UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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DISSERTAÇÃO DE MESTRADO

GUSTAVO FÜHR HARTMANN

Toxidade de microplásticos em plantas: uma revisão crítica e boas práticas para experimentação

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Toxidade de microplásticos em plantas: uma revisão crítica e boas práticas para experimentação

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SUMMARY

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RESUMO

As facilidades advindas do uso de produtos plásticos têm um alto custo ambiental: os elevados níveis de poluição devido à degradação e fragmentação dos polímeros sintéticos, resultando em pequenas partículas denominadas microplásticos (MP, > 5 μm). As consequências da crescente contaminação ambiental por estas partículas estão entre os grandes desafios que a humanidade terá que enfrentar nas próximas décadas. Uma vez disponíveis no ambiente, estas partículas podem interagir com o solo e alterar sua a estrutura físico-química, afetando negativamente a biota local. Ambientes agrícolas são especialmente afetados devido às práticas utilizadas atualmente, como o uso de fertilizantes à base de lama sedimentada durante o tratamento de água e o uso de coberturas plásticas. As pesquisas com MP e plantas terrestres ainda são incipientes, iniciando apenas em 2018, e assim os métodos de estudo ainda estão em desenvolvimento, o que dificulta a padronização dos experimentos. A presença de MP no ambiente pode afetar a produtividade das espécies vegetais, causando alterações metabólicas, citológicas e genéticas, diminuindo o tamanho e número de sementes, sendo absorvidas e transferidas para os diferentes órgãos. O objetivo desta dissertação foi apresentar o estado da arte de experimentos com plantas terrestres e MP, elencando potenciais efeitos destas partículas nas plantas e em sistemas agrícolas e apresentando uma análise cienciométrica das publicações da área. Dada a problemática encontrada na não padronização dos experimentos, e as lacunas de informações nas metodologias e publicações disponíveis, este trabalho também objetivou apresentar uma sessão com boas práticas necessárias para a montagem e condução de experimentos com MP, trazendo uma visão crítica formada a partir das leituras e observações, além de um estudo de caso abordando um experimento realizado por nosso grupo de pesquisa. A contaminação por MP pode acarretar a danos severos às colheitas e à saúde alimentar de seres humanos, porém, mais estudos na área precisam ser desenvolvidos para melhor avaliar o potencial tóxico destas partículas sobre a o crescimento, desenvolvimento e nutrição das plantas, além de efeitos ecológicos e agronômicos em larga escala.

Palavras-chave: cienciometria; fitotoxidade; plantas cultivadas; plástico; poluição ambiental.

ABSTRACT

The facilities arising from the use of plastic products have a high environmental cost: the high levels of pollution due to the degradation and fragmentation of synthetic polymers, resulting in small particles, called microplastics (MP, > 5 μm). The consequences of the increasing environmental contamination by these particles are among the great challenges that humanity will face in the coming decades. Once available in the environment, these particles can interact with soil and change its physical-chemical structure, negatively affecting the local biota. Agricultural environments are especially affected due to the practices currently used, such as the use of sedimented mud-based fertilizers during water treatment and the use of plastic covers. Research with MP and land plants is still incipient, starting only in 2018, thus the study methods are still under development, which makes it difficult the standardization of the experiments. The presence of MP in the environment can affect the productivity of plant species, causing metabolic, cytological and genetic changes, reducing the size and number of seeds, being absorbed and transferred to the different organs. The objective of this dissertation was to present the state of the art of experiments with terrestrial plants and MP, listing potential effects of these particles on plants and agricultural systems and presenting a scientometric analysis of publications in the area. Given the problems found in the non-standardization of experiments, and the information gaps in the methodologies and publications available, this work also aimed to present a session with good practices necessary for the setting up and conducting of experiments with MP, bringing a critical view formed from the readings and observations, in addition to a case study addressing an experiment carried out by our research group. MP contamination can cause severe damage to crops and human food health, however, more studies need to be developed to better evaluate the toxic potential of these particles on growth, development and nutrition of plants, in addition to effects on ecological and agronomic systems on a large scale.

Keywords: crops; environmental pollution, phytotoxicity; plastic; scientometrics.

General introduction

The use of plastic products is deeply associated with the consumption habits of today's society. The growing use of synthetic polymers in diverse areas such as construction, medicine and clothing is due to their versatility, lightness, durability, malleability, thermal and electrical insulation capacity, in addition to their low cost. The annual global production of plastic is estimated to have increased about 200-fold from the 1950s to 2015, accounting for about 380 million tons in 2015 alone and totaling almost 8 billion tons over these years (Ritchie and Roser, 2019). The great production of these materials generates lots of residues, which in most cases do not receive adequate destination, such as reuse or recycling, and ends up being deliberately discarded in natural environments (Geyer et al., 2017). Plastic pollution has been identified as the biggest and most lasting human transformation on the planet (Barnes et al., 2009; de Souza Machado et al., 2018a; Thompson et al., 2009) being defined as a geological marker of the Anthropocene period (Crutzen, 2006; Zalasiewicz et al., 2016).

Small plastic particles called microplastics (MP, > 5 µm) and nanoplastics (NP, size < 100 nm) have drawn the attention of scientists and environmentalists during the last years. MP can be artificially produced (in small sizes and different shapes) or naturally generated by many processes of fragmentation and degradation of larger plastic pieces (Gigault et al., 2018). The wide distribution of MP/NP, and the small size of these particles facilitates their transport (Glaser, 2015), making these particles being found in almost all places of Earth, such as the marine environment (Lebreton et al., 2017), freshwater (Wagner et al., 2014), terrestrial soils (Chae and An, 2018), and agricultural lands (Henseler et al., 2019). It is important to highlight their presence in remote environments, such as snow of the Artic (Bergmann et al., 2019) and in remote mountains (Allen et al., 2019) as well. Moreover, the small size makes MP extremely difficult to identify and remove on a large scale. Therefore, MP pollution is virtually irreversible (de Souza Machado et al., 2018a).

Effects of MP/NP in terrestrial environments were first described in 2012 (Rillig, 2012). Important plastic particles sources to soils are dumping of untreated sewage, leaching from garbage dumps, wear and tear of tires and roads, the use of fertilizers produced from by-products of sewage sludge, degradation and disintegration of plastic materials used in agriculture, and water used for irrigation (de Souza Machado et al., 2018b; Jambeck et al., 2015; Nizzetto et al., 2016a, 2016b; Rillig et al., 2017; Rochman, 2018). In soil, MP promotes changes in structure, increasing soil particles aggregation and bulk density and decreasing water retention (de Souza Machado et al., 2018b). It was observed changes in community and in the metabolism of soil microbiota, due to the release of chemical compounds from plastics and to changes in soil physical and chemical properties (Rillig et al., 2019a; Zhu et al., 2019). Although it is a recent research area, studies already show that MP/NP can affect plant development, promoting metabolic disorders - mainly in energetic processes - cito-genotoxicity, and oxidative stress (Lian et al., 2020; Lu et al., 2020; Zhao et al., 2016; Zhou et al., 2021). In addition, changes in plant growth were observed, such as increased root length and decreased diameter (Z. Li et al., 2020a, 2020b; Lian et al., 2020; Rillig et al., 2019b), which are similar effects to those observed in some species exposed to drought (Khalil et al., 2020; Lozano and Rillig, 2020). Yield can be negatively affected by MP contamination in rice (Wu et al., 2020), wheat (Qi et al., 2018) and also observed in lettuce leaves (Gong et al., 2021; Z. Li et al., 2020a). MP/NP can be absorbed by plants, specially by roots (L. Li et al., 2020), but also by leaves (Sun et al., 2021), and can be translocated through the entire plant body (Liu et al., 2022; Sun et al., 2021).

Chapter 1 presents a critical review of the effects of MP/NP in plants, highlighting the potential consequences crop species exposure to these pollutants, and how this could affect production and human health. A scientometric study was carried out with the available publications, seeking to analyze metrics and have a clearer view of the state of the art in this research area. In addition to publication metrics, this chapter also presents a summary of the effects observed in terrestrial plants exposed to plastic particles, focusing on delimiting how the differences in the physical characteristics of the plastic particles can change how they will affect the plants.

Aiming to contribute to methodological knowledge gaps in the field, Chapter 2 addresses the main problems that scientists may experience while conducting experiments about MP effects in plants. In this chapter, we reported a case study based on the effects of PVC particles on rice seeds and seedlings. With this chapter, we hope to clarify some important points on how to perform experiments with microplastics and plants, such as the best culture medium, the origin and chemical composition of the plastic, the size of the particles, and the plant species used for exposure.

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CHAPTER 1

Phytotoxic effects of plastic pollution in crops: what is the size of the problem

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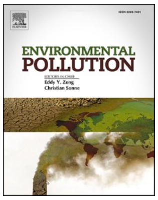

Phytotoxic effects of plastic pollution in crops: what is the size of the problem?☆

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ABSTRACT

Plastic pollution is one of the most impactful human interferences in our planet. Fragmentation of plastic leads to nano- and microplastics (NP/MP) formation, which accumulate in agricultural lands, representing an increasing risk for crop production and food safety. It has been shown that MP promote damage in plant tissues by several direct and indirect ways, and that NP can enter the tissues/cells and accumulate in edible organs. Investigation of the phytotoxic effects of NP/MP in plants started only in 2016, with most of the studies performed with crops. Since contradictory results are often observed, it is important to review the literature in order to identify robust effects and their possible mechanisms. In this review, we discuss the potential of NP/MP in damaging crop species, with focus on the physiological changes described in the literature. We also performed scientometrics analyses on research papers in this field during 2016–2021, to reveal the research situation of phytotoxic effects of plastic pollution in crops. Our review is as a starting point to help identify gaps and future directions in this important, emerging field.

1. Microplastic pollution in terrestrial domains

Plastic pollution is one of the most widespread, lasting and impactful human interference in our planet (Barnes et al., 2009) and a key geological indicator of the Anthropocene age (Zalasiewicz et al., 2016). Due to their durability, malleability, versatility and cost-benefit, plastic polymers are used for several scientific and technological applications (Andrady and Neal, 2009; Thompson et al., 2009), especially in package and construction sectors (Geyer et al., 2017). Around 8,000 billion tons of plastic were produced between 1950 and 2015. A significant part of production was used for disposable products, which are quickly converted in waste, whereas only 20% approximately is for long-term use or reused products (Ritchie and Roser, 2019).

Plastic particles up to 100 nm in size are called nanoplastics (NP), whereas fragments ranging between 100 and < 5 mm are called microplastics (MP) (Gigault et al., 2018; Rochman, 2018). Small plastic particles are industrially synthesized for use in many products (as raw materials and cosmetics) and are named primary plastic particles. NP/MP resulting from fragmentation of larger plastic pieces by mechanical abrasion, ester bonds hydrolysis, photo oxidation by UV exposition, heat and microbial degradation (Maity and Pramanick, 2020; Song et al., 2017) are called secondary plastic particles. These are predominant, accounting for 80% of total particulate plastic found in the environment (Cole et al., 2011; EFSA, 2016).

Terrestrial domains receive larger direct input of plastic than marine environments (Jambeck et al., 2015). The plastic disposed in land areas is carried by rivers, by the atmosphere and by changing tides in coastal areas, accounting for approx. 80% of total MP/NP found in oceans (Horton et al., 2017; Jambeck et al., 2015). The main sources for MP/NP accumulation in environment are urban runoff water (that carries

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☆ This paper has been recommended for acceptance by Da Chen.

