与Hoagland营养液混合,再超声分散一次,配制成 $50\ \text{mg L}^{-1}$ 聚苯乙烯微球暴露试验液。本试验设置两种粒径(0.2和1.0 μm)处理,每个处理4个重复,每个钵(1 L)移入两株生菜苗。移栽后的幼苗在人工气候室继续生长14 d。暴露液每隔2 d更换一次。暴露结束后,借助激光共聚焦显微观察(FluoView FV1000, 奥林巴斯,日本)和扫描电子显微镜(S-4800, 日立,日本)观察植物体内微球的积累与分布状况。

1.4 激光共聚焦显微镜观察

收集吸收富集实验中新鲜生菜根、茎和叶,用超纯水充分清洗干净后,选取根(成熟区)、茎(距茎的基部2 cm)和叶柄,包埋于4%低熔点琼脂糖中。使用振动切片机(VT1200S, 徕卡)将根、茎和叶分别切成40和100 μm 厚的半薄切片,置于载玻片上,滴加磷酸缓冲盐溶液(phosphate buffered saline, PBS)用盖玻片封片。使用激光共聚焦扫描显微镜在激发/发射波长分别为488/515 nm和635/680 nm进行观察。每个样品至少重复3次。图像的后处理经导入成像软件Fluoview FV 10完成。

1.5 扫描电子显微镜观察

收集并清洗吸收富集实验的生菜苗,分别取根和叶,进行扫描电子显微镜观察。取材位置与激光共聚焦显微观察位置相同,切成小块并在液氮中冷冻。然后将样品冷冻干燥,并借助sputter coater(cressington model 108, Ted Pella Inc., 美国)用金涂覆60 s(约1 nm厚的金),用于扫描电子显微镜观察。在具有反向散射检测的高真空模式下,在20 kV的加速电势下观察横截面。以不同的放大率捕获图像。每个样品至少重复3次。

2 结果

2.1 微塑料在生菜中的吸收与分布

研究塑料微球进入植物体内及其在植物体内积累和转运状态对认识其作用影响至关重要. 在利用荧光标记聚苯乙烯微球的试验中, 未发现微米级($1.0\ \mu\text{m}$)微球能被生菜吸收(图2(a)~(f)), 这表明在本实验条件下微米级聚苯乙烯微球难以通过生菜根系细胞间隙的自由空间和质外体屏障进入根系皮层甚至中柱. 本文主要探讨了亚微米级($0.2\ \mu\text{m}$)聚苯乙烯微球在生菜体内的吸收、累积和分布.

红色荧光微球, 由于可避免生菜根部自身背景荧光干扰, 在本研究中被用来示踪微球在生菜根部的蓄积. 植物根冠可以分泌大量黏液来保护植物免受病原体攻击^[18]. 荧光微球处理生菜根尖具有明显肉眼可见“深绿色”(荧光标记微球的颜色), 表明塑料微球能被生菜根冠分泌的黏液(高度水合多糖)捕获并黏附在根表面. 对照组生菜根横切和纵切图中未观察到荧光(图S3). 从荧光微球处理组生菜根的横切(图3(a)~(c))和纵

切(图3(d)~(f))切片图可以看出, 大量荧光存在于细胞壁的间隙中, 表明聚苯乙烯微球能够进入到生菜根里面. 此外, 还发现聚苯乙烯微球能够到达中柱. 在进入中心圆柱体后, 塑料微球就可以在根压和蒸腾拉力的作用下随蒸腾流和营养流通过木质部向地上部分移动^[19,20].

2.2 微塑料在生菜体内的迁移

绿色荧光微球, 由于可避免生菜茎和叶组织自身背景荧光干扰, 被用来研究微球向生菜地上部迁移. 对照生菜茎和叶中未观察到荧光(图S3). 激光共聚焦显微照片显示, 生菜在根部积累大量聚苯乙烯微球后, 通过维管组织可将少量微球输运到茎和叶的脉管系统中(图3(g)~(l)). 为验证聚苯乙烯微球能被生菜吸收和转运, 生菜根叶组织切片被用来在扫描电子显微镜下进一步观察. 扫描电子显微镜照片证明了微球在生菜根部大量存在, 在维管组织中彼此黏附在一起呈现“葡萄”或“链条”聚集状(图4(c), (f)), 在叶片组织中微球粘连在一起呈分散状(图4(i)).

