The review of nanoplastics in plants: Detection, analysis, uptake, migration and risk

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Abstract:
Nanoplastics (NPs) have become an emerging pollutant that has attracted much attention. As plants are the major food sources, it will be of great use to investigate NPs in plants. The crack-entry mode is considered to be the main mode for NPs to enter plants roots. The migration of NPs is feasible, which includes the process of internalization into xylem vessels through the apoplast pathway and migration to the aerial part. The development of chromatography, mass spectrometry, and labeling techniques has made it possible to quantify NPs, although this is difficult to apply in practical settings. How to analyze and quantify NPs in complex environmental media is still an urgent problem to be solved. This article provides a comprehensive overview of NPs detection, uptake, migration, current analytical techniques and ecological risks in plants, bringing together scattered information and analyzing current deficiencies, providing recommendations for future research.

Keywords: Nanoplastics Plants Detection Analysis Uptake Migration Risk

Introduction

Global plastic production was 367 million tons in 2020, [1], and this index is estimated to rise to 3.3 billion tons in 2030 [2], of which about 79% will be landfilled and abandoned in the natural environment [3]. With a large number of plastic products flowing into the natural environment, the resulting plastic pollution in soil has increasingly attracted wider attention [4]. NPs are plastic particles with a size of less than one micron [5-7], which sources include the fragmentation of plastic products in the environment and the inflow of microbeads used as industrial raw materials [8]. Even in our daily lives, large amounts of NPs are released, such as tire wear [9], fabric washing [10,11], and personal care use [12,13]. Due to their potential ecological threat, NPs pollution has become another problem in global environmental pollution. NPs entering the environment can be absorbed by organisms and cause responses by organisms. As the producers of ecosystems and the starting point of bioaccumulation, plants play an important role in maintaining ecological balance [14]. Therefore, it is of great value to explore the interaction between NPs and plants and their mechanisms.

Soil is one of the gathering places of various types of NPs [15,16]. At the same time, due to its poor mobility, NPs accumulate in large quantities in soil [17]. In addition, the accumulation of NPs in farmland soil is greatly accelerated due to the use of plastic film [18,19], sewage irrigation [20] and fertilizer application [21,22]. The accumulation of soil NPs has become a hot spot of global concern, which not only changes the physical and chemical properties of soil [23], but also poses a serious threat to the life activities of soil plants [24]. Just as plants can absorb tiny particles, NPs can also be absorbed by plants. The first study to demonstrate the ability of plant cells to absorb NPs through endocytosis was reported in 2012, driving the heat of plant-microplastic interactions [25]. The root system is the way for plants to absorb NPs from the soil [26], and root exudates have a strong uptake effect on NPs, although root exudates have been shown to promote the adsorption and enrichment of heavy metals in plants [27], its promoting or inhibiting effect on the absorption of NPs by plants has not been clearly proposed. The Kjeldahl band of the root endoderm is considered to be a natural barrier for substances to enter the plant, however the Kjeldahl band has discontinuous areas where the root apical endoderm is immature and where lateral roots grow [28]. The apical meristem is porous due to a high degree of cell division, but the cell wall pores (3.5-5 nm) are not lar.ge enough to support the passage of NPs [29]. These "cracks", which can be several micrometers in length, are known routes of infection by plant pathogens or bacteria [30,31], and have also been shown to be one of the ways plants take up NPs[32]. The current mainstream view is that NPs reach the stele from the root surface through the apoplast pathway, and are mainly concentrated in the vascular column, which is similar to the mechanism by which plants absorb other nanoparticles [33]. Vascular tissue connects the roots and stems of plants through cellular differentiation at different levels. Xylem is the transport site of vascular tissue, through which NPs can be transported into stems and leaves. Root pressure and transpiration pull are considered to be the main driving force [34]. The accumulation and migration of NPs in plants will lead to the transfer of NPs along the food chain and eventually lead to human uptake [35]. The accumulation of NPs in the human body may alter the immune system or may lead to

other clinical complications [36]. In addition, plastic additives or their leached chemicals have the ability to trigger endocrine disruption and even carcinogenesis [37]. Given these potential risks, a detailed understanding and better monitoring of the behavior of NPs in plants is urgently needed.

Early studies focused on the impact of NPs exposure on plants life activities[38,39], until the development of plastic particle analysis technology and process research, the complex mechanism of the interaction between plants and NPs was gradually revealed [40]. The use of high-precision Scanning Electron Microscopes (SEM) makes it possible to observe the morphology of NPs in plants[41]. And the process research of NPs has further promoted the development of this field. Using fluorescently labeled NPs to act on plants, and then observing plant tissues with laser scanning confocal microscope (LSCM) can more effectively verify the absorption of NPs by plants and determine the location of absorption and migration [24]. To further judge the ecological risks of NPs, quantitative analysis of NPs absorbed by plants is necessary. The combination of thermal analysis technology with gas chromatography and mass spectrometry has been proved to be a feasible method to quantify a specific type of NPs in plants [42], and the content of a certain type of NPs in plants can be obtained through the analysis of specific thermal degradation products. In order to estimate the possible uptake of NPs by plants, quantitative analysis of NPs can be achieved by quantifying other substances loaded on NPs. Doping metal elements on NPs to detection NPs in animals by time-resolved fluorescence of lanthanide chelates has been reported earlier [43,44]. Recently identifying and quantifying metal elements by inductively coupled plasma mass spectrometry (ICPMS) to indirectly obtain the amount of NPs absorbed by plants has become another current method [45]. However, these methods all have their own limitations, and they can only be applied to laboratory conditions for the time being, and cannot meet the needs of quantitative analysis of the enrichment of NPs in plants under natural conditions. It is urgent to develop an analytical method with a wide range of applications.