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particles coming from self-care products and clothes wash), wear of vehicles tires, improperly discarded plastic waste and agricultural practices (Kole et al., 2017; Bläsing and Amelung, 2018; Zhang et al., 2020; Isari et al., 2021).

Agricultural lands are extremely susceptible to MP/NP accumulation, due to constant input as a result of crop management practices (Nizzetto et al., 2016; Rillig et al., 2017; Büks and Kaupenjohann, 2020; Isari et al., 2021), such as: degradation of plastic mulch film and plastics used in irrigation systems; use of fertilizers based on sewage sludge extracted from wastewater treatment (up to 56,400 particles Kg^{-1} were found in raw sludge); and the use of contaminated water for irrigation (Zhu et al., 2019) (Fig. 1A). Besides, the proximity of farms to roads represents a potential source of MP/NP from tires wear to agricultural systems (Cao et al., 2021). These particles were already found in carrot (*Daucus carota* L.), lettuce (*Lactuca sativa* L.), broccoli (*Brassica oleracea* L.), apple (*Malus* sp.) and pear (*Pyrus* sp.) found in supermarkets, suggesting a high potential for human ingestion of plastic particles derived from plant products (Waring et al., 2018; Oliveri Conti et al., 2020). The ingestion of plastics poses risk to the gastrointestinal, excretory, respiratory and nervous systems (Waring et al., 2018; Lehner et al., 2019; Toussaint et al., 2019; Yee et al., 2021).

In agricultural areas, plastic particles can be absorbed by roots and translocated to aerial parts (Chae and An, 2020), leading to reduced grain biomass and crop productivity (Qi et al., 2018; Wu et al., 2020). Studies focusing on the phytotoxic effects of MP/NP in terrestrial plants are recent (de Souza Machado, 2018a, 2019; Bosker et al., 2019; Jiang et al., 2019; Dong et al., 2020; Giorgetti et al., 2020; B. Li et al., 2021) and still scarce. Little is known about the future implications for crop production and food safety to humans (Lehner et al., 2019; Oliveri Conti et al., 2020; Dong et al., 2021). Regarding phytotoxicity, we already know that NP/MP affect germination, root and shoot growth, mineral composition (Qi et al., 2018; Wu et al., 2020) and promote oxidative damage and several metabolic changes in plant tissues (Lian et al., 2020a; Zhou et al., 2021) (Fig. 1B).

In this review we discuss how size, type, shape and concentration of nano- and microplastic particles affect crop species. We also performed scientometrics analyses on research papers in this field during 2016–2021, providing an overview of the field.

2. Micro- and nanoplastic pollution and plants: the state of the art

Studies regarding the interaction between MP/NP and terrestrial environments begun to be addressed only in 2016. The effects of these particles were studied in crops species in ~83% of publications comprising the 2016–2021 period, representing 19 out of 27 species (Fig. 2A, Tab. S1). This indicates the big concern about the impact of MP/NP in agriculture, regarding productivity and food safety. Interestingly, most of the research in this field comes from countries that have a large output of plastic waste, such as China and the USA, with 51.5 and 8.5%, respectively, as shown in Fig. 2B (Jambeck et al., 2015; Ritchie and Roser, 2019). It is also important to point out that experiments were performed in either hydroponic system or soil (~53 and 47%, respectively). Thus, we need to be cautious to extrapolate results to agricultural environments, since the dynamics of MP/NP can be quite different depending on the environment, as shown in sections below.

Fifteen types of polymers were used in experiments with plants (Fig. 3A): In soils, the most common polymer types found are those used in packaging and construction industry: polypropylene (PP), polyethylene (PE), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) (Fuller and Gautam, 2016; M. Liu et al., 2018; Wu et al., 2017; Xu et al., 2019). Curiously, the most used polymer in plant experiments, the

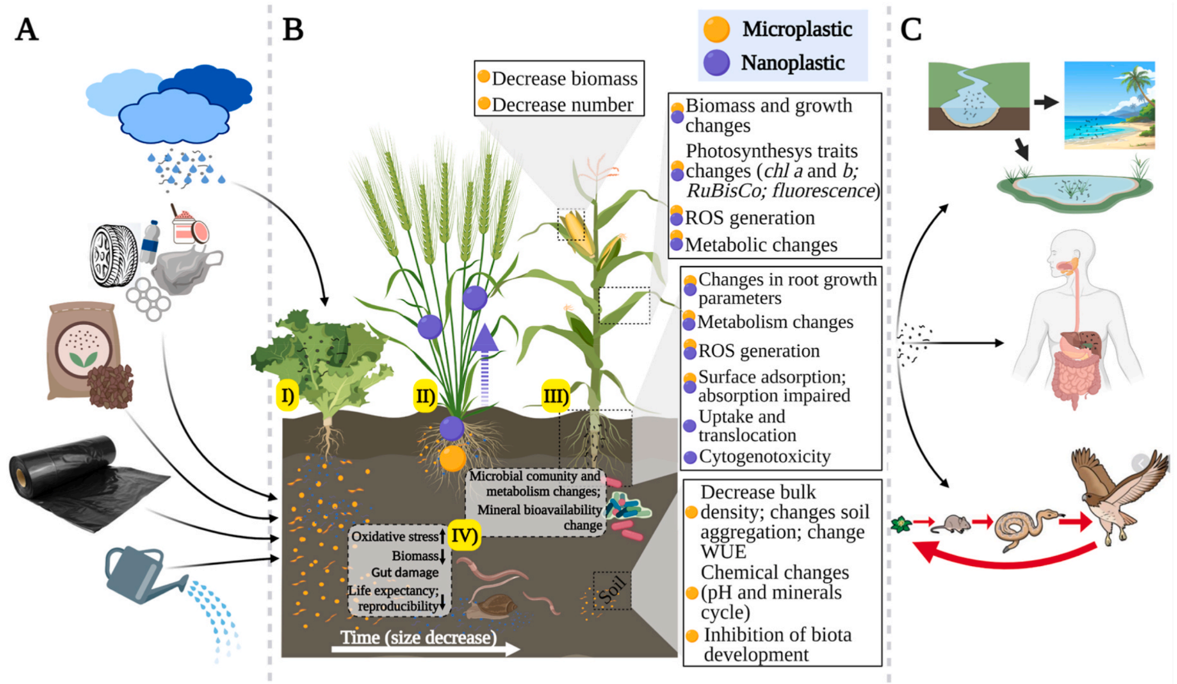

Fig. 1. Plastic particles routes in agricultural lands and effects in crops. (A) Sources of micro-/nanoplastics (MP/NP) contamination to soil: atmospheric circulation, dust and waste, sewage sludge and biofertilizers, plastic mulch and irrigation. (B) Summary of most common effects of MP/NP in crops species (grain, stem/leaf and root, respectively) and soil/microbiota. Image show three most common crops used in experiments (lettuce, wheat and maize). I) shows the ways of MP/NP contamination to vegetables, through soil and air; II) only nanometric particles are uptake and translocated to shoots, and this transport occurs by xylem. Larger particles remain retained in root surface; III) common effects observed in grain, shoot and root; IV) a quick summary of effects in soil structure and in soil invertebrates and microbes. Effects are driven by particles shape, size and type and can be very variable. (C) Agricultural fields as source for MP/NP contamination for: rivers, lakes and ocean; human consumption through food chain; and entry into the trophic chain assisting in the magnification and dispersion of particles. Abbreviations: ROS – reactive oxygen species; WUE – water use efficiency. Image was made in platform Biorender.com.

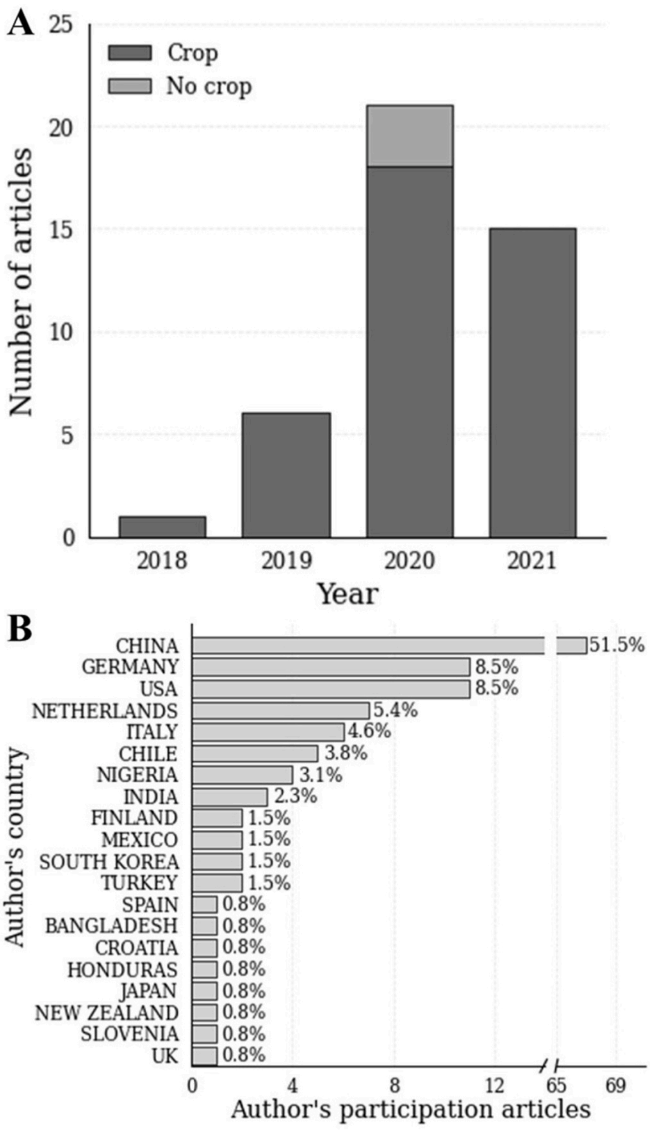

Fig. 2. Publication's metrics of micro- and nanoplastics in terrestrial plants. Scientometrics data regarding publications with MP/NP in terrestrial environments was conducted in Scopus with the following strings words: (TITLE-ABS-KEY ((microplastic OR microplastics) OR (nanoplastic OR nanoplastics)) AND TITLE-ABS-KEY ((soil* OR terrestri*) AND NOT (marine OR sea OR ocean OR freshwater))) AND (LIMIT-TO (SUBJAREA, "ENVI")) AND (LIMIT-TO (DOCTYPE, "ar")), filtering by Environmental Sciences category and original articles. Search was performed on September 15th, 2021. Analyses were performed using the package Bibliometrix in R version 4.0.2 (Aria and Cuccurullo, 2017; R Core Team, 2017). **(A)** Number of research articles per year analyzing the effects of micro- and nanoplastics contamination in crop and non-crop species. **(B)** Number of authors' participation and relative frequencies of each country in articles included in the analysis performed in Fig. 2A.

polystyrene (PS), does not show elevated concentrations in soils (Xu et al., 2019), which can be related to the fact that PS is the most common polymer type found in scientific products stores, having options with standard sizes, shapes and fluorescence. Approx. 74% of the experiments evaluated here used synthesized MP/NP, especially those with PS. The rest used hand-fragmented particles from large pieces of plastic.

Experiments covered a range from 0.02 to 5000 μm (Fig. 3B; Table S1), but there is a prevalence of experiments using particles from 110 nm to 100 μm. In the natural environment, a range of 10 mm–5 mm plastic particles were found in different soil types. However, due to constant particle fragmentation and movement (Rillig et al., 2017), coupled with methodological difficulties (for example, there is still no reliable, reproducible protocol for extraction of submicrometric particles) it is challenging to predict particle size distribution pattern (Möller et al., 2020). Regarding particles shape, 53.6% of MP/NP used in experiments were spherical (including beads), 39.3% were fragments (undefined shape) and 7.1% were fibers (Fig. 3B; Table S1). Fibers and fragments are more commonly found in soils, probably due to plastic waste disposal such as packaging and clothing products (Xu et al., 2019).