图2 荧光标记聚苯乙烯微球($1.0\ \mu\text{m}$, $50\ \text{mg L}^{-1}$)处理14 d后生菜根部横切(a)和纵切(d)的激光共聚焦显微成像图. 其中, (a)和(d)分别为荧光(b), 明场(c)和荧光(e), 荧光(f)的合成图. 在生菜根部横切和纵切片中未观察到红色荧光, 指示在根组织中没有聚苯乙烯微球积累

Figure 2 Confocal images of cross and longitudinal section of lettuce root treated for 14 d with a $50\ \text{mg L}^{-1}$ solution of $1.0\ \mu\text{m}$ fluorescently labelled polystyrene (PS) microbeads. (a) and (d) are the corresponding merged images of band (c), (e) and (f). No red fluorescence was observed in cross-sections or longitudinal-section of the lettuce root, indicating no or very few PS beads accumulated in root tissue

图 3 荧光标记聚苯乙烯微球($0.2\ \mu\text{m}$, $50\ \text{mg L}^{-1}$)处理14 d 后生菜根部横切((a)~(c))、纵切((d)~(f))和茎((g)~(i))、叶((j)~(l))的激光共聚焦显微成像图。其中, (a), (d), (g), (j)分别为(b)和(c), (e)和(f), (h)和(i), (k)和(l)的合成图

Figure 3 Confocal images of cross ((a)~(c)) and longitudinal ((d)~(f)) section of lettuce root, stem ((g)~(i)) and leaf ((j)~(l)) treated for 14 d with a $50\ \text{mg L}^{-1}$ solution of $0.2\ \mu\text{m}$ fluorescently labelled polystyrene (PS) microbeads. (a), (d), (g), and (j) are the corresponding merged images of (b) and (c), (e) and (f), (h) and (i), (k) and (l)

3 讨论与结论

陆地环境微塑料来源广泛, 产生量大, 由于尚缺乏统一的土壤和土壤孔隙水微塑料分析检测标准方法, 其在土壤中浓度难以估测。目前已有不少关于农业土壤中微塑料污染的报道^[8,9], 但仍无法确定其实际暴露

水平。需要指出的是由于很难从实际环境中分离出足够量的微塑料用于其生态环境效应的评价, 本研究选用不同尺寸的亚微米级聚苯乙烯微珠模拟微塑料污染, 指示了高等植物的可吸收和积累性。由于聚合物类型、尺寸、表面性质和形状等的差异, 商品化的微塑料可能与环境中微塑料存在一定差异。未来的研究不

图4 荧光标记聚苯乙烯微球($0.2\ \mu\text{m}$, $50\ \text{mg}\ \text{L}^{-1}$)处理14 d 后生菜根部((a)~(f))和叶片((g)~(i))的横切面扫描电子显微镜照片。其中, (a), (d), (g)分别为根和叶的全局图, (c), (f), (i)分别是(b), (e), (h)中红色方框处的扩大图

Figure 4 Scanning electron microscopy (SEM) images of $0.2\ \mu\text{m}$ polystyrene microbeads localization in the root and leaf of a lettuce plant. The one-month-old lettuce plants were exposed to PS beads for 14 d. ((a)~(f)) The microbeads were detected as systematically aggregated clumps in a “grape-like” or “chain-like” shape in the root of lettuce. (i) PS microbeads were observed and dispersed in the leaf vein of the lettuce plant. (c), (f), and (i) show an enlargement of the pane in (b), (e), and (h), as indicated by the red square

仅包括微珠或近似球形的塑料颗粒, 还应包括环境中经常检测到的纤维和其他形状塑料颗粒^[21]。此外, 未来需进行更接近微塑料环境浓度和环境条件的暴露实验, 以此来评估其对生态系统的真实影响。

值得关注的是, 可直接食用高等植物生菜是受国内外消费人群喜爱的绿叶蔬菜之一, 同时也是世界上无土栽培的常见蔬菜之一。本试验研究证明, 生菜不仅可吸收微塑料, 而且可将其运输、积累和分布在茎叶之中。因而, 在实际生产过程中, 特别是当无土栽培的营养液受微塑料污染时, 很有可能产生生菜农产品安全风险, 继而通过食物链影响人体健康, 因而需要引起重视。无土栽培过程中营养液可能会受到塑料装置、塑料大棚以及大气沉降等影响受到微塑料污染。与有土栽培介质相比, 微塑料在水溶液中移动性更强, 在蒸腾拉力作用下更加容易积累到植物体内。不同作物或者蔬菜由于根系分泌物、细胞壁空隙度、蒸腾速率和根系水力传导率等影响因素的差异, 其吸收富集微塑料颗粒的能力也可能存在差异, 需要进一步进行比较

