

禾本科作物小麦能吸收和积累聚苯乙烯塑料微球

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摘要 农用地土壤中微塑料的积累及分布已有报道, 食用蔬菜在溶液培养下能吸收微塑料也已被发现, 但微塑料能否在固相培养条件下进入禾本科作物中并在体内传递积累尚未被证实。本研究选用小麦作为模式植物, 以0.2 μm荧光标记聚苯乙烯微球为供试微塑料材料, 采用真实河砂盆栽培养实验, 结合激光共聚焦荧光显微和扫描电子显微技术, 发现小麦幼苗在砂培条件下能吸收和传输0.2 μm聚苯乙烯微球。小麦幼苗在含有荧光标记微球的河砂中生长21 d后, 其根部维管柱和外皮层细胞壁间隙组织中呈现较强的荧光分布, 表明这种亚微米级塑料微球能被小麦吸收进入根部外皮层质外体空间和维管组织。塑料微球进入根部维管柱后, 可通过维管组织运输到地上部的茎部维管束和叶片的脉管组织中。研究结果为进一步认知土壤-作物系统中微塑料的传递与积累机制提供了方法学和科学依据。

关键词 小麦幼苗, 聚苯乙烯微球, 砂培, 吸收, 积累

微塑料已被证实广泛存在于海洋、湖泊等水体环境中, 并能通过摄食作用被鱼类、贝类、虾蟹类等生物吸收积累, 甚至还可以通过食物链向更高营养级生物迁移^[1,2], 对人体健康构成潜在风险。近期研究表明, 陆地尤其是农地土壤中微塑料污染也应该引起重视。农用地膜破碎、污泥和有机肥施用、污水灌溉、大气沉降以及地表径流等均能导致微塑料在土壤中积累^[3~6]。有研究者估计, 每年向欧洲和北美农田土壤中输入的微塑料分别达到110000和730000 t, 这一数字远超过全球海洋表层微塑料的输入量^[3,7]。目前, 我国已有滨海潮滩土壤和农田土壤中微塑料的类型、丰度及分布的研究报道^[8,9], 但对其在土壤动植物中的积累及其生物生态、食物链的风险尚缺乏研究与了解。因此, 亟待加强微塑料的农用地土壤污染研究, 为我国农田土

壤微塑料污染的风险管控与治理提供科学依据^[10]。

微塑料进入土壤后可改变土壤的理化性质, 进而对土壤生物产生影响^[11~13]。土壤中微塑料不仅可通过食物链传递、富集带来潜在的健康风险^[14], 而且也可影响作物的生长发育。已有研究发现微塑料能降低小麦种子发芽率, 损害小麦叶片的光合系统, 并抑制其生长^[15,16]。但是, 土壤中微塑料能否进入作物体内有待证实。我们前期的研究表明, 水培条件下生菜能吸收和积累聚苯乙烯塑料(0.2 μm)微球, 并能将其运输到可被直接食用的茎叶之中^[17]。Jiang等人^[18]则报道了水培条件下聚苯乙烯微球(0.1 μm)可以被蚕豆根系吸收和积累, 并且能干扰营养物质运输, 产生遗传毒性。小麦作为全球广泛种植的粮食作物之一, 是中国第二大粮食作物, 在我国北方地区种植和食用尤为普遍^[19]。同时, 小麦作

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为一种禾本科作物，其具有不同于双子叶植物的根系结构和根系分泌物组成^[20,21]，这些都可能影响其对塑料微球的吸收。因此，微塑料能否被禾本科作物吸收和传递更值得关注。此外，目前有关微塑料的植物吸收研究都是基于营养液水培实验，而水培环境与植物正常生长的固-液相环境差异较大，难以反映植物生长与吸收的真实状态。因此，在模拟固-液相介质生长条件下探讨微塑料的植物吸收和积累更具有实际意义。