In natural soil environments, MP concentrations reaches up to 67,000 mg kg^{-1} in industrial soils and up to 223 mg kg^{-1} in agricultural soils (Xu et al., 2019). MP concentrations used in hydroponic and soil experiments vary from 0.000001 to 0.025 w/v % and 0.001 to 10 w/w %, respectively, while in experiments with NP the concentration used ranges from 0.000001 to 0.1 w/v % and 0.001 to 0.01 w/w % (Table S1). In addition, some papers presented particles concentration as 'particles number', which in MP/NP crop species experiment vary to 10^4 from 10^7 particles Kg^{-1}. The particle number as a concentration measure is useful as it gives information on the quantity of particles used; however, the non-standardization of the methodology can make it difficult to compare the available data. Concentrations used in experiments exceed those found in natural environments; however, it can be useful in order to generate more accurate models to understand and predict future effects of the increasing accumulation of MP/NP.

3. Phytotoxic effects of micro- and nanoplastics on crops

Plastics are highly hydrophobic polymers with potential for leaching and absorbing organic and inorganic compounds (such as polychlorinated biphenyls, polycyclic organochlorine pesticides, aromatic hydrocarbons, antibiotics and heavy metals) (de Souza Machado et al., 2018a; Dong et al., 2020; Li et al., 2018; Zhang et al., 2020). Some studies observed that biodegradable plastic presents strong negative effects on plant growth and chlorophyll content, which can be related to quick degradation and consequent bioavailability of plastic residues for plant (Qi et al., 2018; Wang et al., 2020a).

Soil changes caused by plastic particles can favor spread of some plant species, especially invasive ones, as shown by Lozano and Rillig (2020) in soils exposed to MP fibers, which can lead to crop production losses and increase in the use of herbicides in agricultural lands. In addition, larger particles seems to be sharper and cause more mechanical damage to external structures, while submicrometric particles can be absorbed, interacting within the cell (de Souza Machado et al., 2018a). Thus, besides the potential of NP/MP pollution to damage crops being genotype- and species-dependent (Gong et al., 2021; Wu et al., 2022), their toxicity is specially related to the type (chemical composition), shape, concentration in the environment, but especially to the *size* of particles, which in turn determine whether the effects are direct or indirect.

Direct effects of plastic particles in plants (Fig. 1B) are related to the interaction between these contaminants and plant surface or internal structures. Evidence suggest that larger particles are more harmful to plant surfaces, especially roots, causing abrasion. They can also adhere on root epidermis and accumulate in the cortex, inhibiting the uptake of water and nutrients (Bosker et al., 2019; Lian et al., 2020a; Wu et al., 2020) by promoting the occlusion of the spaces between cellulose microfibrils in cell wall matrix. The term 'clog' has been used as a reference to this blockage of water and minerals uptake by organic or inorganic particles adsorbed in seed and roots surface (Asli and Neumann, 2009), including MP/NP. Smaller particles such as NP can be absorbed by cells, potentially causing molecular and metabolic changes (de Souza Machado et al., 2018a). In this case, PVC, PP and biodegradable plastic showed more prominent effects (Z. Li et al., 2020a; Pignattelli et al., 2020; Qi et al., 2018) probably due to chemical composition.

Indirect effects are related to changes in soil physical-chemical properties and microbiota, thus affecting plant growth and development (Fig. 1B). Changes in soil properties seem to be related to the size

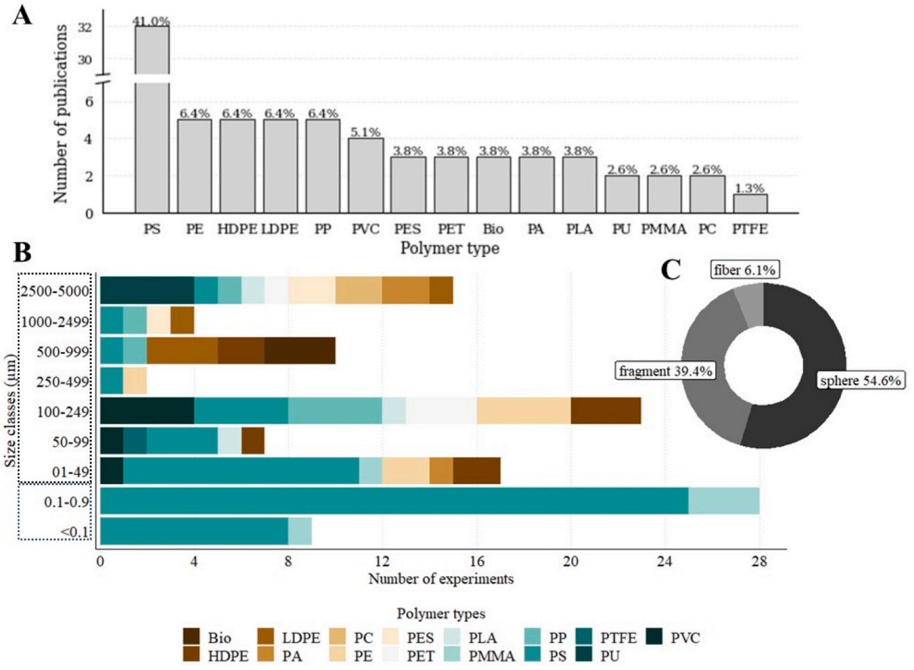

Fig. 3. Characteristics of micro- and nanoplastics used in experiments with terrestrial plants. **A)** Particle's type used in plant experiments (absolute and relative frequencies); **B)** Size classes of particles used in experiments. Some publications used a mixture of particles of varying sizes, so the median size of the particles was considered in the experiments (Boots et al., 2019; de Souza Machado et al., 2019; Z. Li et al., 2020a; Lian et al., 2021a; Lozano et al., 2021; Lozano and Rillig, 2020; Pehlivan and Gedik, 2021; Pignatelli et al., 2020; Qi et al., 2020, 2018; Verla et al., 2020; Wang et al., 2021, 2020a; 2020b; Yang et al., 2021). Polystyrene (PS), polyethylene (PE), low-density polyethylene (LDPE), high-density polyethylene (HDPE), polypropylene (PP), polyvinyl chloride (PVC), polyester (PES), polyethylene terephthalate (PET), starch-based biodegradable polymer (Bio), polyamide (PA), polylactic acid (biodegradable polymer; PLA), polyurethane (PU), polymethylmethacrylate (PMMA) and polycarbonate (PC). Black and blue dashed squares are microplastics and nanoplastics size classes (respectively); **C)** Shape of particles used in plant experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

of plastic particles, in a way that plastic particles larger than soil's affects soil properties more severely. In addition, authors observed variable effects in differ soil textures. Loamy sand soil exposed to polyester (PES) fibers decreased soil bulk density and water stable aggregates while increased water holding capacity and evapotranspiration. On the other hand, in clay soils exposed to PE plastics the loss of water through evaporation was severe, causing cracking due to desiccation in soil. Variable data warns of the need to understand the effects of MP/NP in soils (de Souza Machado et al., 2018b; Xu et al., 2019; Zhang et al., 2020). MP/NP promotes changes in soil pH and it is related to the presence of chemical compounds in MP/NP (such as in biodegradable ones, where chemicals are leached faster) that can influence nutrient availability and rhizosphere quality, affecting crop development negatively (Wang et al., 2020a; Xu et al., 2021). Finally, soils treated with MP/NP shows an inhibition in macro invertebrates growth and biomass, probably due to ingestion of these particles (Boots et al., 2019; Huerta Lwanga et al., 2016; Qi et al., 2018), affecting negatively crop development and production (van Groenigen et al., 2015). Little is known about NP effects in soil physical and chemical properties, and therefore this is an area which should be further investigated in the future.

In the recent years, it has been demonstrated that MP/NP pollution affects several steps of plant growth and development, such as germination and photosynthesis, but results are still controversial in some cases. In view of this, the main phytotoxic effects of plastic pollution on crop physiology are discussed in the sections below.

4. Germination

Seed germination is a critical stage in plant life that strongly affects crop development and yield (Barnard and Calitz, 2011). This process is delayed by large plastic particles, since they accumulate in seed coat, blocking pores (pits) and decreasing water uptake, as showed by Bosker et al. (2019) in garden cress (*Lepidium sativum* L.) treated with PS-MP of 4,8 μm. In this work, although a few particles reached the endosperm during imbibition, water deficit induced by plastic delays starch granules hydrolysis and leads to high levels of reactive oxygen species production, inhibiting germination and decreasing seedling vigor. In the same species, a 55% inhibition in germination of seeds exposed to PE, PVC and PP (<125 μm) was detected in the first six days. In 21 days, PE and PP reach to 7 and 14.3% of germination inhibition (Pignattelli et al., 2020). Likewise, germination of ryegrass (*Lolium perenne* L.) was inhibited by large polyamide (PA) fibers (>2 mm), even after 30 days (Boots et al., 2019). In lettuce, germination index reduced significantly with NP and MP exposure in both high and low concentrations, but no differences were detected for the three other species studied – radish, wheat and maize (Gong et al., 2021). Seedling malformations and decreased root size were also observed in seeds exposed to plastic debris, probably due to cytotoxic effects and slow energy generation caused by water deficit (Bosker et al., 2019; B. Li et al., 2021).

On the other hand, an improvement on germination was observed in wheat (*Triticum aestivum* L.) seeds exposed to NP. They entered the seed and improved germination rate, root elongation and seed vigor. The authors attributed this opposite effect to the changes in metabolic pathways involved in energy production (TCA cycle, starch, sucrose and galactose metabolism). NP used in the experiment also seem to stimulate seed germination and seedling development by improving starch granule hydrolysis, acting as a "*nanocatalyst*" for plants development in initial life stages (Lian et al., 2020a).

In general, most of germination experiments showed inhibition of germination due to increased plastic concentration in the environment, which can be especially dangerous for agricultural systems over the decades. Delayed seed germination and malformed seedling can jeopardize plant development and growth, resulting in yield losses, especially in annual species (Rice and Dyer, 2001). There is no data about viability of seeds produced from plants exposed to plastics, but decreases in grain number and biomass were already reported (Qi et al., 2018; Wu et al., 2020).

5. Plant growth and development

Edible parts of crops have their growth decreased by MP/NP exposure, as shown in carrot and maize roots (Dong et al., 2021; Urbina et al., 2020; Fu et al., 2022), in spring onion (*Allium fistulosum* L.) bulbs (de Souza Machado et al., 2019), in lettuce dry weight, leaf biomass/area and height (Gao et al., 2021; Lian et al., 2021a), in chinese cabbage fresh weight (Yang et al., 2021), and in the number and biomass of rice (*Oryza sativa* L.) and wheat grains (Qi et al., 2018; Wu et al., 2020). These data highlight the potential harm of MP/NP to crop production and yield quality, especially in the next decades, when plastic accumulation in land soils is likely to increase.