研究。目前已有不少关于植物对金属和碳基纳米颗粒吸收转运的研究报道^[22,23], 而微塑料由于其较强的黏附性和可形变性, 更容易被植物黏附并吸收到体内, 进而产生潜在的生态和健康风险。可食作物中微塑料的积累, 还可能会增加人体对塑料中添加化学品(包括可浸出添加剂和黏附污染物)的直接暴露, 并可能进一步对人类健康造成危害。需要指出的是目前尚缺乏食品中微塑料含量、膳食暴露以及微塑料对人体毒性的基础数据, 其健康风险评估方法也亟需建立。未来应亟待加强微塑料颗粒及其添加剂和黏附污染物毒性的研究和基础数据积累。

本文报道了生菜吸收、传输和积累微塑料的研究成果, 可为研究高等植物对微塑料的吸收和积累机制及生态效应提供科学依据, 对于评估土壤中微塑料对农作物及蔬菜的潜在安全及健康风险具有重要意义。基于更细小的微塑料更易被生物吸收, 因而未来需要加强对亚微米甚至纳米微塑料在食用性作物中积累和食物链传递机制研究, 并关注其对生态系统和人体健康的影响。

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补充材料

图S1 红色和绿色荧光标记的聚苯乙烯微球(0.2 μm)激光共聚焦显微成像图

图S2 聚苯乙烯微球(0.2 μm)的红外光谱图及其与标准谱图的对比

图S3 对照组(未添加聚苯乙烯微球)处理14 d后生菜根部横切、纵切和茎、叶的激光共聚焦显微成像图

图S4 不同激光波长下生菜根、茎和叶的激光共聚焦显微成像图

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Summary for “食用蔬菜能吸收和积累微塑料”

Uptake and accumulation of microplastics in an edible plant

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Microplastic (MP, 100 nm–5 mm) may present an attributable risk to ecosystem and human health, and its pollution has become a global environmental concern. Despite a wealth of information on the accumulation of MPs in aquatic species, there is no information on the uptake and accumulation of MPs by higher plants. Terrestrial edible plants are directly exposed to MPs when agricultural soil was applied with organic manure, sewage sludge as fertilizer or plastic mulching. In this paper, the uptake of two sizes of polystyrene (PS) microbeads (0.2 and 1.0 μm) and then their distribution and migration in an edible plant lettuce were firstly investigated based on laboratory experiments. We used fluorescent markers to track PS microbeads in plant tissues and found fluorescence to be a sensitive and reliable detection method. Sections from untreated control lettuce showed no autofluorescence. When roots were treated with fluorescently labeled PS microbeads, the microbeads could be identified by its fluorescence. Our main study investigated the uptake of 0.2 μm beads, as few luminescence signals were observed in lettuce roots for 1.0 μm beads in our experiment. We observed that 0.2 μm fluorescent microbeads were extracellularly trapped in the root cap mucilage (which is a highly hydrated polysaccharide) and a “dark green tip” (which was typical of lettuce roots exposed to label PS beads) was usually visible to the naked eye. Confocal images revealed that the PS luminescence signals were mainly located in the vascular system and on the cell walls of the cortex tissue of the roots, indicated that the beads passed through the intercellular space via the apoplastic transport system. Once inside the central cylinder, the 0.2 μm PS beads were transferred from the roots to the stems and leaves via the vascular system following the transpiration stream. We also observed that the PS beads adhered to one another and self-assembled systematically into “grape-like” and “(chain) string-like” clusters in the intercellular space of the root and stem vascular tissue of lettuce plant. In contrast to the root and stem, PS beads were dispersed in the leaf tissue. Here, for the first time we provide evidence of the adherence, uptake, accumulation, and translocation of submicrometer MPs within an edible plant. Our findings highlight the previously underappreciated human exposure pathway to MPs through the consumption of contaminated crops and emphasize the need for new management strategies to control the release of MPs waste products into the terrestrial environment. Ultimately, the potential impacts of low range sized MPs on food safety of crop plants and human health need to be urgently considered.

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