NPs pollution has become one of the most pressing environmental problems globally, threatening the health of ecosystems [46]. Due to the effect of NPs on plants, NPs may also affect plant community structure while affecting plant productivity, which is caused by different species' different responses to NPs [47,48]. Changes in community structure will affect ecosystem function. In particular, the impact of NPs on crops may have a direct impact on global food production and security. To address this threat, the study of NPs interaction with plants needs to be further explored. While studying the effects of NPs on plants, we need to explore the behavior of NPs in plants, and relevant detection and analysis techniques need to be further improved to meet the requirements. But to date, there are few comprehensive and detailed reviews on the behavior and analysis of NPs in NPs-plant system. Therefore, in this article, in order to gain a more comprehensive and in-depth understanding of the fate of NPs in the NPs-plant system, this article aimed to: 1) summarize recent methods for analyzing NPs in plants, especially for quantitative analysis; 2) review the mechanism of NPs uptake and migration by plants, and explore the influencing factors; 3) discuss the current gaps in the research on the micro(nano)plastic-plant system, and analyse the direction that needs to be further explored in the future.

Material and methods

All materials in this paper were obtained from Internet databases. In order to obtain more comprehensive data to accurately reflect the current research progress, multiple databases such as Web of Science, Science Direct, and Nature Springer are used for retrieval. The keywords "MPs", "NPs", "absorption", "analysis", "quantification" were used to search for publications from various databases. Not all relevant literature could be found by these keywords, so we screened NPs and plant-related studies individually to ensure comprehensiveness of the data. We collected information about NPs used in related studies, including particle size, charge and fluorescence characteristics of NPs. We also collected information about the interaction between NPs and plants, including the migration location of NPs, research methods and conclusions. This information is integrated into tables and figures. In addition, based on the shortcomings of the current research, we also searched for keywords such as "nanoplastic" and "plant" in an attempt to make up for the shortcomings of the current research through the interaction process of other nanoparticles with plants.

Detection of NPs in plants

Verification and observation of NPs in plants

In the current study, microscopes are commonly used to observe MPs in soil and water. This is a non-destructive method that can be used to observe the characteristics of the color, shape, surface texture and size of plastic particles, which can distinguish possible plastics particles and other particles [49,50]. However, it is difficult to identify particles with a diameter of labeling technology provides new ideas for verifying whether plants can absorb NPs. Cultivate plants in a culture medium containing a certain concentration of fluorescently labeled NPs. After culturing for a certain period of time, take different plant tissues to make slices, and observe the fluorescence in the plant slices with the help of a LSCM at a certain wavelength [55,56]. This can not only prove the internalization of NPs by plants, but also can be used to observe the migration path of NPs which is shown in Fig. 1. It should be noted that attention should be paid to avoid the interference of the background fluorescence of plants. To avoid interference from background fluorescence, green fluorescence is commonly used to observe plant stems and leaves, while red fluorescence is used to observe plant roots.

Recently, the use of lanthanide metal chelates to replace traditional fluorescent dyes has been successfully applied to achieve background-free fluorescence imaging by analyzing the characteristic time-resolved fluorescence of chelates [45]. Not only by directly observing the NPs, it has also become a feasible way to verify the existence of NPs by observing other substances carried by the NPs. In the present study, SEM and LSCM are the two most important means to observe NPs in plants. The advantage

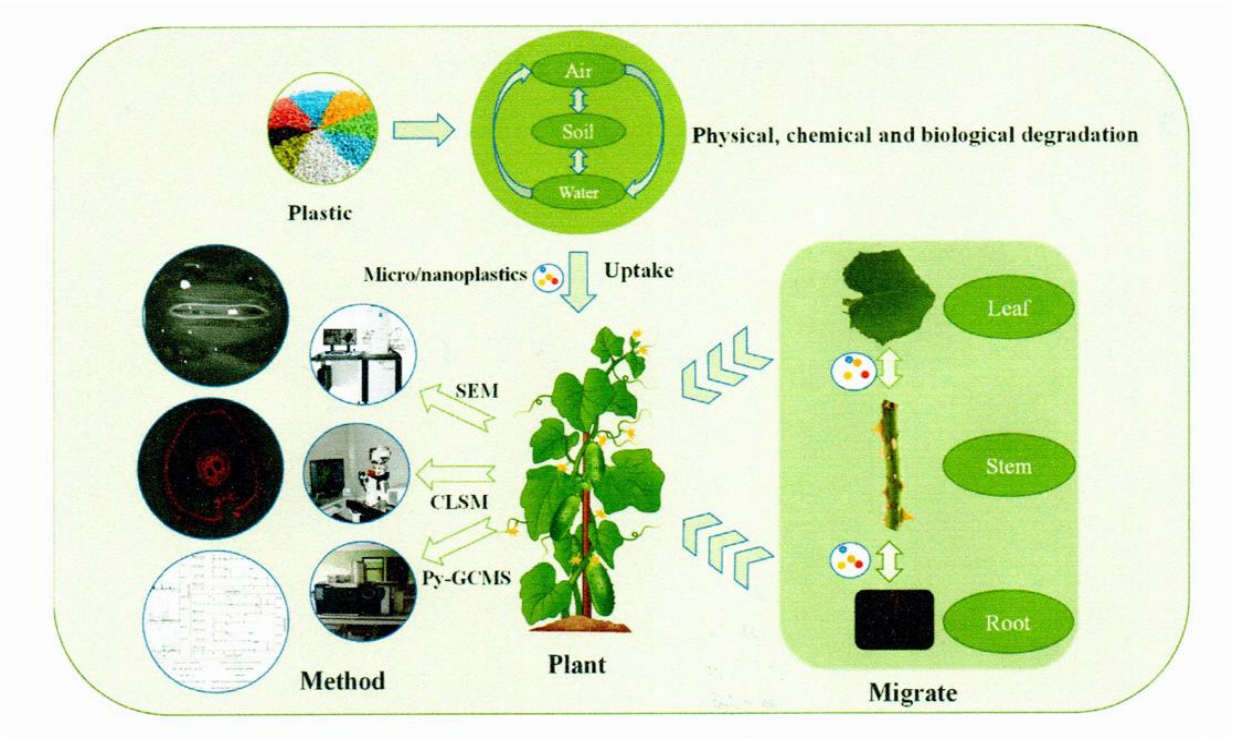

Fig. 1. Schematic diagram of the detection of NPs in plants.

of SEM is that the shape and size of NPs can be accurately observed, but only one section can be observed, and making slices is destructive, which cannot be continuously observed. The advantage of LSCM is that the transport path of NPs can be observed by fluorescence, and the process of NPs absorption by plants can be observed by continuous monitoring, but it will be interfered by background fluorescence.