Most of experiments regarding MP/NP has been conducted in soils and hydroponics, and plant responses varies according to particles features and experimental system. In soil experiments, roots generally show increased elongation, reduced diameter, increased surface area, and higher root-to-shoot biomass index. These effects are similar to those exhibited by plants experiencing water deficit (Khalil et al., 2020), and by affecting root water and nutrient uptake. In fact, plants treated with MP show more pronounced decreases in fresh biomass than dry biomass, indicating decreased tissue hydration (de Souza Machado et al., 2019). In shoots, it has been observed a decrease in leaf length and number, in number and biomass of fruits, and in tiller production. These effects were observed in plants exposed to both classes of plastic particles sizes (Boots et al., 2019; de Souza Machado et al., 2019; Dong et al., 2021; Z. Li et al., 2020a; Lozano and Rillig, 2020; Maity and Pramanick, 2020; Meng et al., 2021; Pignattelli et al., 2020; Qi et al., 2018; Sun et al., 2020; Lian et al., 2021a).

In hydroponic system plastic particles interactions are expected to be distinct, making MP/NP more susceptible to directly contact with root tissues, while in terrestrial medium, MP/NP interact with soil particles and may be less bioavailable. Arabidopsis (*Arabidopsis thaliana* L.), maize, onion and rice plants present reduced biomass and reduced root and shoot length, specially under high MP/NP concentrations (Dong et al., 2020; Giorgetti et al., 2020; Maity et al., 2020; Sun et al., 2020; Urbina et al., 2020; Zhou et al., 2021).

Rice seedlings exposed to NP particles show an interesting pattern, decreasing main root length and biomass while increase lateral root density (Zhou et al., 2021). Authors described the down-regulation of *OsOFP2* (*Ovate Family Protein 2*), a protein linked to root elongation, while observed an up-regulation in genes *OsMADS25* (MADS-Box Transcription Factor 25) and *OsNAAT1* (Nicotianamine Aminotransferase 1), related to lateral roots development and mineral acquisition, respectively (Ogo et al., 2011; Yu et al., 2015). Root changes are possibly a response to alterations in water and nutrients uptake (Zhou et al., 2021). On the other hand, lettuce and wheat showed increased root growth after MP and NP exposure, respectively (Z. Li et al., 2020a; Lian et al., 2020a). Improvement on root growth is a response to external stressors and the shoot to root biomass reallocation can be a response to inhibition of nutrient acquisition (Lian et al., 2020a). However, as mentioned before, some results are still controversial and more studies are needed to understand the dynamics of MP/NP in plant growth and development, as well as to identify consistent, robust responses across different plant species.

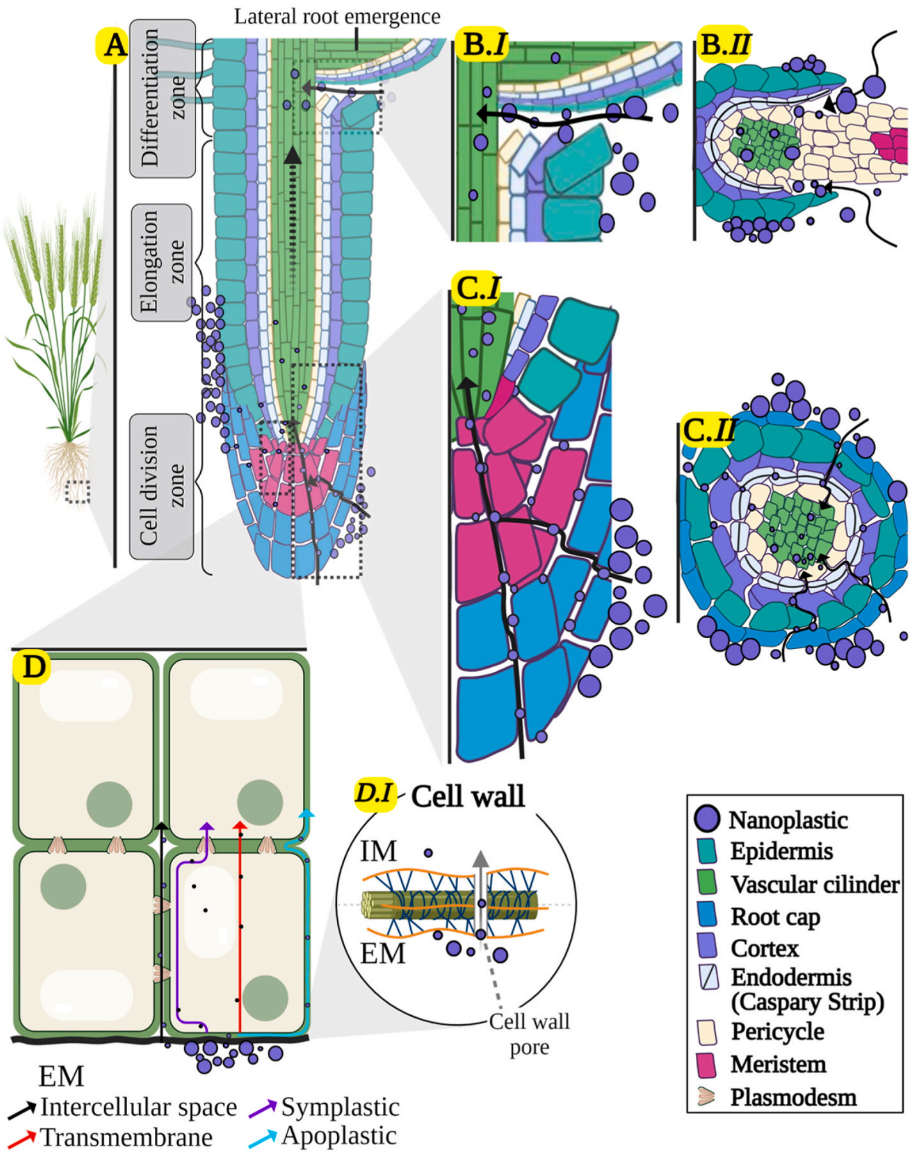

Fig. 4. Mechanisms of nanoplastics (NP) entry in vascular system. **A)** Root tip with an emergence of lateral root. The figure shows NP accumulation in root surface, possible causing inhibition of water and mineral uptake, and the three possible routes to NP entry in vascular system; **B)** In crack-entry mode, particles entering the vascular system through cellular gaps created in the emergence region of the lateral root. Larger NP particles (~200 nm) can be transported by this route (L. Li et al., 2020); I) longitudinal section of lateral root emergence point; II) transversal section of lateral root emergence point; **C)** Entry by interspace of meristematic cells, only small NP can enter by this route. NP reach the vascular cylinder moving through the space between the meristematic cells, the lack of Caspary strip in developing endodermis, allows the entry of NP into the vascular system (L. Li et al., 2020); I) longitudinal section of root apex; II) transversal section of root apex (above meristematic region). In both B.I and C.I it is possible see areas where the Caspary' strip is discontinuous. **D)** Meristematic root cell. Transport of NP by intracellular mechanisms (apoplastic, symplastic and transmembrane) is restricted to the size of the cell wall pore opening (approx. 6 nm), the entry of this particles in cytoplasm is more size restricted. These mechanisms were not observed in experiments with NP particles, but these particles have already been observed internalized in the cytoplasm (Giorgetti et al., 2020). Although it was observed cellular internalization of carbon nanoparticles by phagocytose (Bandmann et al., 2012), we not include this mechanism in illustration due to the lack of more information; I) Detail of cell wall pore. EM: External medium, IM: Intracellular medium. Image was made in Biorender.com.

6. Uptake and translocation

Particle size can differently affect plant nutrient uptake and translocation: particles smaller than 2 μm were found in root stele, but only nanometric particles were translocated to shoots. MP/NP accumulation in roots and/or leaves has been reported in Arabidopsis, carrot, lettuce, maize, mung bean (*Vigna radiata* L.), rice and wheat (Chae and An, 2020; Dong et al., 2021; Z. Li et al., 2020b; Sun et al., 2021, 2020; Zhou et al., 2021) and it can be taken up by crop species even at a very early growth stage - less than seven days after sowing (Gong et al., 2021).

The main sites of particle accumulation in roots are the root cap, the subapical meristem and the differentiation zone (Fig. 4A). The entry in root seems to occur through two main pathways: by crack-entry mode, an apoplastic pathway, where the emergence of lateral roots in the primary root creates cracked areas, exposing the intracellular space (Fig. 4B), or through intercellular spaces created in regions with high cell division activity, such as the root cap and apical meristem (Fig. 4C) (L. Li et al., 2020). Uptake is higher under higher transpiration rates, suggesting that movement close to rhizosphere occurs through mass flow. Under low transpiration rates, only the crack-entry pathway seems to be possible, as observed in wheat and lettuce. In addition, mucilage secreted by root cap seems to facilitate absorption (L. Li et al., 2020).

Once inside the root, particles move via apoplast towards the stele (Liu et al., 2022). The access of stele seems to be achieved through zones where the Casparian strips are discontinuous (named passage cells), located in apical meristem and lateral roots emergence zones (L. Li et al., 2020). Only particles smaller than 5 nm penetrate the pores of the epidermal cell wall and are transported via the apoplastic route (through the cell wall) or internalized in the cell. Higher molecular weight particles are not transported by the xylem to shoots. In fact, while MP (>1 to <2 μm) were only observed in outermost root tissues (fewer particles were observed in root stele), 1 μm MP (Liu et al., 2022) and NP particles were translocated to shoots via xylem, accumulating in leaf tissues (Chae and An, 2020; Dong et al., 2021; Z. Li et al., 2020b). In addition, authors hypothesize that PS-NP (100 nm) can be degraded in cucumber leaves, resulting in toxic benzene rings accumulation in plants (Z. Li et al., 2020c).

Cellular internalization of NP (<25 nm) has been detected, probably due to plasma membrane disruption (Giorgetti et al., 2020). Internalization of very small particles suggests the possibility of cell-to-cell movement through plasmodesmata connections (diameter of approx. 60 nm) by symplastic pathway. Rice seedlings exposed to an aquaporin inhibitor ($HgCl_2$) show less accumulation of NP in the roots, while plants not exposed to the inhibitor accumulated high levels of these particles. Aquaporins appear to play an important role in cell internalization of very small NP particles (Zhou et al., 2021). This hypothesis needs to be carefully tested, as the aquaporins opening is, on average, 3 nm (reach to 0.2 in narrowest point) (Agre, 2006). The relation between NP absorption and aquaporins activation, observed by Zhou et al. (2021), can be explained by a greater mass movement force, pulling the particles towards the root. Although the lack of evidence on the accumulation of NP in flowers, grains and fruits, the high potential of particles translocation to the aerial part of the plants through the xylem suggests that particles can translocate to aerial organs, resulting in NP accumulation in the edible parts of vegetables that can be directly ingested by humans.

Recent evidence indicate that NP can also access plant tissues via stomata pores (Tripathi et al., 2017; Sun et al., 2021; Lian et al., 2021a). Sun et al. (2021) demonstrated leaf-to-root translocation of NP in maize by entering the plant through stomata and descending to the roots through vascular tissues. The authors tested positively and negatively charged particles. Both NP could accumulate on maize leaves, but due electrostatic attraction to the negatively charged cell walls, positively charged NP association with leaf surface was more prominent. This leaf-to-root pathway was also showed in lettuce exposed to PS-NP, which were detected in leaf and root cells through electron transmission microscopy (Lian et al., 2021a).

The uptake of NP in plants has attracted increasing attention due to its potential toxicity to organisms at higher trophic levels, such as humans. However, the mechanism remains ambiguous due to the lack of quantitative methods for NP uptake in plants. Recently, a new method incorporating alkaline digestion, cellulose precipitation and ultrasonic leaching, followed by gas chromatography analysis of pyrolysis – mass spectrometry was developed to quantify NP uptake in cucumber (*Cucumis sativus*) plants (C. Li et al., 2021). Other methods, such a hydroponic experiment was conducted to verify whether nano-sized (80 nm) and micro-sized (1 μm) fluorescence-labeled PS microspheres can enter rice roots and translocate to the part aerial (Liu et al., 2022). These developments hold promise to make the mechanism of NP transport inside plants better understood in the near future.