聚苯乙烯是全球使用量较大的一种塑料聚合物类型，同时也是在环境中经常被检出的微塑料污染物类型^[22]。本研究基于前期工作的基础，在实验室河砂基质砂培条件下，以荧光标记聚苯乙烯塑料微球为供试微塑料材料，运用激光共聚焦荧光显微和扫描电子显微技术，研究了微球在小麦幼苗体内的吸收、积累、传输和分布。研究结果可为进一步认知土壤-作物系统中微塑料的传递与积累机制提供方法学和科学依据。

1 材料与方法

(i) 荧光聚苯乙烯塑料微球。本研究采用两种不同荧光标记(Nile blue荧光染料标记的红色荧光微球，4-氯-7-硝基-1,2,3-苯并氧杂恶二唑标记的绿色荧光微球)0.2 μm聚苯乙烯塑料微球，均在水相中分散、保存，固含量为1%，通过天津大鹅科技有限公司定制合成。红色荧光标记的聚苯乙烯塑料微球在激发(620 nm)/发射(680 nm)波长下可观察到高亮度荧光。绿色荧光标记的聚苯乙烯微球在激发(488 nm)/发射(518 nm)波长下可观察到高亮度荧光，两者均具有良好的荧光稳定性^[17]。通过激光粒度仪动态光散射(Zateseries Nano-ZS90, Malvern Panalytical, 英国)分析其水合粒径为(0.24±0.06) μm，利用扫描电子显微镜(S-4800, 日立，日本)观察其微观形貌，其外观呈规则球形(图S1)。

(ii) 供试植物培养。小麦(*Triticum aestivum*)种子由中国农业科学院提供。挑选饱满且大小一致的小麦种子，先用0.5% NaClO溶液浸泡处理5~8 min，进行表面灭菌。随后用超纯水将种子洗涤5次以去除残留的NaClO溶液。将种子置于培养盒湿润的灭菌滤纸上，在25°C下避光催芽3 d。将小麦幼苗取出洗净，转移至1/5 Hoagland营养液中^[23]，在人工气候室(温度为(25±2)°C，光照时间:黑暗时间为12 h:12 h，相对湿度为55%)继续培养3 d后供使用。

(iii) 塑料微球吸收实验。本研究所用砂培基质为取自烟台鱼鸟河的原状河砂。砂砾取回后仅过筛除去大粒

砂子，为反映真实砂体性质对作物吸收塑料微球的影响，砂体未做淋洗处理去除其中的黏粒等天然有机无机胶体物质。所用河砂粒径分布为0.5~0.05 mm: 34%; 2~0.5 mm: 64%; 2~0.5 mm: 2%，pH为7.9，可溶性有机碳(DOC)含量为11.2 mg/L，河砂pH和DOC含量均为在水砂比1:1的浸提液测得。水砂悬液以250 r/min震荡0.5 h，在5500 r/min离心10 min，上清液用0.45 μm针式过滤器过滤后，分别用pH计(S220, 瑞士，梅特勒)和有机碳分析仪(TOC-VCPh, 日本岛津)测定。将红色和绿色荧光标记的聚苯乙烯微球原液25°C超声(KQ-500DE, 昆山市超声仪器有限公司，中国)分散3 min后，取一定量原液与1/5 Hoagland营养液混合均匀后加入到400 g砂砾中，砂液质量比约为10:1，塑料微球浓度为0.5 mg/g。

实验设置两个处理，对照处理组和荧光微球处理组，每个处理设置两盆重复，每个盆钵(250 mL)移入株高、株重无明显差异的6株小麦幼苗。移栽后的幼苗在人工气候室继续生长21 d。每隔2 d补充一次1/5 Hoagland营养液。暴露结束后取小麦根、茎、叶，运用激光共聚焦显微镜(FluoView FV1000, 奥林巴斯，日本)和扫描电子显微镜观察植物体内塑料微球的积累与分布。

(iv) 激光共聚焦显微镜观察。塑料微球吸收实验结束后，采集新鲜小麦根、茎和叶，用超纯水充分清洗干净。选取根、茎(距茎的基部2 cm)和叶，用4%低熔点琼脂糖包埋。使用振动切片机(VT1200S, 徕卡，德国)将根、茎和叶分别切成70和100 μm厚的半薄切片，平铺在载玻片上，滴加磷酸盐缓冲溶液(PBS)，盖玻片压片。使用激光共聚焦扫描显微镜在激发/发射波长分别为488/518和620/680 nm下进行观察。每个样品至少重复3次。