Isolation and purification of NPs in plants

Since organisms are composed of complex mixtures, usually containing organic matter, inorganic matter, cells, and water, it is very challenging to separate plastics in organisms [2,57]. Isolation and purification of NPs in plants is a prerequisite for analysis and quantification, and current research has not developed a simple and applicable method [58]. Traditional digestion methods such as the use of nitric acid and hydrogen peroxide to digest plant tissue may destroy the NPs themselves, while Fenton oxidation may not remove the interference of the plant tissue [59,60]. Separating and purifying the NPs absorbed by plants without destroying the NPs is a problem waiting to be solved. In recent research, a method was developed for quantitative analysis and extraction of NPs in plants (Fig. 2a). First, tetramethylammonium hydroxide is added to the ground plant powder to digest the tissue, and then ethanol is added to make it precipitate. After centrifugation and drying the precipitate, the NPs are ultrasonically washed with dichloromethane, and the supernatant is reconstituted with dichloromethane and evaporated to dryness to obtain NPs in plants [42]. The results show that this is a feasible method, but the complicated preprocessing and high detection limit of NPs the application of this method. If an effective enrichment and concentration technique can be developed, the problem of high detection limit can be effectively eliminated. In a recent study, NPs in water samples were agglomerated by alkylated ferric oxide, filtered through an inorganic microporous membrane, and analyzed by pyrolysis gas chromatography/mass spectrometry (Py-GC/MS), and NPs in natural water were successfully detected [61]. Due to the interference of plant samples, this enrichment method cannot be directly applied to the detection of NPs in plants, but its reference value cannot be ignored. Furthermore, based on the hydrophobic properties of NPs, utilizing cloud point extraction and protein corona mediation are also potential methods to isolate and purify NPs, although these methods are also affected by the plant itself [62-64].

Identification and quantification of NPs in plants

Based on the known infrared absorption band of polymers, Fourier Transform Infrared Spectrometer (FTIR) and its optimized technology are the most commonly used techniques for chemical characterization of plastic types [65,66]. But FTIR technology also has a certain lower detection limit, which is about 20 mm [67]. Compared with FTIR, Raman spectroscopy has a higher resolution [52,68]. When coupled with a microscope, plastic particles with a size of species of NPs. It has been reported that after NPs were detected by SEM, NPs particles in fish intestines were successfully identified using m-FTIR and m-Raman [70]. However, similar methods have not been applied to the identification of NPs in plants.

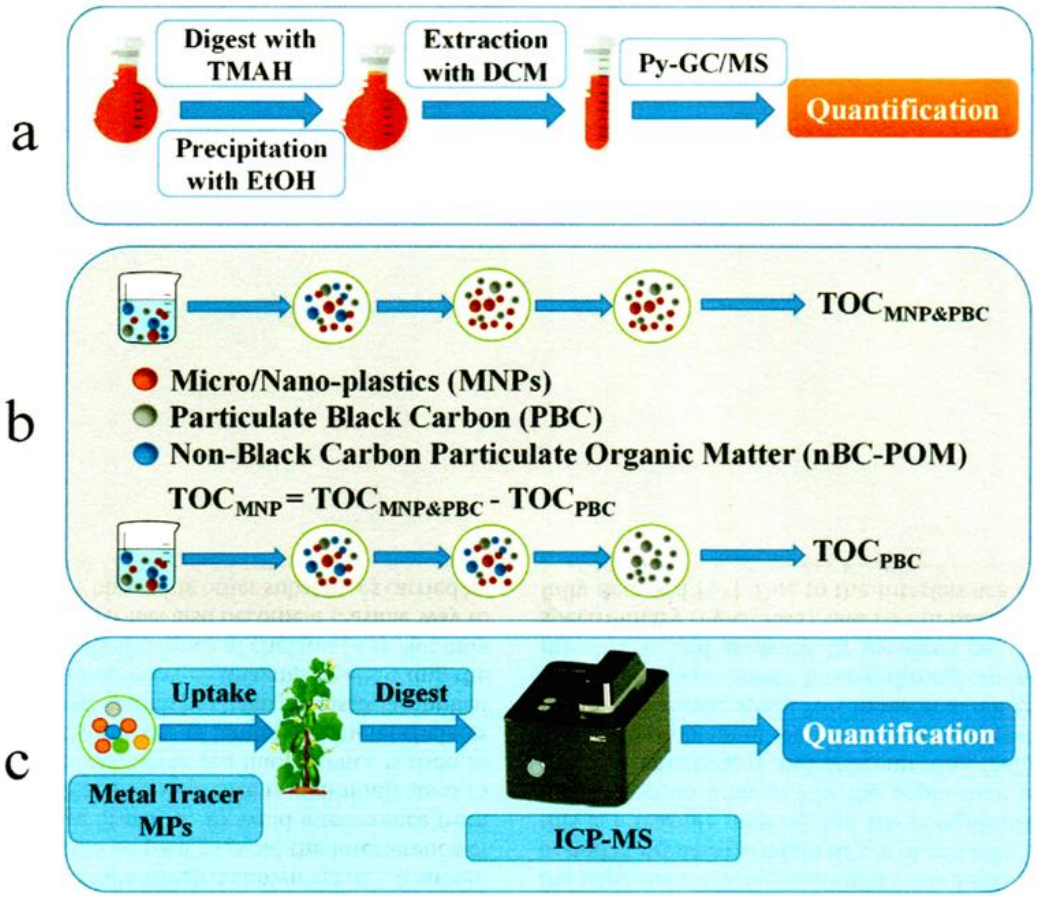

Fig. 2. Quantitative flow chart of NPs. (a. Quantitative determination by Py-GC/MS method; b. Quantitative determination by TOC method; c. Metal tracer method for quantitative determination. TMAH, tetramethylammonium hydroxide; DCM, dichloromethane)

Thermal analysis is a promising technique for analyzing NPs that are too small to be analyzed by other techniques [71,72]. In recent years, the use of thermal analysis techniques to analyze the characteristic degradation products of plastics to identify and quantify plastic particles in environmental samples has been demonstrated, including soil [2,49,73,74], water [75], animals [72,76,77], and plants [42]. Different types of plastics in environmental samples can be identified by selecting specific decomposition products of polymers, their respective indicator ions and retention time [78]. In a recent study, based on Py-GC/MS technology, NPs in plants were successfully detected, and the recovery rate was verified to be more than 90% and the process is shown in Fig. 2a [42]. However, this technology has extremely high requirements for sample pretreatment and requires extensive purification procedures to reduce organic matter and concentrate plastic materials [76,79]. And it is difficult to manually add plastic samples to the pyrolysis cup [80].