7. Nutrition and pollutants bioavailability and uptake

Nitrogen (N) is the macronutrient needed in larger amounts to sustain crop growth. There is still scarce data regarding MP effects on N nutrition, and the evidence available seems to be contradictory for biodegradable and conventional plastic. Maize plants exposed to ~3 μm conventional PE particles in hydroponic system showed decreased N concentration in roots and leaves, probably due to accumulation of plastic on root surface and decreased root area available for absorption (Urbina et al., 2020). Likewise, N concentration in spring onion leaves was negatively affected by microfibers of PES (<5 mm) and corroborating these results, genes related to N metabolism was down-regulated in rice plants exposed to PS-MP (Zhang et al., 2021). However, exposure to PA particles (~15 μm) increase leaf N ~2-fold concentration in this species, probably due to the presence of amines and carboxylic acids in polymer composition, which is made available to the environment during fragmentation and quickly metabolized by biota (de Souza Machado et al., 2019).

NP exposure in hydroponic system increased N concentration in wheat and lettuce leaves (Lian et al., 2020a; Lian et al., 2021a). The N concentration was higher possibly due to increased uptake and improved conversion from inorganic to organic N in plants. Nitrate accumulation were also observed in rice root, probably due to *OsMADS25* up-regulation upon NP exposure, inducing nitrate transporters synthesis (Zhou et al., 2021). The MADS-box genes are related to nitrate transport and accumulation in plants (Kong et al., 2021), and changes in regulation of these genes can explain the enhanced N concentration observed in plants exposed to NP (Lian et al., 2020a; Zhou et al., 2021).

The C:N ratio was also affected by plastic type in spring onion, being decreased by PA and increased by PES exposure (de Souza Machado et al., 2019). In lettuce, this ratio was decreased after PS-NP exposure (Lian et al., 2021a). In general, increased C concentration in plant tissues has been observed after MP/NP exposure (Lian et al., 2020a; Urbina et al., 2020; Zhou et al., 2021), but it may be due to adsorption, absorption and translocation of plastic particles in plants, and not “metabolic” carbon itself. Studies using carbon isotopes are need to shed light into this issue.

Dynamics of micronutrients in the presence of MP/NP are still unclear and has varying effects. Cucumber (*Cucumis sativus* L.) exposed to 100 nm–700 nm PS showed increased Fe and decreased Zn concentration in leaves (Z. Li et al., 2020c). In the same way, wheat exposed to 100 nm PS showed decreased Cu, Fe and Zn and increased Mn and Mg concentration in roots, but no correlation to particles concentration was found. In leaves, however, concentration of these three elements decreased significantly ($p < 0.01$) with plastic concentration (Lian et al., 2020a). Zhou et al. (2021) observed that exposure of rice roots to PS-NP can affect Fe uptake due to upregulation of the Fe uptake-related genes *OsIRO2* (Iron-Related Transcription Factor 2) and *OsNAAT1*. The former is a transcription factor involved in regulation of Fe uptake transporters (Ogo et al., 2011) and the later promotes the synthesis of mugineic acid family phytosiderophores, which are important Fe chelators in the uptake pathway (Inoue et al., 2008).

Variation in minerals' concentration can be harmful for production and nutrition of crops and should be further studied to understand possible long-term effects of exposure to plastic particles. Studies on the interactions between plastic polymers and minerals are needed. Little is known about relations of polymers and essential minerals and trace elements to plants, which can also become unavailable and promote deficiencies.

The addition of toxic elements normally found in agricultural soils to MP/NP treatments shows different effects depending on the species and element. Co-exposure of arsenic and MP resulted in a reduction in leaf biomass and in total chlorophyll content of rice seedlings, and an inhibition of arsenic uptake by carrot under MP exposure (Dong et al., 2021, 2020; Lian et al., 2020b; Wang et al., 2020b). In rice, MP interacted with root exudates and reduced the formation of iron plaques, which in turn inhibited arsenic uptake (Dong et al., 2022). The presence of PE-MP increased bioavailability of cadmium in soil, as well as its concentration and toxicity in lettuce (Wang et al., 2021). On the other hand, co-exposure of PE-MP, cadmium and copper resulted in decreased accumulation and a mitigating effect on the toxicity of these heavy metals (Zong et al., 2021).

Plastic particles also affect the accumulation of organic compounds by plants. Recently, it has been shown that the co-exposure of PS (0.1–100 μm) and phenanthrene decreased the accumulation of this organic pollutant in roots and shoots of soybean. Besides the lower concentration of phenanthrene in tissues, the co-exposure promoted higher oxidative stress and genotoxicity than phenanthrene treatment alone (Xu et al., 2021). The attenuation of pollutants effects in plants is, at least in part, probably due its adsorption on plastics surface, decreasing their bioavailability, in a similar mechanism that affects uptake in general. However, by continuous fragmentation, 'particles + pollutant' complex can be absorbed, increasing the potential toxicity.

In opposite to the negative effects of MP and NP reported above, no phytotoxic effects were detected in maize genotypes cultivated in the presence of polymer-coated fertilizers (Lian et al., 2021b), which are composed by a soluble nutrient core surrounded by a polymeric coating, designed to release nutrients in a progressive way.

8. Photosynthesis

MP can lead to *stomatal limitation* of photosynthesis by indirectly decreasing water availability and leading to stomatal closure. This happens due to cell wall and aquaporins blockage in roots, inhibiting water absorption. In addition, MP changes in soils can alter water cycle, influencing root water uptake (Boots et al., 2019; de Souza Machado et al., 2019; Dong et al., 2020; Lian et al., 2020a; Urbina et al., 2020). Reductions in photosynthetic gas exchange variables were observed in maize, lettuce and rice leaves upon MP treatment (Dong et al., 2020; Gao et al., 2019; Urbina et al., 2020; Fu et al., 2022). On the other hand, NP (100 nm) promotes hormesis effect in wheat leaves, increasing gas exchange variables at low concentrations (0.01 and 0.1 mg L^{-1}) and decreasing under higher concentrations (1 and 10 mg L^{-1}) (Lian et al., 2020a).

Plastic particles also drive *nonstomatal limitations* of photosynthesis (Z. Li et al., 2020b; Pignattelli et al., 2020; Wang et al., 2020a; Wu et al., 2020). Lettuce's RuBisCO (the enzyme responsible for carbon fixation) had its activity reduced under NP exposure in a dose dependent manner (Gao et al., 2019). RuBisCO synthesis in lettuce seems to be inhibited by oxidative stress promoted by NP particles (Gao et al., 2019; Parry et al., 2008). Due to the importance of this protein in photosynthesis, reduction in RuBisCO abundance and activity can decrease crop yield (Parry et al., 2008).

Decrease in chlorophyll content was observed under MP and NP exposure (mainly under NP exposure or smaller MP particles) in lettuce, maize and chinese cabbage (Gao et al., 2019; Wang et al., 2020a; Pehlivan and Gedik, 2021; Yang et al., 2021). However, increased concentration has been detected as well in leaves of cress, lettuce, wheat and ryegrass in other studies (Boots et al., 2019; Z. Li et al., 2020a; Lian et al., 2020a; Pignattelli et al., 2020; Qi et al., 2018; Wang et al., 2020a), illustrating that chlorophyll responses are not consistent. Effects in chlorophyll synthesis can be related to metabolic disorders and/or oxidative damage generated directly or indirectly by MP/NP.

Plastic particles seem to damage the photosystem II reaction center, apparently in a dose dependent manner. This can be seen by a decrease in electron transport rates (Pehlivan and Gedik, 2021) and in the maximum quantum efficiency (commonly referred as F_v/F_m), affecting the efficiency of light energy conversion (Dong et al., 2020; Gao et al., 2019; Z. Li et al., 2020c), which leads to electron accumulation in thylakoids and increased oxidative stress, which is reviewed in the next section.

9. Oxidative stress

Elevated levels of malondialdehyde (MDA - a byproduct of lipid peroxidation), reactive oxygen species (ROS, e.g., $O_2^{\bullet -}$, ^{-}OH, H_2O_2) and antioxidant enzymes (catalase - CAT, peroxidase - POD and superoxide dismutase - SOD) have been found in roots and/or leaves of cress, cucumber, lettuce, maize, onion, rice and wheat exposed to plastic particles (Gao et al., 2021; Jiang et al., 2019; Z. Li et al., 2020c; Lian et al., 2020b; Maity et al., 2020; Wu et al., 2020; Yang et al., 2020; Gong et al., 2021; Zhou et al., 2021). Increases in these compounds and enzymes indicate excessive ROS generation and insufficiency of ROS scavenging system, which can result in oxidation of plant tissues, damaging plants due to membrane lipids damage, organelles disintegration and dysfunction, proteins and nucleic acids oxidation, and metabolic pathways destabilization (Demidchik, 2015). In fact, oxidative stress has been considered the main mechanism of toxicity of PS particles - MP and NP - to crops (Gong et al., 2021).

Although the mechanisms of MP/NP-induced oxidative stress are still unclear, increased reactive oxygen species generation seems to occur by injuries on plant surface due to MP abrasion (Kalčíková et al., 2017); leaching of chemical compounds from absorbed NP (Jiang et al., 2019; Z. Li et al., 2020c; Pignattelli et al., 2020; Verla et al., 2019); water deficit resulted from changes in soil structure (de Souza Machado et al., 2019; Wan et al., 2019); and photosynthesis disruption (Dong et al., 2020).

Plastic particles physical and chemical characteristics affect oxidative stress level. Due to higher potential of interaction with internal tissues and cellular compounds, NP are more prone to induce ROS generation in plants, causing a greater ROS production, an increase in activity of antioxidant enzymes, an increase in electrolyte leakage rate, and an decrease in total antioxidant capacity in cress and lettuce (Jiang et al., 2019; Z. Li et al., 2020a; Lian et al., 2021a). Regarding MP, smaller particles (75–150 μm *versus* 150–212 μm) showed to induce higher concentrations of H_2O_2 and increased membrane instability in maize seedlings exposed to different particle types. They also presented higher transcript levels of the Heat Shock Protein 1 (*HSP1*) after exposure to PET, PVC and a mix of different plastics. In general, the most stressful conditions were promoted by treatment with mixed MP (Pehlivan and Gedik, 2021). In rice, ROS-staining analyzes revealed a significant accumulation of $O_2^{\bullet -}$ and H_2O_2 in roots of seedlings treated with PS-MP. Transcriptome analysis revealed that genes related to flavonoid and flavonol biosynthesis were up-regulated in these plants (Zhang et al., 2022), indicating the activation of antioxidant mechanisms, since these secondary metabolites have a strong capacity to donate electrons and hydrogen atoms (Hernández et al., 2009).

In addition to size, particle shape influences the degree of damage caused: sharper particles (fragments) can cause more damage to the root/cell surface than fibers or spheres, inducing oxidative stress (Rozman et al., 2021). The chemical compounds inherent in the polymer composition also seem to be related to the level of oxidative damage, as observed in cress, by higher levels of ROS generated in PVC and PE exposure, than in PP exposure (Pignattelli et al., 2020).

10. Metabolic and molecular changes

MP/NP affects the expression of several genes related to amino acids, organic acids, fatty acids, and polysaccharides biosynthetic pathway (Lian et al., 2020a; Wu et al., 2020).