(v) 扫描电子显微镜观察。扫描电子显微镜取材位置与激光共聚焦显微观察位置保持一致。将各部位切成小块并迅速在液氮中冷冻，然后将样品冷冻干燥(Scientz-10N, 宁波新芝生物科技股份有限公司，中国)48 h，使用Sputter Coater(Cressington model 108, Ted Pella Inc., 美国)对样品切面喷金60 s(厚度约为1 nm)，用于扫描电子显微镜观察，每个样品至少重复3次。

2 结果

2.1 小麦幼苗根系对微塑料的吸收及分布

在前期利用荧光标记聚苯乙烯微球的实验中发现，水培条件下亚微米级(0.2 μm)聚苯乙烯微球能被可食

蔬菜生菜根部吸收并传输到地上部茎叶之中，而微米级微球未能被生菜吸收^[17]。基于前期工作基础，本研究主要探讨了砂培条件下亚微米级($0.2\text{ }\mu\text{m}$)聚苯乙烯微球在小麦体内的吸收、累积和分布。

通过对小麦幼苗不同部位组织自发荧光检测发现，小麦幼苗根部组织分别在405 nm(蓝色)、488 nm(绿色)、559 nm(橙色)激发光波长下均有一定强度的自身背景荧光，在633 nm激发光波长下自身背景荧光较弱(图1(a))。因此，在激发(620 nm)/发射(680 nm)波长下的红色荧光微球可有效避免小麦根部自身背景荧光干扰，可用于指示微球在小麦幼苗根部的累积。在相同条件下，小麦幼苗地上部组织则在激发(488 nm)/发射(518 nm)波长下自身背景荧光较弱(图1(b), (c))。激发(488 nm)/发射(518 nm)波长下的绿色荧光微球可有效避免小麦幼苗茎和叶组织自身背景荧光干扰，被用于指示微球向小麦地上部的迁移。

实验期间所有小麦幼苗生长状况良好，微球处理组与对照组小麦长势无显著性差异，添加微球未对小麦生长产生影响。小麦根部暴露在含有聚苯乙烯塑料微球的砂砾中后，观察到根表附着大量根系分泌物；微球处理组小麦根部经超声清洗后，其表面仍观察到明显的红色荧光，这表明塑料微球能被小麦根系分泌物捕获并黏附在根表面。如图2所示，对照组小麦根组织

切片(图2(a))中未观察到荧光；从荧光微球处理组小麦根组织切片(图2(d))来看，荧光主要分布在根表皮、外皮层和维管柱木质部中，少量存在于根的内皮层。这表明聚苯乙烯微球能被小麦根吸收，并主要分布在根外皮层和维管柱中。为进一步明确塑料微球在小麦根部的富集，通过扫描电子显微镜观察小麦根组织切片发现塑料微球以聚集体形式分布于根部木质部及外皮层的细胞间隙中(图3)。

2.2 微塑料在小麦幼苗体内的迁移

激光共聚焦显微图片显示，对照组茎(图4(a))、叶(图4(g))中未观察到绿色荧光，而荧光微球处理组小麦幼苗茎的维管束(图4(d))以及叶的脉管系统中(图4(j))均呈现不同强度的绿色荧光，这表明小麦根部吸收的塑料微球可通过木质部导管输送到地上部。

3 讨论与结论

土壤中微塑料在生物和非生物作用下可破碎为粒径更小的塑料颗粒或者碎片，这可能会进一步增大其对土壤生态系统的潜在危害。目前，已有关于微塑料对植物生长发育毒效应的研究报道^[16,24]，但对其作用过程和机制尚不清楚。Bandmann等人^[25]发现烟草细胞能通过内吞作用吸收纳米级的聚苯乙烯颗粒。本研究则