It is also a possible way to quantify NPs in plants by measuring total organic carbon (TOC) (Fig. 2b). In a recent study, removal of organic matter from water using Fenton digestion and quantitative estimation of NPs using TOC was used [81]. Currently, the use of TOC to quantify NPs in water has been obtained. Further validation, in this study, TOC in water was divided into MPs and NPs, granular black carbon, and non-black carbon particulate organic matter, a group of which was treated by potassium peroxodisulfate oxidation and Fenton digestion to eliminate non-black carbon particles. For organic matter, the TOC of MPs and granular black carbon was measured, and the other group was sequentially digested by sulfonation and Fenton treatment to eliminate non-black carbon particles and MPs to measure the TOC of black carbon particles, and the two were subtracted to obtain the TOC of MPs [82]. But unlike water samples

rich in organic matter, the digestion of plant samples presents more difficult challenges. How to eliminate the interference of plants without destroying NPs is an area that needs to be further explored in the future.

The technological research of NPs can largely overcome the technical challenges of NPs analysis [83]. Molecular labeling with fluorescent dyes is a common method for visual tracking of NPs in living organisms. The use of fluorescent NPs to quantify biological uptake of NPs is currently a promising method. The NPs content in the tissue can be obtained by infecting the organism, digesting the biological tissue, and then measuring its fluorescence intensity. The key to this approach is finding a way to efficiently digest biological tissue without destroying NPs and fluorescent dyes. However, due to the interference of the autofluorescence of biological tissues and the influence of the leakage of dye molecules, the experimental results often have artifacts, which is also the reason why the existing fluorescent labeling methods cannot be applied to the quantification of NPs in vivo [84,85]. Doping particles with metal tracers is an efficient method with the main advantage that complex samples do not quench the tracer during homogenization or digestion, and standard methods for trace metal analysis are now available. There is also no need to worry about high background interference when working with rare metals [86]. This approach was recently used to study the fate and behavior of NPs in sludge from municipal wastewater treatment plants [83]. In a recent study, rare earth metal-organic fluorescent complexes were used for the quantification of NPs in vivo (Fig. 2c). Due to its long fluorescence lifetime, the interference of biological autofluorescence can be effectively reduced when using time-resolved technology to collect fluorescence. Moreover, the fluorescence of the shed or leaked rare earth complexes will be quenched by environmental impurities, which effectively avoids false fluorescence signals generated by the shedding. The rare earth elements contained in rare earth complexes have low background and high sensitivity in plasma mass spectrometry (ICP-MS) analysis. Based on this characteristic, rare earths can be used as element labels for the precise quantitative analysis of labeled plastic particles by ICP-MS, which has the advantages of high sensitivity, fast analysis speed and good selectivity. The problem that conventional fluorescent labeling methods cannot be accurately used for quantitative analysis of NPs is solved [45]. Research on NPs in plants has made some progress and Table 1 exhibits the research conclusions on the mechanism of plant uptake of microplastics.

Factors affecting the absorption of NPs by plants It is indisputable that plants can absorb NPs, and there is evidence that the external environment or the plants themselves can affect the absorption of NPs by plants. Here, we summarize the influencing factors into three categories for discussion: size, charge, and secretion. Table 2 summarizes the factors that affect the absorption of NPs by plants.

Size

Experiments have shown that the main channel for plants to absorb NPs is through the pore openings on the leaf surface or the cracks in the lateral roots of the root system. Therefore, the particle size of the NPs directly determines whether the NPs can enter the plant. In recent research, nano-level PS can enter the lettuce, while micro-level PS is not observed in the lettuce [24]. In addition NPs smaller than 200 nanometers can be absorbed by Arabidopsis and migrate in plants [56]. Most of the current studies on the absorption of plastic particles by plants are based on NPs, but some studies have shown that plants can also absorb MPs with a particle size of more than 1 mm. In another study, both nano-sized and micro-sized plastic particles can be absorbed by the roots of rice plants and migrate to the above-ground parts [89]. Epidermal cells also have certain size requirements for the endocytosis of NPs. It is found that 20 nm NPs are quickly internalized by the wall BY-2 cells, while NPs larger than 100 nm cannot be internalized, this result may be related to the size of endocytic vesicles produced by the cell. However, compared with parietal cells, BY-2 protoplasts can internalize larger nanospheres with a diameter of up to 1000 nm. This difference can be explained by the lack of turgor pressure in the protoplasts in principle. The turgor of the walled BY-2 cells can prevent the formation of larger vesicles [25]. In a recent study, it was found that PS NPs were first attached to the surface of protoplasts, and then small-sized PS NPs were absorbed, while only a few large-sized PS NPs were absorbed. Whereas in the same study on callus it was found that only small particle size PS NPs were absorbed, while large particle size was hardly absorbed [90]. This also reflects the size-selective uptake of NPs by the cell wall and cell membrane.