Organic acids are key molecules in carbon metabolism, being the central actors in photosynthesis and cell respiration (Drincovich et al., 2016). MP exposure decreased organic acids content in rice leaves in a dose dependent manner. Malic, succinic and fumaric acids, important intermediates of the Krebs cycle in mitochondria, showed decreased concentration in rice plants exposed to MP, indicating a disruption in plant energy production, which in turn affects crop growth and development.

Exposure to MP also resulted in decreased leaf concentration of proline, phenylalanine, glycine, serine, threonine, alanine, aspartate and glutamate, important amino acids for protein production, biotic and abiotic defense, electrical signaling and auxin production (Hayat et al., 2012). These effects are probably due to oxidative stress, leading to macromolecules oxidation and interreference in metabolites pathways (Wu et al., 2020). Additionally, foliar-applied PS-NP drastically reduced the levels of essential amino acids in lettuce leaves, such as lysine, tryptophan, threonine, isoleucine, leucine, and valine. The levels of semi-essential and non-essential amino acids proline, tyrosine, serine, aspartate, arginine, asparagine, and ornithine also declined (Lian et al., 2021a).

Plastic particles also affect plant defense against biotic and abiotic stressors, being possible to infer the increased potential for biotic stressors due to depletion of defense systems (Kim and Hwang, 2014; Neilson et al., 2013). Jasmonic acid (JA) is an important signaling molecule for plant biotic and abiotic stress defense, and it also act as a growth regulator (Ruan et al., 2019). JA concentration decrease in rice under NP exposure, regardless particles concentration (Zhou et al., 2021), resulting in growth reduction and increased oxidative damage in rice roots. Exogenous supply of JA alleviated the adverse effects (Zhou et al., 2021). The negative effects of NP on JA metabolism suggests that crops exposed to NP pollution may be more susceptible to biotic stressors.

It is interesting to highlight the decrease in the lignin hydroxybenzoic acid concentration, a phenolic compound present in secondary cell walls, suggesting an adverse effect in cell wall composition and functioning (Wu et al., 2020). NP exposure also affected lignin biosynthesis in rice roots by downregulation of many enzymes and upregulation of laccases-coding genes related to lignin degradation, contributing to lower levels of this natural polymer and weakening cell walls (Rico et al., 2014; Zhou et al., 2021). Altered content and composition of lignin in cell walls components due MP/NP exposure can seriously compromise crop yield by facilitating infection by pathogens due to weakening of the cell wall barrier; reducing tolerance to drought; increasing heavy metals in cytosol due to decreased metal binding capacity of cell wall; decreasing thermal tolerance due to negative effects in transpiration rate; reducing stiffness, mechanical stability and lodging resistance due to lower accumulation of this polymer in cell wall (Q. Liu et al., 2018; Moura et al., 2010).

The metabolomic and transcriptomic profile of rice under PS-MP exposure was analyzed in a field study, and distinct responses were obtained in two rice subspecies, showing that metabolites and gene regulation/interaction act in a cultivar-dependent manner (Wu et al., 2021).

11. Cyto- and genotoxicity

Effects in root growth have been used as biomarker for cyto- and genotoxic effects (Bonciu et al., 2018). Giorgetti et al. (2020), Gopinath et al. (2019) and Maity et al. (2020) demonstrated an inhibition in growth of onion roots exposed to NP, concomitantly with cytogenotoxic effects. These effects result from decreased Mitotic Index (MI) and high potential for causing chromosomic abnormalities, especially in regions with high cell division rates (e.g. root tips) (Bonciu et al., 2018).

Cytogenetic effects are related to inhibition of cell cycle and repair regulation genes, which result in cell malformation and impacts in cell division, triggered by cellular oxidative damage (Giorgetti et al., 2020; Gopinath et al., 2019; Klaunig et al., 2010). Genotoxicity effects include chromosomes and nuclear abnormalities (CA and NA, respectively). Cytotoxic and genotoxic effects were observed in crops exposed to plastic particles, especially under high particles concentration (Giorgetti et al., 2020; Gopinath et al., 2019; Jiang et al., 2019; Maity et al., 2020). These effects are related to intoxication by chemicals absorbed from the particle surface (Gopinath et al., 2019; Lu et al., 2020), such as organic and inorganic pollutants (Yadav et al., 2019). NP particles are potentially more cytotoxic than MP due to higher induction of oxidative damage and easier absorption (Giorgetti et al., 2020; Jiang et al., 2019).

Effects of MP/NP cytotoxicity are mainly triggered in a ROS dependent pathway. However, mitotic index (MI) reduction was observed in onion root cells without oxidative stress (at low concentration of 100 nm PS, 0.01 g L^{-1}), raising the hypothesis of cytotoxicity independent of ROS overproduction. Decreased MI seems to be time and dose dependent (Giorgetti et al., 2020; Gopinath et al., 2019; Jiang et al., 2019; Maity et al., 2020). Mitodepressive activity of plastic particles can be attributed to inhibition of cell cycle regulators and DNA replication, especially due the decreased expression of *CDK2* (Cyclin-dependent Kinase 2) gene. It acts directly on G2/M transition, blocking cells in G2 phase and delaying all mitotic processes (Maity et al., 2020). In addition, cytotoxic effects could be a result of direct effect of NP particles internalized in cells or of the leached chemicals from its surface (Giorgetti et al., 2020; Gopinath et al., 2019). Decreases in MI can inhibit root growth, mainly in regions of high cell division rates (Bonciu et al., 2018).

Interestingly, Gopinath et al. (2019) showed that PS particles isolated from health care products induce higher CA than primary particles, likely due to chemicals adsorbed in polymer surface. Plants exposed to NP presents CA in all mitotic phases, except in prophase. The most common abnormality was clumped chromosomes, laggard chromosomes, ring chromosomes, vagrant chromosomes, multipolarity, precocious movement, stick bridge and disoriented pole. These effects can be a result of malfunctioning or inactivation of spindle or by clastogenic effects (Giorgetti et al., 2020; Maity et al., 2020). CA index decreased with time, suggesting an enhancement in mitotic control system, although this pattern was not consistent in all treatments (Gopinath et al., 2019).

NA include micronuclei formation, nuclear bud and binucleated cells. These abnormalities can be formed as a defense system, in case of micronuclei and nuclear bud, to exclude the excess of genetic material (Fenech et al., 2003), yet binucleus (or polynucleus) formation is a response to inhibition of cytokinesis in telophase, due to spindle disruption (Nefic et al., 2013). Micronuclei formation increased approximately 10-fold under high NP (100 nm) concentration (Giorgetti et al., 2020; Jiang et al., 2019; Maity et al., 2020). Nuclear bud and polynucleated cells also significantly increase upon NP exposure (Maity et al., 2020). NP are more prone to induce genotoxicity than MP, due to higher interaction with internal tissues. In addition, NA occurrence seems to be time and dose dependent, becoming more harmful with time and in higher doses of NP (Giorgetti et al., 2020; Gopinath et al., 2019; Jiang et al., 2019; Maity et al., 2020).

12. Future perspectives in plastic particles effects on plants

So far, many results are still descriptive, and further studies are needed to better understand the mechanisms of MP/NP-induced toxicity to plants. Most experiments were carried out in short-term exposures and in controlled environments, which mitigates the effect of external influences, such as interactions with the microbiota, existence of chemical compounds from different sources and environmental variation. These variables have to be carefully analyzed in order to know the real effects of plastic pollutants in natural environments, especially focused on the impact in food production.

Plastic particles negatively affect important food crops, inhibiting their growth and development by many direct and indirect mechanisms. The continuous increment in the concentration of these particles in agricultural fields around the world is likely to result in losses of food production globally. In addition, negative effects of MP/NP in nutrients acquisition, which can affect human diet quality due to decreased nutritional value, and the translocation of NP particles to plant edible parts, are worrying points to food security, once these particles can be harmful to humans (Lehner et al., 2019). Although there is relevant information regarding the pollution of plastic particles and their effects on soil and plant species, this topic is recent and has several important gaps: the effects of MP/NP in species with different ecological types (annual and perennial herbs, trees and ecological implications); the potential for translocation and accumulation of NP particles, and possible adsorbed toxic compounds in leaves, grains and fruits; and how plastic particles interact with plant physiologic and anatomic traits.

Another important topic to explore in the future is how to mitigate plastic accumulation in agricultural fields. These actions can be divided into: i) use of alternative techniques and materials for agricultural activities, such as reuse of materials, responsibility for the plastic material use and choice of less toxic polymers (Galati and Scalenghe, 2021); and ii) development of technologies and efficient methods to eliminate plastic particles from organic fertilizers (wastewater sludge), water used in irrigation and others potential sources of plastic particles to agricultural fields, such as removal techniques by bioreactor system, ultrafiltration and oxidation (Liu et al., 2021).

Future research should focus not only on the effects of these particles on the growth, development, translocation and accumulation in edible organs, but also on possible effects on food production and on the ingestion of contaminated plants by animals and humans, since the presence of MP/NP has already been confirmed in farmland and in the atmosphere (Allen et al., 2019; Xu et al., 2019). In order to understand the real effects of these particles in organisms on natural environments, different size, origin and concentration should be analyzed due to increasing use of plastic and continuous accumulation and fragmentation of these pollutant in terrestrial domain. This will help determine the distinct effects related to size, type and concentration, which are still mixed and not clearly defined.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Appendix: Article supplementary table.

Table S1. A summary of publications selected for this review

Tested plants		Plastic particles					Experiment medium	Effects	Source
Species	Family	Type	Size (µm)	Concentration	Shape	Origin			
Lolium perenne L. (Ryegrass)	Poaceae	HDPE; PLA; PA	0.4 - 360	0.01; 1 g kg^{-1}	Sphere; Fiber	Secondary	Soil	Decreases germination and plant shoot; increase root:shoot and *chla*/*chlb* ratios.	Boots et al., 2019
Lepidium sativum L. (Cress)	Brassicaceae	PS	0.05; 0.5; 4.8	10^3; 10^4; 10^5; 10^6; 10^7 particles mL^{-1}	Sphere	Primary	Hydroponic	Delay of germination and primary root and shoot growth; particles accumulate in seed testa and in root hairs.	Bosker et al., 2019
Vigna radiata L. (Mung bean)	Fabaceae	PS	0.02	0.01; 0.1 g kg^{-1}	Sphere	Primary	Soil	Decreases root length and leaf weight; particles accumulate in leaves.	Chae and An, 2020
Oryza sativa L. (Rice)	Poaceae	PS; PTFE	10	0.04; 0.1; 0.2 g L^{-1}	Fragment	Primary (milled)	Hydroponic	Decreases roots and shoot biomass; Reduce photosynthetic net, chlorophyll fluorescence and *chla* concentration; promote ROS overproduction.	Dong et al., 2020
Lactuca sativa L. (Lettuce)	Asteraceae	PE	0.2	0.25; 0.50; 1 mg mL^{-1}	Sphere	Primary	Hydroponic	Decrease root and shoot weight and length; promote ROS overproduction; reduce RuBisCo activity; decrease of *chla* and *chlb* concentration and chlorophyll fluorescence; effects are more severe in MP + di-n-butyl phthalate treatment.	Gao et al., 2019
Lactuca sativa	Asteraceae	PS	0.1 - 1; >10	0.25; 0.50; 1 mg mL^{-1}	Sphere	Primary	Hydroponic	Decrease plant biomass and height; promote ROS overproduction; effects are more severe in MP + di-n-butyl phthalate treatment.	Gao et al., 2021
Allium cepa L. (Onion)	Amaryllidaceae	PS	0.05	0.01; 0.1; 1 g L^{-1}	Sphere	Primary	Hydroponic	Decrease root length; increase cytological anomalies and micronuclei formation, while reduce mitosis index; particles internalized in cells; damages in root tissue; promote ROS overproduction.	Giorgetti et al., 2020
Raphanus sativus L. (Radish); *Lactuca sativa* L. (Lettuce); *Triticum aestivum* L. (Wheat); *Zea mays* L. (Maize)	Brassicaceae; Asteraceae; Poaceae	PS	0.1; 5	1; 10 mg L-1	Sphere	Primary	Hydroponic	Inhibits germination, mainly in lettuce; Decrease root length and weight, mainly in lettuce and maize; MP and NP promote oxidative stress; Fluorescent signal in roots, suggesting uptake of NP particles.	Gong et al., 2021