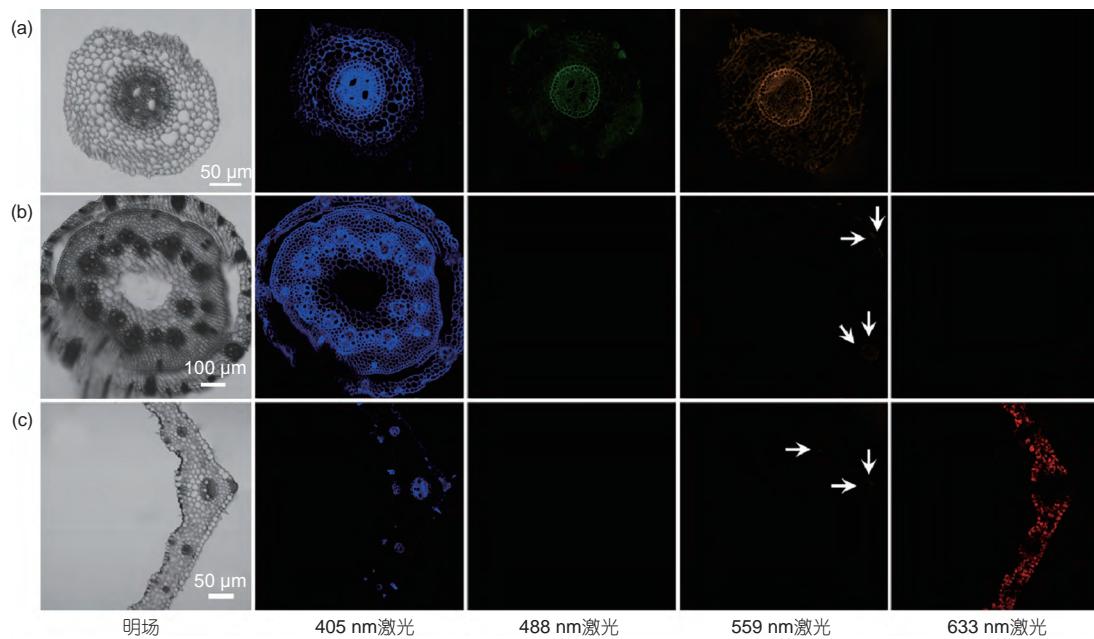


图 1 不同激光波长下小麦根、茎和叶的激光共聚焦显微成像图

Figure 1 Confocal images of cross section of wheat root, stem, and leaf with various excitation wavelengths