Electric charge

NPs in nature tend to be charged with a certain amount of charge under the action of physics, chemistry and biology [92], which may affect the absorption of NPs by plants. In studies on Arabidopsis, it was found that the uptake and transport pathways of NPs in root tissue were affected by different electrical charges. The uptake and internalization of positively charged PS-$_{NH2}$ by plants is lower than that of negatively charged PS-SO_3H, and $PSNH_2$ will be more adsorbed on the root surface [56]. Further research found that the possible reason for the above results is that PS-NH_2 stimulates the root system to produce more secretions, which promotes the aggregation of PS-NH_2, thereby hindering plant internalization. In another study on maize plants, both positively charged PS-NH_2 and negatively charged PS-COOH could migrate from leaves to roots. Similarly, the positively charged $PSNH_2$ was more likely to attach to the plant leaf surface, while the negatively charged PS-COOH was more likely to enter the plant, and PS-NH_2 was observed to form larger aggregates [87]. A more subtle mechanism was shown by a recent report, which found that positively charged PS-NH_2 was more capable of transporting in plant protoplasts and callus than negatively charged PS-COOH, although

negatively charged PS-COOH can be migrated to deeper locations, which may better explain the above results [90]. The reason for this result may be due to the composition of plant cell walls, negatively charged cell walls have a higher affinity for positively charged $PSNH_2$ [93,94], and plant cell membranes have been shown to have stronger adsorption to positively charged particles [95]. Therefore, it can be inferred that negatively charged NPs are more capable of migrating to the depths of the plant, but plant cells have a stronger ability to absorb positively charged NPs, which is closely related to the properties of secretions and plant cells.

Table 1 Research conclusions on the mechanism of plant uptake of NPs.

Plant	Type	Size (mm)	Concentration (mg/L)	Duration (Day)	Result	Reference
Zea mays	PS-NH2 PS COOH	20, 50, 100, 200	100	7	NPs undergo a translocation from leaf to root.	[87]
Lactuca sativa	PS	200, 1000	50	14	Micron-sized PS microspheres are difficult to absorb by lettuce, while PS NPs can be transported to the vasculature of stems and leaves in small amounts through vascular tissue.	[24]
Triticum aestivum	PS	200	0.5	21	The plastic microspheres absorbed by wheat roots can be transported to the ground through xylem catheters.	[88]
Cucumis sativus	PS, PMMA	50, 100	50	7, 14	Developed a method for quantitative analysis of NPs in plants.	[42]
Riticum aestivum	PS, PMMA	200, 2000	50	10, 20	PS and PMMA particles penetrating the stele of both species using the crack-entry mode.	[32]
Oryza sativa	PS	80, 1000	40	14, 40	Both nano- and micro-sized MPs could be absorbed by rice roots and subsequently translocated to aerial parts.	[89]
Lactuca sativa, Raphanus sativus, Triticum aestivum and Zea mays	PS	100, 5000	1, 10	7	In the very early growth stage (7 days after sowing), NPs can also be absorbed by plants.	[55]
Arabidopsis thaliana	PS-SO_3H, PS-NH_2	55, 71	10, 50, 100	7, 10, 49	Regardless of the surface charge, Arabidopsis can absorb and transport NPs smaller than 200 nanometers in size.	[56]
Triticum aestivum	PS-NH_2 PS COOH	50, 100, 200	20	6	Few small-sized PS NPs are transported into the cytoplasm, large-sized PS NPs are only present on the epidermis.	[90]
Triticum aestivum, Lactuca sativa	PS	200	50	14	MPs are mainly concentrated in the xylem and cell walls of root cortical tissues and can migrate to stems and leaves.	[45]

Table 2 Factors affecting the uptake of NPs by plants

Factors	Plant	Type	Size (nm)	Results	Reference
Size	Nicotiana tabacum (BY-2 cells)	PS	20, 40, 100	20 and 40 nm nano PS beads were taken up by BY-2 cells. 100 nm beads were excluded from uptake into turgescent and plasmolysis cells.	[25]

	Vicia faba	PS	100, 5000	A large number of 100 nm PS-MPs particles entered the Vicia faba root tips. 5 μm PS-MPs whose presence in the V. faba root tips was very scarce.	[91]
	Lactuca sativa	PS	200, 1000	0.2 μm polystyrene microspheres are observed in the roots, stems and leaves of plants.	[24]
	Oryza sativa	PS	80, 1000	Both nano- and micro-sized MPs could be absorbed by rice roots and subsequently translocated to aerial parts.	[89]
	Triticum aestivum	PS	50, 100	Few small-sized PS NPs are transported into the cytoplasm, large-sized PS NPs are only present on the epidermis.	[90]
Charge	Zea mays	PS-NH_2 PS COOH	20	The positive charge is conducive to the binding of PS NPs to the leaf surface and greater aggregation on the leaf surface.	[87]
	Arabidopsis thaliana	PS SO_3H, PS NH_2	200	NPs with negative charges on the surface can be internalized into the roots, while positively charged.	[56]
	Triticum aestivum	PS NH_2 PS-COOH	50, 100	The proportion of PS-COOH being transported to the deeper part was higher, the amino-modified PS NPs seemed to have stronger adsorption capacity on the root surface, and the amino-modified PS-NH2 had stronger translocation capacity in cellular uptake.	[90]
Exudates	Triticum aestivum	PS	200	Plastic microspheres can be captured by wheat root exudates and adhere to the root surface.	[88]
	Arabidopsis thaliana	PS SO_3H PS NH_2	200	The roots of Arabidopsis thaliana exudate mainly oxalate, and oxalic acid will change the size of the positively charged NPs aggregates.	[56]