Tested plants		Plastic particles					Experiment medium	Effects	Source
Species	**Family**	**Type**	**Size (μm)**	**Concentration**	**Shape**	**Origin**			
Allium cepa	Amaryllidaceae	PS	0.1	1; 2.5; 5; 7.5; 10; 25 μm mL^{-1}	Sphere; fragment	Primary; secondary (cosmetic product)	Hydroponic	Decrease root length; Promote chromosome aberrations and decrease mitotic index.	Gopinath et al., 2019
Vicia faba L. (Faba)	Fabaceae	PS	0.1; 5	10; 50 100 mg L^{-1}	Sphere	Primary	Hydroponic	Decrease root length and biomass; promote ROS overproduction; increase micronuclei formation and reduce mitotic index; particles accumulation in surface tissue.	Jiang et al., 2019
Triticum aestivum L. (Wheat); *Lactuca sativa*	Poaceae; Asteraceae	PS; PMMA	0.2 - 10	0.5; 5; 50 mg L^{-1}	Sphere; Fragment	Primary; Secondary (Wastewater sludge)	Hydroponic	Particles accumulate in root surface; NP particles can enter in root vascular system by root tip (cellular interspace) or by crack-entry mode (lateral roots formation).	L. Li et al., 2020
Cucumis sativus L. (Cucumber)	Curcubitaceae	PS; PMMA	0.05; 0.1; 0.5	*	Sphere	Primary	*	Validation of a methodology for extracting plastic particles from lettuce plants.	Li et al., 2021
Triticum aestivum	Poaceae	PS	0.1	0.01; 0.1; 1; 10 mg L^{-1}	Sphere	Primary	Hydroponic	Increase seed vigour, seed water uptake and root elongation; increase root and shoot biomass, but decrease shoot:root ratio); increase photosynthetic net, stomatal conductance, intracellular carbon concentration, transpiration rate and SPAD value; increase carbon and nitrogen concentration; change minerals composition.	Lian et al., 2020a
Triticum aestivum	Poaceae	PS	0.1	10 mg L^{-1}	Sphere	Primary	Hydroponic	Decrease plant biomass; decrease photosynthetic rate and chlorophyll concentration; promote ROS overproduction; change minerals composition.	Lian et al., 2020b
Zea mays L. (Maize)	Poaceae	PU (PCF)**	~ 3.8	0.01; 0.1; 1% (w/w)	Fragment	Primary	Soil	Significantly improve of plant performace; alterations in rhizosphere metabolites; changes in soil microbial community and soil chemical properties.	Lian et al., 2021
Lactuca sativa L. (Lettuce)	Poaceae	PS	0.1	0.1; 1 mg L-1**	Sphere	Primary	Hydroponic*	Decrease weight, height and foliar area of leaves; reduces in chlorophyll A and B, in addition to carotenoids; decreases of antioxidant capacity; reduce amino acids and micronutrients concentration; Possible	Lian et al., 2021a

Tested plants		Plastic particles					Experiment medium	Effects	Source
Species	**Family**	**Type**	**Size (µm)**	**Concentration**	**Shape**	**Origin**			
								absorption and transport of particles through the leaves.	
Festuca brevipila Tracey; *Holcus lanatus* L.; *Calamagrostis epigetos* (L.) Roth; *Achillea millefolium* L.; *Hieracium pilosella* F.W.Schultz & Sch.Bip.; *Plantago lanceolata* L.; *Potentilla argentea* L.	Poaceae; Poaceae; Poaceae; Asteraceae; Asteraceae; Plantaginaceae; Rosaceae	PES	~1000	4 g kg^{-1}	Fiber	Secondary	Soil	In general, increase shoot and root biomass and increase root:shoot ratio; decrease root length and root surface area, while increase root diameter; favored the development of certain species, changing plant community structure.	Lozano and Rillig, 2020
Daucus carota L. (Carrot)	Apiaceae	PA; PES; LDPE; PET; PP; OS; PU; PC	~5000	1; 2; 3; 4 g kg^{-1}	Fragment; Fiber	Secondary	Soil	Increase root and shoot biomass; promote changes in soil structure.	Lozano et al., 2021b
Allium fistulosum L. (Spring onion)	Amaryllidaceae	PA; PES; HDPE; PET; PP; PS	17 - 5000	0.2; 2 % (w/w)	Fragment; Fiber	Secondary	Soil	Increase root biomass, bulb biomass, root length, root surface area and root:shoot ratio (in general); PES increase arbuscular mycorrhizal hyphae; Changes in nitrogen, carbon and C:N ratio.	de Souza Machado et al., 2019
Allium cepa	Amaryllidaceae	PS	0.1	25, 50 100, 200, 400 mg L^{-1}	Sphere	Primary	Hydroponic	Decrease root length; increase chromosome and nuclear abnormities, while decrease mitotic index; promote ROS overproduction; inhibit expression of *cdc2* gene.	Maity et al., 2020
Phaseolus vulgaris L. (Bean)	Fabaceae	LDPE, Bio	50 - 1000	0.5; 1; 1.5; 2; 2.5 % (w/w)	Fragment	Primary	Soil	Decrease root, leaves and fruits biomass; increase root length, also decrease shoot:root ratio and leaf area; decrease chlorophyll concentration	Meng et al., 2021
Zea mays L. (Maize)	Poaceae	PVC; PVC; PE; PS; PET	75-150; 150-225	0.02(%) w/w	Fragment	Secondary	Soil	Changes in antioxidant system genes expression; decreased photosynthetic pigments concentration; biochemical imbalances involved in cell membrane instability.	Pehlivan and Gedik, 2021

Tested plants		Plastic particles					Experiment medium	Effects	Source
Species	**Family**	**Type**	**Size (µm)**	**Concentration**	**Shape**	**Origin**			
Lepidium sativum	Brassicaceae	PC	~3000	0.1; 1; 10 % (w/w)	Sphere	Primary	Soil	Decrease germination speed and root and shoot length.	Pflugmacher et al., 2020
Lepidium sativum	Brassicaceae	PE, PVC, PP	125	0.2 g kg^{-1}	Fragment	Secondary	Soil	Delay germination rate; decrease shoot biomass and length; promote ROS overproduction; increase pigments and proline concentration.	Pignattelli et al., 2020
Triticum aestivum	Poaceae	LDPE, Bio	50 - 1000	1 % (w/w)	Fragment	Secondary	Soil	Decrease root and shoot biomass and tillers, leaves and fruits number; negative effects in associated microbiota.	Qi et al., 2018
Triticum aestivum	Poaceae	LDPE, Bio	50 - 1000	1 % (w/w)	Fragment	Secondary	Soil	Decrease plant biomass; changes rhizosphere microbiota community.	Qi et al., 2020
Brassica rapa L. (Flowering cabbage)	Brassicaceae	PS; PLA	0.07; 5; 5000	0.1 g kg^{-1}	Sphere; fragment	Primary; Secondary (mulch film)	Soil	Increase plant weight; changes in photosynthetic parameters; changes rhizosphere microbiota community.	Ren et al., 2021
Arabidopsis thaliana (L.) Heynh	Brassicaceae	PS (with different superficial charge)	0.5; 0.7	10; 50; 100 µm mL^{-1}; 0.3; 1 g kg^{-1}	Sphere	Primary	Soil; Hydroponic	Decrease root (organ and cells) and shoot length and biomass; decrease chlorophyll concentration; particles accumulate in root surface (accumulation facilitated by exudates released in the root).	Sun et al., 2020
Triticum aestivum L. (Wheat)	Poaceae	PS	0.02	0, 10, 50, 100, 200, 400 and 500 ng/spot **	Sphere	Primary	*	Accumulation in leaves; Absorption of NP particles through stomatal opening; transport of particles to plant body through vascular bundle; inhibitory effect on photosynthesis and activation of antioxidant system.	Sun et al., 2021
Arabidopsis thaliana; Triticum aestivum	Brassicaceae; Poaceae	PS	0.04; 1	29 mg L^{-1}	Sphere	Primary	Hydroponic	Particles accumulate in root surface; no negative effects were observed in plant development.	Taylor et al., 2020
Zea mays	Poaceae	PE	3	0.0125; 100 mg L^{-1}	Sphere	Primary	Hydroponic	Decrease in root and shoot biomass and length; increase of nitrogen and carbon concentration in roots.	Urbina et al., 2020
Citrus aurantium L. (Lime)	Rutaceaea	LDPE; PP; PS	< 5000	10 g kg^{-1}	Fragment	Secondary	Soil	Variable effects in plant growth (plant height, leaf number and area and branch number).	Verla et al., 2020
Zea mays L. (Maize)	Poaceae	PLA; PE	100 - 154	0.1; 1; 10 % (w/w)	Sphere	Primary	Soil	Decrease root and shoot biomass (in 10% PLA root weight increase); treatments with MP and cadmium reduce chlorophyll concentration.	Wang et al., 2020a

Tested plants		Plastic particles					Experiment medium	Effects	Source
Species	Family	Type	Size (µm)	Concentration	Shape	Origin			
Zea mays	Poaceae	PS; HDPE	100 - 154	0.1; 1; 10 % (w/w)	Sphere	Primary	Soil	Decrease in root biomass (increase in 10% HDPE); Effects are more severe in treatments MP + cadmium.	Wang et al., 2020b
Lactuca sativa L. (Lettuce)	Asteraceae	PE	8.5-500	0.1; 1; 10 g kg-1	Fragment	Primary	Soil	Changes in soil physico-chemical properties; decrease plant biomass; increase cadmium bioavailability and accumulation in plants;	Wang et al., 2021
Oryza sativa	Poaceae	PS	<50	50; 250; 500 mg L^{-1}; 0.05; 0.25 mg kg^{-1}	Sphere	Primary	Soil; Hydroponic	Decrease in root biomass, shoot length and biomass and in grain number; changes in metabolic profile; promote ROS overproduction.	Wu et al., 2020
Glycine max L. Merrill (Soybean)	Fabaceae	PS	0.1; 1; 10; 100	10 mg kg-1	Sphere	Primary	Soil	Decrease root activity, mainly when exposed to MP particles; MP/NP decrease phenanthrene root uptake and leaves concentration; higher plant toxicity observed in plants exposed to MP.	Xu et al., 2021
Brassica rapa L. (Chinese cabbage)	Brassicaceae	PS; HDPE	25; 25-48; 48-150; 150-850	2.5; 5; 10; 20 g kg-1	Fragment	Primary	Soil	Changes in soil chemical properties; reduction of fresh weight; changes in leaf soluble sugar and starch concentration in leaves; decrease of chlorophyll concentration in leaves;	Yang et al., 2021
Lactuca sativa	Asteraceae	PVC	18; 150	0.5; 1; 2 % (w/w)	Fragment	Primary	Soil	Increase root parameters; promote ROS overproduction; no significantly effects in photosynthetic system.	Z. Li et al., 2020a
Cucumis sativus L. (Cucumber)	Curcubitaceae	PS	0.1; 0.3; 0.5; 0.7	50 mg L^{-1}	Sphere	Primary	Hydroponic	Decrease plant biomass; increase root activity; promote ROS overproduction; reduce in Mg, Ca and Fe concentration; decrease protein and soluble sugar concentration.	Z. Li et al., 2020b
Cucumis sativus	Curcubitaceae	PS	0.1; 0.3; 0.5; 0.7	50 mg L^{-1}	Sphere	Primary	Hydroponic	Decrease root and shoot biomass and length; promote ROS overproduction; decrease chlorophyll concentration; increase chlorophyll fluorescence parameters; increase protein and Vitamin C concentration, while decrease soluble sugar; change minerals concentrations in leaves.	Z. Li et al., 2020c
Oryza sativa L. (Rice)	Poaceae	PS	0.2	0.1; 10; 1000 mg·L−1	Sphere	Primary	Hydroponic	Promotion of root elongation; reduction of antioxidant enzymes activity and ROS accumulation in roots; upregulation of genes involved in flavonoids and flavonol synthesis and down regulation of those involved in nitrogen metabolism.	Zhang et al., 2021