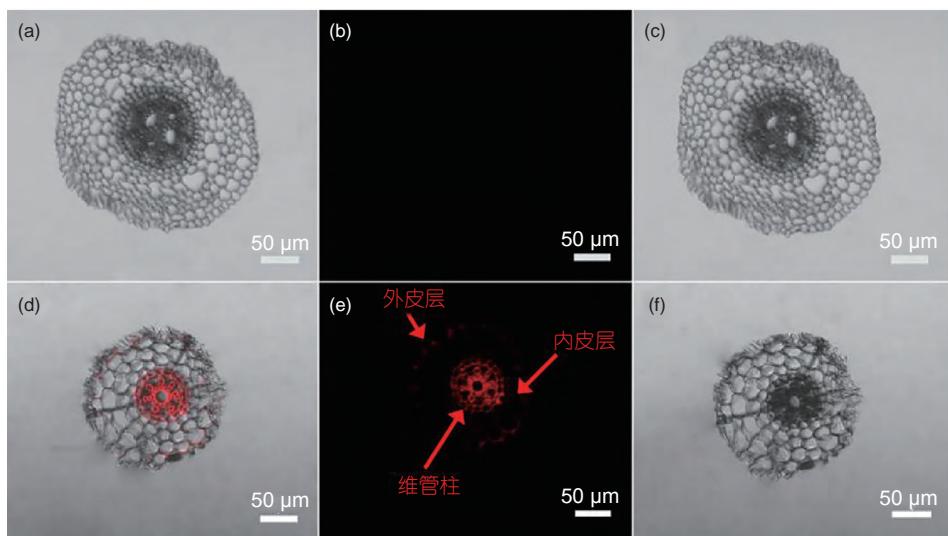


图 2 对照组小麦根部位横切(a)~(c)和0.2 μm荧光标记聚苯乙烯微球(0.5 mg/g河砂)处理21 d后小麦根部横切(d)~(f)的激光共聚焦显微成像图. (b), (e) 激发波长为633 nm的荧光照片; (c), (f) 明场照片; (a), (d) 分别为(b)和(c), (e)和(f)的合成图
Figure 2 Confocal images of cross section (a)–(c) of wheat root in control group, and for wheat grown for 21 d in the sand with 0.5 mg/g 0.2 μm fluorescently labelled polystyrene (d)–(f). (b), (e) The fluorescent images with excitation wavelength of 633 nm; (c), (f) the bright field; (a) and (d) are the corresponding merged images of (b), (c), and (e), (f)

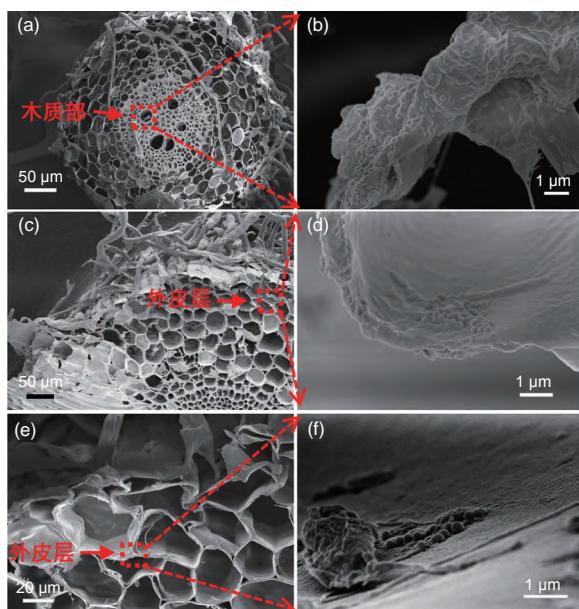


图 3 在含有0.5 mg/g 0.2 μm荧光标记聚苯乙烯微球的河砂中生长21 d后的小麦根横切面扫描电子显微镜照片. (b), (d), (f)分别是(a), (c), (e)中红色方框处的放大图
Figure 3 Scanning electron microscopy images of cross section of wheat root. The wheat plants were grown for 21 d in the sand with 0.5 mg/g 0.2 μm fluorescently labelled polystyrene microbeads. The microbeads (0.2 μm) were detected as aggregated state in the root of wheat. (b), (d), (f) is an enlarged view of the red square of (a), (c), (e)

是在更接近植物真实生长环境的砂培条件下观察到了亚微米级聚苯乙烯塑料微球能被小麦幼苗根系吸收到

外皮层甚至到木质部，并进一步传输到地上部，但对于其进入根系的机制和传输途径仍需深入研究。纳米颗粒能通过植物根尖、根毛或者侧根吸收到体内，并通过质外体途径从根表皮内化到皮层，甚至到达木质部导管^[26]。植物结构上的相互贯通可确保各种生理功能的正常进行。比如，茎与根相互联系共同组成植物体的体轴，而茎与根通过过渡区维管组织不同水平部位上细胞的分化而连接起来^[27]，形成一个地下部与地上部物质运输的通道。由于木质部是维管植物的运输组织，可将根部吸收的水分及营养传输到植物的各个器官。纳米塑料到达根部中柱可进一步转移到茎、叶之中^[28]。然而，塑料微球与纳米塑料在植物吸收机制和传输途径方面的异同尚需进一步研究。

在前期营养液培养的工作基础上^[17]，在更加接近植物真实生长环境的固-液相介质培养中，证实了亚微米级(0.2 μm)聚苯乙烯塑料微球能被植物体吸收并转移到地上部。值得注意的是，本研究所用的商品化塑料微球为单一材质粒子，形状规则，与真实环境中的微塑料形貌、材质、老化程度会有所不同。另外，实验所用的河砂培养基质与真实土壤环境仍存在一定差异，但河砂培养基质中含有的天然有机质、无机离子以及矿物胶体等物质，很可能会影响塑料微球的表面性质和存在状态，进而影响植物根系对微球的吸收。因此，真实土壤环境中植物对微塑料的吸收、传输及其量化评