Root exudates

Root exudates are mainly composed of low molecular weight organic compounds, including organic acids, fatty acids and specific metabolites, which are also one of the indicators of response to environmental changes [96,97]. Especially in the face of extreme temperature, heavy metal and other pollutants stress, root secretions will change to cope with [98,99]. It was found that the exudation of low molecular weight organic acids such as oxalic acid and malic acid is an important reaction mechanism to toxic elements [100,101]. Plants also respond to high levels of heavy metal pollution by increasing their excretion [102]. Different conditions will affect plant root exudates, and plant root exudates will also affect the absorption of NPs by plant roots. NPs also have a direct impact on plant root exudates. A recent study found that tomato roots secrete a large amount of low molecular weight organic acids to resist MPs stress, and the process is affected by the type of MPs. In addition, compared with the positively charged PS-NH_2, the negatively charged PS-SO_3H has more findings in Arabidopsis. The results of this experiment show that PS-NH_2 stimulates roots to produce a large amount of exudate, which contains a large amount of oxalate. Compared with PS-SO_3H treatment, PS-NH_2 treatment is associated with greater oxalate exudation, indicating that root exudation may be affected by the surface charge of NPs. At the same time, DLS data showed that the size of the positively charged NPs aggregates increased with the increase of the oxalic acid concentration, while the size of the negatively charged NPs remained unaffected [56]. Therefore, the charge of NPs may affect their own agglomeration by affecting the production of plant root exudates, and the large agglomerate size will directly affect the absorption of NPs by the roots. Due to the complexity of soil environment, many pollutants may exist simultaneously. This also leads to the possibility that root exudates may affect the NPs absorption process of plants by responding to pollutants such as heavy metals. The current research on this content is not enough to reveal the mechanism, which needs further exploration

Materials

Plant uptake of NPs should be affected by the type of NPs, possibly due to the different materials of NPs. Although the present study did not directly prove the effect of NPs material on the absorption of NPs by plants, it was found in the study of other nanoparticles that different surface coatings had an important effect on the absorption and translocation

of nanoparticles by plants [103]. It was found that the pores of the cuticle can absorb nanoparticles larger than their own pore size, which may be influenced by the physical and chemical properties of the cuticle and nanoparticles [104,105]. The properties of leaf surface such as lipophilicity may be related to it. Stoma, trichoid or cuticle show extensive lipophilicity, and stoma protection cells have protein-like hydrophilicity or amphiphilicity [103,106]. Therefore, the amphiphilic and lipophilic properties of the NPs surface may play a role in the absorption of NPs through the leaf surface. After passing through barriers such as stomata and cuticle, NPs makes contact with epidermal and mesophyll cells. Mesophyll cells have a wide range of functional groups that can interact with the surface of the NPs [107], while the NPs may have hydrogen bonds with the cell wall, which may also affect the translocation of the NPs [103]. The coating of nanoparticles can profoundly affect their translocation in leaves, so we speculate that different types of NPs have an effect on the uptake of NPs by plants, although this part of the study needs to be further explored.

Plant internalization

Uptake

NPs, like other nanoparticles, have a higher specific surface area [108]. At the same time, due to the strong adhesion and deformability of NPs, it is easy to adhere to plants and be absorbed by plants through certain channels [24]. Soil and the atmosphere are the source of NPs absorbed by plants. The existing experimental results have proved that plants can absorb NPs through the atmosphere and soil [51,87,109]. Many results have proved the existence of NPs in plants. Table 3 summarizes the current absorption and migration of NPs in plants. As early as 2012, plant BY-2 cells cultured in vitro demonstrated the absorption of NPs by plant cells. They found that BY-2 cells can absorb 20 nm NPs after 15 min of exposure [25]. But this only represents the absorption of NPs at the cell level. For a complete plant body, what kind of response will it have to the uptake of NPs? This idea was verified in an experiment on lettuce plants. Under hydroponic conditions, 200 nm PS NPs can be absorbed by the spinach root system and migrated to the stem and leaves, while 1 mm PS MPs hasn't been detected in plants [24]. Since plant cells have a natural barrier, the cell wall, that prevents large particles from entering the cytoplasm. According to previous reports, carbon or metal nanoparticles can pass through the cell wall and be taken up by plant cells [110-112], so whether NPs have a similar uptake mechanism has aroused the interest of scholars.

Crack entry mode

The crack entry mode is considered to be the main mode of NPs uptake by plants. In a study of wheat callus with cell walls, PS NPs were shown to pass through the cell wall and into the cytoplasm. Although the cell wall pore size (1.5–5 nm) of plants is much smaller than the particle size of NPs [114]. In studies of the interactions of other nanoparticles and plants, nanoparticles up to 50 nm have been found to cross this hurdle by expanding pores or altering cell wall structure [115,116]. Furthermore, since NPs have poorer mechanical properties than metal particles and cell walls, this may also lead to softer NPs that are more likely to penetrate cell walls and enter the cytoplasm of plants than metal particles [32]. But how plants absorb NPs is still an area that needs to be explored. A recent study about wheat and lettuce has made great progress in exploring how plants absorb NPs. In this study, NPs in 200 nm were observed in the lateral root caps and apical meristems of roots of wheat and lettuce. They believed that the retention of root cap mucus promoted the penetration of microbeads into the cell wall. Active cell division causes the meristem to be porous, but the diameter of the cell wall pores (1.5–5.0 nm) and plasmodesmata (midpoint of 50–60 nm) is smaller than the particle size of the PS NPs used in this study. Therefore, the microbeads can only enter the root apical meristem through the complete epidermal layer of the root apex. In lettuce plants, fluorescence was observed mainly along the cell wall and intercellular zone, indicating that 200 nm PS NPs entered the cortical zone through the gap between epidermal cells, but could not penetrate the endothelial layer of the continuous area of the casparian zone. At the same time, strong PS luminescence signals were detected in the cracks in the lateral root area (50–100 mm from the apex). Since the lateral root penetrates the endothelial layer and cortex, it indicates that these cracks are the main places to enter the endothelial layer. The crack entry mode is regarded as an important mode of interaction between NPs and plants, and what needs to be mentioned is the root openings caused by aging, underground herbivores and mechanical damage, which may also provide places for NPs to enter[32].

Apoplast transport

Apoplast transport is also considered to be a way for roots to absorb NPs[56,117,118]. NPs can be internalized from the root epidermis to the cortex through the apoplast pathway, and even reach the xylem vessel [33]. The possible mechanism of apoplast transport is that NPs are captured by mucus secreted by plant roots, gather on the root surface, and migrate under the action of plant transpiration and root pressure [56,119]. In the experiment of rice hydroponics, both nano-/micro-sized PS can be absorbed by rice roots and then transferred to the above-ground part. These microbeads mainly gather on the cell wall of the root cortex tissue, indicating that apoplast transport may be the main way of absorption and the dominant factor for PS translocation in rice tissue [89]. Limited by the size exclusion limit of the cell wall, the apoplast pathway has certain requirements on the size of the plastic microbeads [117]. Due to limited diffusion through the cell wall pores, PS MPs with a size of 1 mm cannot penetrate the cell wall [25]. However, some studies have shown that MPs

can deform the cell wall so that MPs of larger particle size can pass through [120]. And their other study found 1 mm PS particles in the intercellular layer of carrot roots [121].