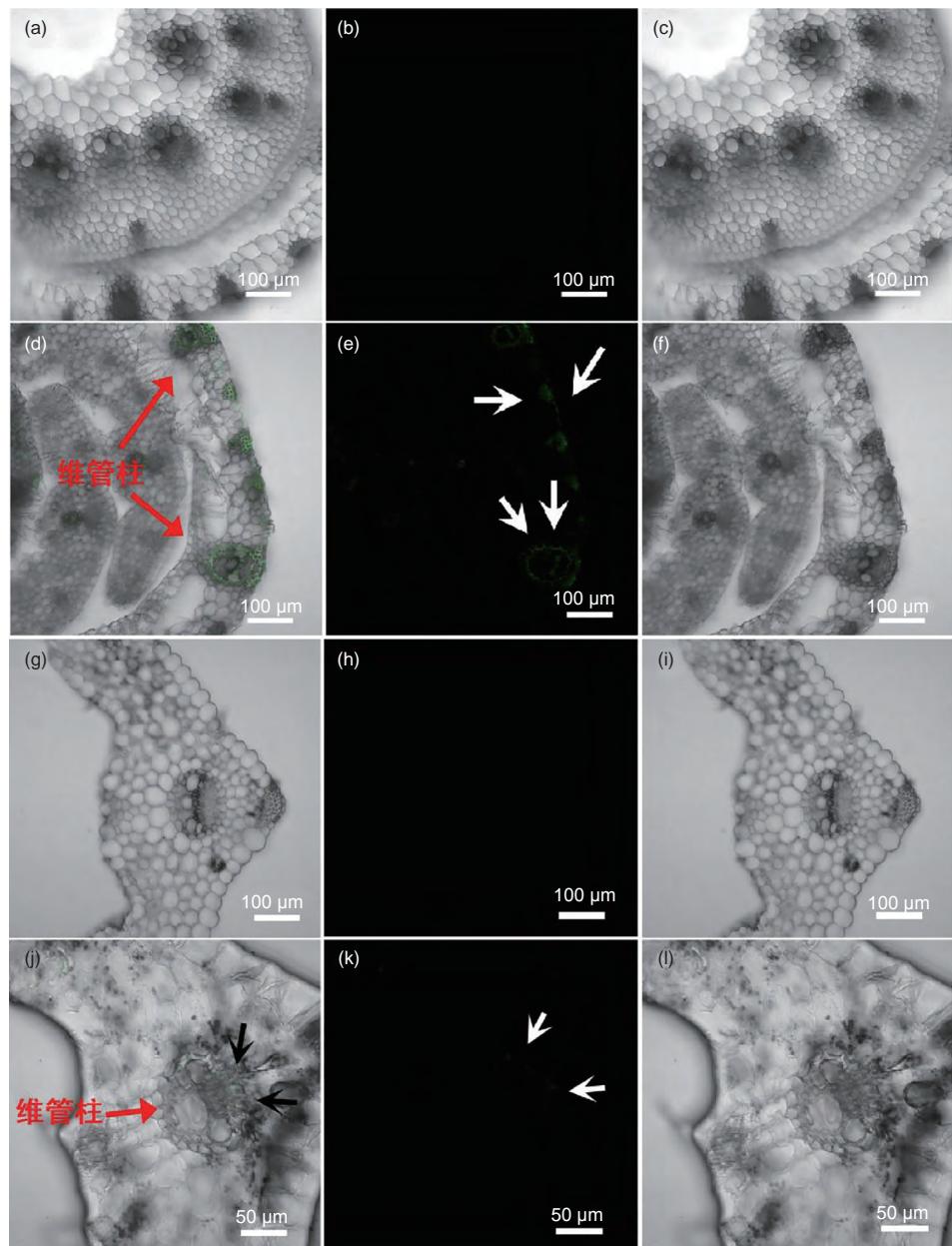


图 4 对照组小麦茎部(a)~(c)与叶横切(g)~(i)和0.2 μm荧光标记聚苯乙烯微球(0.5 mg/g河砂)处理21 d后小麦茎部(d)~(f)与叶横切(j)~(l)的激光共聚焦显微成像图。(b), (e), (h), (k) 激发波长为488 nm的荧光照片;(c), (f), (i), (l) 明场照片;(a), (d), (g), (j) 分别为(b)和(c), (e)和(f), (h)和(i), (k) 和(l)的合成图

Figure 4 Confocal images of cross section of wheat stem (a)–(c) and leaf (g)–(i) in control group, and of stem (d)–(f) and leaf (j)–(l) for wheat grown for 21 d in the sand with 0.5 mg/g 0.2 μm fluorescently labelled polystyrene microbeads. (b), (e), (h), (k) The fluorescent images with excitation wavelength of 488 nm; (c), (f), (i), (l) the bright field; (a), (d), (g), and (j) are the corresponding merged images of (b) and (c), (e) and (f), (h), and (i), (k) and (l)

估将是未来值得研究的重要科学问题。另外, 微塑料一旦能被作物吸收积累, 其表面吸附的常规污染物及其本身的化学添加剂, 均有可能随着微塑料的吸收而在植物体内积累^[29,30], 从而同步提高其健康风险。

本研究报道了在砂培条件下亚微米级的聚苯乙烯

微球能进入到小麦幼苗根部, 主要分布在表皮层及维管束。积累在根部的微球可被转移到地上部, 主要分布在茎部维管束, 甚至能到达叶片的脉管系统中。研究结果为评估微塑料在土壤-作物系统吸收、积累与传输提供了参考依据。

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补充材料

图S1 0.2 μm聚苯乙烯微球SEM图片

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Summary for “禾本科作物小麦能吸收和积累聚苯乙烯塑料微球”

Uptake and accumulation of microplastics in a cereal plant wheat

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Microplastics pollution is becoming a global environmental concern, and growing evidence has demonstrated the accumulation and distribution of microplastics in terrestrial ecosystems. Once entering into soil, microplastics can change the physical, chemical and biological properties of soil, and then affect the growth of plants. Currently, most attentions have focused on the toxic effects of microplastics on terrestrial plants, only very limited report showed the uptake of microplastics by higher plants under hydroponic culture conditions. The nutrient solution is useful in understanding the mechanism of microplastics uptake, however, it does not account for the importance