Table 3 Research results of plant uptake of NPs

Plant	Type	Size (nm)	Exposed location	Migrate location	Reference
Nicotiana tabacum (BY-2 cells)	nano-beads (fluorescent)	20, 40, 100	Cell culture medium	Turgescent and plasmolysed cells	[25]
Lepidium sativum	plastic particles	50, 500, 4800	Seed	Plant surface and Root hair	[113]
Lactuca sativa	PS (fluorescent)	200, 1000	Root	Root, stem and leaf	[24]
Triticum aestivum	PS (fluorescent)	200	Root	Root, stem and leaf	[88]
Cucumis sativus	PS, PMMA	50, 100	Root	Root, stem and leaf	[42]
Riticum aestivum	PS, PMMA (fluorescent)	200, 2000	Root	Root, stem and leaf	[32]
Oryza sativa	PS (fluorescent)	80, 1000	Root	Root, stem and leaf	[89]
Lactuca sativa, Raphanus sativus, Triticum aestivum and Zea mays	PS (fluorescent)	100, 5000	Root	Root	[55]
Arabidopsis thaliana	PS-SO_3H, PS-NH_2 (fluorescent)	55, 71	Root	Root	[56]
Zea mays	PS-NH_2, PS-COOH (fluorescent)	20, 50, 100, 200	Leaf	Root, stem and leaf	[87]
Triticum aestivum	PS-NH_2, PS-COOH (fluorescent)	50, 100, 200	Root	Root	[90]

Symplast transport

Contrary to apoplast transport, cell endocytosis may also be one of the absorption methods of NPs [108]. In one study, fluorescent plastic particles of 20 nm were rapidly internalized by BY-2 cells cultured in vitro [25]. In addition, another study observed that 50 nm PS was internalized in the vacuole and cytoplasm of root cells, but rarely appeared in the nucleus [122]. Therefore, the symplast transport is the third possible way for root cells to absorb NPs. However, this route is only based on independently cultured cells, and there is no research on the endocytosis of NPs by the root cells of intact plants. This is still an area that needs to be explored.

Migrate

After being absorbed by plants, NPs may migrate, accumulate, biomagnify and eventually lead to accumulation in organisms and humans [88]. Therefore, studying the migration of NPs in plants is of great significance for ecological environment and health risk assessment [131]. Early studies believed that NPs would be trapped in the epidermal cells of plants after being absorbed by plants. However, with the development of granular plastic analysis technology, in recent years, there has been tremendous progress in detecting the absorption and migration of MPs and NPs in plants [108]. In an experiment, lettuce plants were exposed to fluorescently labeled polystyrene NPs. And it can be observed from the section of the root system that a large number of PS NPs of 200 nm are present in the gaps of the plant cell wall, which indicates that the PS NPs can enter the interior of the root system. It was also observed that the PS NPs could reach the central pillar, and the PS NPs that reached the central pillar would migrate upward from the xylem to the above-ground part of the plant under the action of transpiration pulling force [24,88]. Moreover, a recent study found that 0.2 mm PS NPs and 2.0 mm PS MPs exist in the xylem sap of wheat and lettuce, indicating that both submicron and micron plastic beads migrate from root to shoot through transpiration. In addition, under high transpiration conditions, the fluorescence intensity at the junction of the primary and secondary roots of wheat, which is regarded as the microbead entry channel, is stronger than under low transpiration conditions, indicating that the increase in transpiration rate enhances the absorption of beads by plants [32]. The migration process starting with the leaves is similar. The nanoparticles need to penetrate the cell wall and membrane to achieve further translocation, and this process is thought to be accomplished through apoplast and the symplast transport. Nanoparticles were shown to be able to transfer from leaves to adjacent leaves, stems, and roots [132]. What's more, clumps of nanoparticles were observed mainly in the vascular region,

suggesting that nanoparticles may be able to achieve ectopic placement within the plant through the vascular region [128,133].

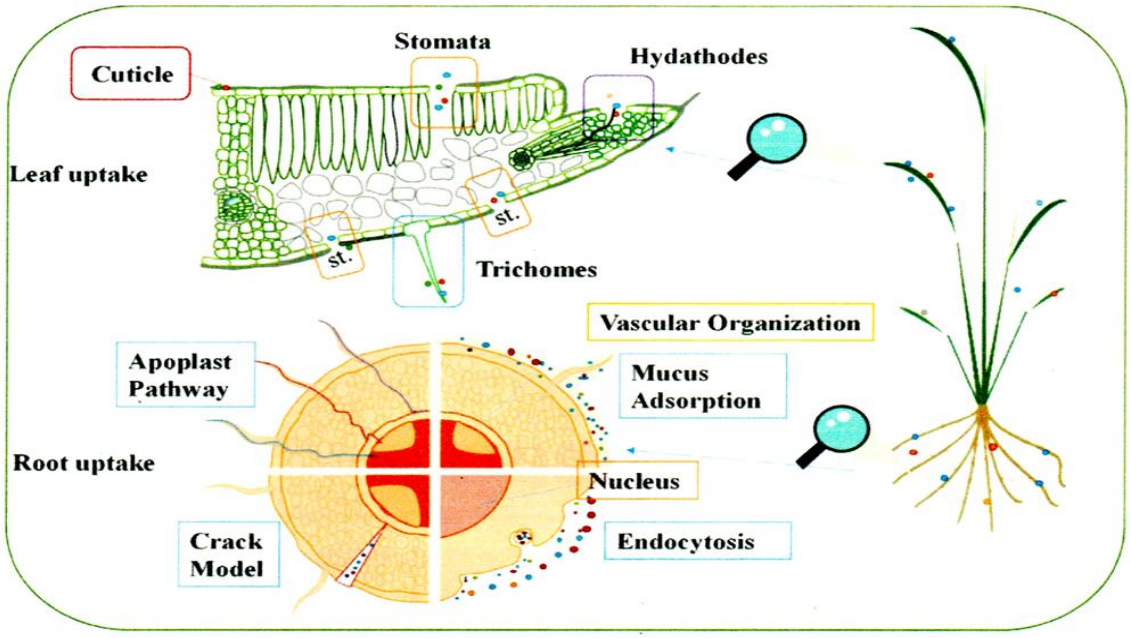

Fig. 3. Possible pathways for plant roots and leaves to absorb NPs.

Risks in agriculture

Toxicity of plants

NPs can not only interact with plants, but also have an important effect on plant growth and development. Current studies have extensively demonstrated the effects of NPs on plants. Germination rate is one of the important indicators to evaluate the plant toxicity of NPs [134]. Studies have shown that NPs can reduce the germination rate of seeds [113]. This effect may be caused by the mechanical blockage of seeds by NPs. In addition, plasticizers, as one of the components of plastics, may be released in the environment [135]. Studies have shown that plasticizers can also inhibit the germination process of seeds and cause oxidative damage [136]. NPs may also cause root cell clogging, physical damage to algal cell walls, and even limit the transfer of energy and matter between cells and the environment [41,137]. In addition, NPs can reduce the metabolism of ROS, and ROS stress may lead to the obstruction of plant energy metabolism, and may also produce genetic toxicity [56,138].

A large number of higher plants have shown a response to NPs [134,139]. NPs could affect the growth of wheat root system, decrease root biomass and reduce root/branch ratio [140,141]. In addition, NPs inhibits peanut vegetative growth and nitrogen uptake by damaging root cells and interfering with soil nitrogen cycling[142]. Plant enzyme activities and hormones are also affected by NPs, and thus affect carbohydrate metabolism and ROS metabolism in barley [51].

NPs may also indirectly affect plants. Changes in soil and microbial properties due to NPs may have indirect effects on plants. For example, NPS-mediated changes in soil structure may affect soil fertility and rhizosphere processes [139,143,144]. In addition, the toxic effects of NPs on soil fauna will affect soil porosity and water content [143,145]. All of these processes indirectly affect plant growth. Although NPs is considered an emerging pollutant and its phytotoxicity has been demonstrated [56]. It is possible that NPs may have some positive effects on plants. For example, MPs fiber can reduce soil bulk density, which can be directly translated into reduced penetration resistance of plant roots and better soil aeration, thus promoting root growth [146–148].

Potential threats in agriculture

NPs not only affects individual plants, but also poses potential threats to plant communities and even ecosystems [47,48,149]. Different plants have different responses to NPs [55]. Due to different stress effects, the homogeneity of plants in the community will be affected [48], even affecting plant diversity and community composition [47]. This potential risk may be due to changes in soil structure, evaporation of soil water and changes in microbial communities caused by NPs. Accelerated water evaporation will enhance the advantages of drought-tolerant plants [150], while changes in microbial communities will strongly affect the composition and diversity of plant communities [151,152].

Farmland is one of the hardest hit areas for plastic pollution due to agricultural inputs [153]. The continued influx could make NPs another problem affecting agricultural production. Especially in today's climate disasters, the protection of global food security has become an urgent problem for us to solve. As an emerging pollutant in agricultural soils, NPs

can pose a threat to crops and can also act as a carrier for other pollutants. Therefore, it is urgent to explore the unknown risks of NPs and put forward reasonable measures.

Conclusion

This article reviews the current methods used to detect NPs in plants, the commonly used NPs and their modifications in research, the absorption mechanism and migration mechanisms of NPs by plants and the risk in agriculture. This report shows that plants can absorb NPs and NPs can migrate in plants, and this migration can occur simultaneously in roots and leaves. The uptake of NPs by plants may be affected by the size, material, charge and plant secretions of the plastic particles. It is possible to verify and observe the absorption of NPs by plants simply and effectively through SEM and LSCM. According to existing research, plant roots may absorb NPs in the following possible modes: apoplast transport pathways, crack entry modes, and endocytosis of epidermal cells. The absorption channels of NPs by leaves may be stomata, cuticle, trichomes and hydathodes. In addition, NPs has become a new type of pollutant threatening agricultural production. However, the current research on the interaction between plants and NPs still has shortcomings. The transfer and enrichment of NPs in the food chain caused by plant absorption and the impact on food production and food safety still need to be studied in depth.

On this basis, the following issues are worthy of further attention by researchers in future work.

(1) Although studies have shown that plants can internalize MPs and NPs, the qualitative and quantitative analysis of MPs and NPs in plants is still blank. At present, quantitative techniques for MPs and NPs in plants cannot be applied in real environments. Therefore, a widely applicable, accurate and effective method for qualitative and quantitative analysis of plant MPs and NPs needs to be proposed.

(2) The current research is basically carried out in an environment with high concentration of NPs under human interference. However, in the natural environment, except for some extreme conditions, the concentration of NPs in most areas does not exceed 0.1%. Therefore, the uptake and interaction of NPs by plants under natural conditions need to be explored.

(3) The combined effect of NPs and other environmental pollutants needs to be paid attention to. Under natural conditions, the surface of NPs in the environment tends to adsorb other pollutants, leading to increased toxicity. Therefore, it will be of great use to study the combined effect and the influence mechanism of NPs and other pollutants on plants.

(4) Most of the existing studies use granular plastic microspheres, but under natural conditions, the shape of NPs is ever-changing, and the degree of aging of NPs is also different. Further studies are needed to determine the effects of NPs with different morphology and aging degrees on plant uptake and on plant growth.

(5) Nanomaterials can stimulate the defense system of crops and enhance crop stress resistance. The application of nanomaterials in agricultural production is promising. Therefore, whether NPs can be wisely applied to crops to enhance crop adaptability needs to be further explored.

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