of affecting factors in the real environment (e.g., the presence of soil organic matter) and therefore do not represent the actual uptake of microplastics in the real-world. Here, we aim to determine whether wheat plants growing in a sand matrix are able to take up 0.2 μm polystyrene (PS) microbeads and translocate these particles from roots to shoots. Wheat was chosen as a representative of cereal crops because it is one of the main staple foods worldwide. A simple and rapid approach for the imaging of fluorescently labelled PS microbeads within plant tissues by confocal laser scanning microscope (CLSM) was used to investigate the uptake, accumulation, translocation and distribution of microspheres in the wheat plant. Two different fluorescent dyes were encapsulated into the PS microbeads matrix and they were used to detect the localization of PS beads in the root and the green tissue respectively. The presence of PS microbeads in plant tissue was then verified using scanning electron microscopy (SEM). 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 wheat seedling roots after exposure in sand matrix with a concentration of 0.5 g kg⁻¹ of PS beads for 21 d, indicated that the beads passed through the intercellular space via the apoplastic transport system. Microbeads clusters were observed in the intercellular space of epidermal tissues and the steles by SEM. 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. Here, for the first time, we provide evidence of the adherence, uptake, accumulation, and translocation of submicrometer (0.2 μm) PS within the cereal plant in real sand matrix. Our findings provide a methodology and scientific basis for study of the accumulation mechanism of microplastics in soil-crop systems and their potential risk in food chain transfer.

wheat seedling, polystyrene microbeads, sand culture, uptake, accumulation

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