Tested plants		Plastic particles					Experiment medium	Effects	Source
Species	**Family**	**Type**	**Size (µm)**	**Concentration**	**Shape**	**Origin**			
Oryza sativa	Poaceae	PS	0.02	10; 50; 100 mg L^{-1}	Sphere	Primary	Hydroponic	Decrease root length and biomass and shoot height; increase lateral root formation (upregulation of genes involved in this process); promote ROS overproduction; inhibit of jasmonic acid and lignin synthesis; increase of carbon metabolism; changes in genes expression; absorption of NP particles.	Zhou et al., 2021

* Plastic particles applied directly in leaves; ** Polymer coated fertilizer

CHAPTER 2

Experiments with microplastics in plants:
how should they be performed?

ABSTRACT

Microplastics are widely distributed in world, and the contamination by these pollutants can cause serious negative effects to existing biota. Experiments with terrestrial plants exposed to microplastics are recent, first published in 2018. Thereby, there is a lack of standardization, both in experimentation methods and in data presentation, in addition to the lack of essential information for experiment replication. The choice of plant species, as well as the size and the type of plastic particles and culture medium must be carried out considering the questions to be addressed. In this chapter we present methodological information that we deem important for setting up and conducting experiments in this topic, seeking to clarify points that may be unclear.

Keywords: experimentation; phytotoxicity; plants; plastic pollution.

Introduction

Since the 50's plastic production has been increasing at an amazing pace, reaching ~8 billion tons in 2015 (Geyer et al., 2017). The versatility and high cost-effectiveness of synthetic polymers have made this material usable in practically all sectors of the industry (Andrady and Neal, 2009; Thompson et al., 2009). However, the wide use of these materials generate a large amount of waste improperly discarded in natural environments (Ritchie and Roser, 2019). Microplastics (MP, size < 5 mm) and nanoplastics (NP, size < 100 nm) (EFSA, 2016) have become one of the main challenges in environmental conservation and, due to their worldwide distribution and difficult detection and removal, have been identified as the largest and most long-lasting human intervention on Earth (Barnes et al., 2009). MP/NP can be classified as primary, when they are synthesized in millimetric sizes for different industrial uses, e.g. pellets and beauty products, and as secondary, when they come from the degradation of larger pieces of plastic (Klingelhöfer et al., 2020). The degradation of plastic in the environment occurs due to the action of abiotic agents, such as UV radiation and weathering. Polymer degradation also occurs via biotic pathways, with the action of bacteria that degrade matrices with strong carbon bonds (Moharir and Kumar, 2019).

Small plastic particles accumulation and their effects in terrestrial areas started to be addressed in 2012 only (Rillig, 2012); nowadays it is known that agricultural lands accumulate a large part of all the plastic waste in terrestrial domains, which poses a risk to the productivity and food health of animals and humans (Pflugmacher et al., 2020; Toussaint et al., 2019; Wong et al., 2020; Wu et al., 2020). MP/NP negatively affect soil structure and plant physiology, resulting in reduced germination and growth rates (Boots et al., 2019; Bosker et al., 2019; de Souza Machado et al., 2019; Z. Li et al., 2020b; Lian et al., 2020a; Wu et al., 2020). In addition, the uptake and translocation of plastic particles to aerial parts of plants have been confirmed (L. Li et al., 2020a; Liu et al., 2022; Sun et al., 2021), representing a direct source of this pollutant to humans. Micro and macrobiota are also negatively affected when exposed to MP/NP, presenting changes in the community, decreased organism size, and reproduction potential (B. Gao et al., 2021; W. Wang et al., 2020). In humans, MP/NP contamination promotes inflammation of the exposed tissues such as the guts and lungs, promoting oxidative stress and other important metabolic changes (Yee et al., 2021). Different plastic particles were found in human blood as well (Leslie et al., 2022).

The methods chosen for experimentation are crucial to obtain satisfactory results. Experimental methods with plants exposed to plastic particles are still under development; therefore, the procedures lack standardization, which can lead to bias and low reproducibility. Crucial information such as soil type used, hydroponic system set up, the reason for choosing the growth medium, and the selected species are often absent in several papers (Hartmann et al., 2022). For example, both hydroponic and soil mediums have been already used for germination studies (Boots et al., 2019; Bosker et al., 2019; Jiang et al., 2019; Lian et al., 2020a), and several problems while replicating these experiments were found by our group, as reported below.

Which plastic particles to use?

Shape, size, and type of MP/NP must be chosen accordingly to what scientists want to address in the experiments. Primary MP purchased for scientific purposes generally has uniform shape and size, whereas secondary MP presents varied forms and sizes, that are the majority found in natural environments (Zhu et al., 2019). A scientometric study performed by our group pointed out the use of 13 different types of plastic particles in studies with terrestrial plants exposed to MP/NP. Most particles used were polystyrene (PS) of the primary origin (spheric particles) and in nanometric sizes, although this plastic type was largely used in micrometric sizes. Interestingly, PS plastic was used in 41% of the analyzed experiments, even though this type of polymer is not the most commonly found in the environment (Hartmann et al., 2022). Companies that sell primary MP/NP for scientific purposes have a portfolio mostly with a large number of PS-based products, explaining a large number of experiments with this polymer type. On the other hand, in experiments in which secondary polymers have been used, a larger variation in the type of polymer used was observed, with emphasis on PE and its variations (LDPE and HDPE). This polymer is commonly used in disposable materials such as cups and bags (Chae and An, 2018).

The use of fluorescent particles is another interesting tool in experiments with plants. By using this material, it becomes possible to observe in a confocal microscopy how the MP/NP can be absorbed and accumulate in plant body, mainly into its roots. Some studies have been using this method to analyze the potential of these particles to accumulate around the root, to infer routes and limits of the size of particles that can be absorbed into the roots, the places where they accumulate within the plant, and also their translocation mechanisms through the plant body (Jiang et al., 2019; L. Li et al., 2020b, 2020a; Liu et al., 2022; Taylor et al., 2020). Manufactured MP/NP have a homogeneous format, allowing to analyze with greater precision the effects of size and shape that particles will exert. On the other hand, secondary particles can be used to infer general effects promoted by MP, not focused on size and shape, but on concentration. The use of these particles allows us to observe phytotoxicity effects more similar to those in natural environments, since in these places mainly particles of secondary origin are found (Qi et al., 2018).

Manual griding is a cheap way to obtain small plastic particles. This can be achieved by using liquid nitrogen to break plastic particles (de Souza Machado et al., 2019; Qi et al., 2018). However, this method was replicated in our experiment to obtain MP from larger LDPE fragments, but the result was not satisfactory. Manual fragmentation of plastics creates particles more similar to those found in natural environments, with heterogeneous size and shape. In addition to this variability, particles originating from these methods can be pointed, sharp, and grooved, allowing to observe effects related to surface physical damage in the plants (Kalčíková et al., 2017). Experiments that used this method to obtain MP from larger plastic pieces showed that it negatively affect plant development (de Souza Machado et al., 2019; Qi et al., 2018). The presence of spikes can harm bellowground plants organs (e.g. roots), injuring the cell wall and enhancing ROS production (Kalčíková et al., 2017). In the same way, grooves in particles' surface increase contact and can favor the growth of bacterial colonies and increase the release and/or adsorption of chemical compounds harmful to the biota (Zhang et al., 2020).

The use of homogeneous particles (e.g., primary ones) is indicated in experiments that analyze the effects of MP/NP size and shape with accuracy, assisting to acquire theoretical knowledge about contamination by these pollutants. Small primary particles facilitate the understanding of the threshold of how these particles can enter and translocate in plant organs, in addition to how MP/NP can affect cells and other components of the plant body. In addition, the use of these primary MP, allow the analysis of isolated effects of particle properties. As we increase the particles' size, the effects become external, enabling to analyze how MP can affect the medium and how this will affect plant development (Bosker et al., 2019; L. Li et al., 2020a; Z. Li et al., 2020a; Lian et al., 2020a; Liu et al., 2022; Zhou et al., 2021). On the other hand, secondary particles allow a more realistic analysis of the general effects of MP/NP in natural environments, without requiring great accuracy in terms of size and shape, since there is great heterogeneity between particles. This type of experiment is more realistic on how these pollutants will affect plant crops (Boots et al., 2019; de Souza Machado et al., 2019; Lozano et al., 2021; Wu et al., 2020). The composition of the particles is also an important issue, as the chemical components from particles can leach and interfere in plant development (Jemec Kokalj et al., 2019).

Which species to select?

When studying the effects of pollutants in plants, the choice of species used in the experiments is key. So far, most experiments with MP/NP used major crop species, especially those in which the aerial parts are consumed (rice, wheat, and lettuce), but also carried out with species where belowground organs are of commercial interest (onion and carrot) (Hartmann et al., 2022). The analysis of crop species is of great importance for the evaluation of potential negative effects of plastic particles in agricultural lands and on food health and safety. Oliveri Conti et al. (2020) extracted plastic particles from apple, pear, carrot, lettuce, broccoli, and potato purchased in supermarkets, inferring that the contamination occurs in all stages of the production chain, from planting, harvesting, selection, storage, and distribution of these vegetables. Although these authors show the presence of microplastic in fruits and vegetables, the mainly stage of contamination of plants by these particles it is not clear yet, being necessary more studies focused on this topic. In this experiment, mainly small particles (NP) were found, as they are more likely to translocate between cells and through the plant's vessels. Oliveri Conti et al. (2020) reported that carrots had the major plastic concentration and minor mean MP size in their sample, while lettuce presented a lower concentration of particles but the largest particles. In the same way, Dong et al. (2021) observed the absorption and accumulation of PS particles with 1 µm in bellowground organs, and translocation of particles with 0.2 µm to leaves. This pattern can be explained due to the mechanism of plastic accumulation, that in carrots occurs by root/tuber uptake, and in lettuce mainly by surface deposition, although the root-to-shoot translocation can also occur, the translocation of these particles to leaves in this plant was observed with NP (Gao et al., 2019; Lian et al., 2021a). The tuber can be a good model to study absorption and accumulation of MP/NP due to characteristics such as high accumulation in below-ground organs, high surface in contact with particles, and the higher potential to be ingested (Dong et al., 2021; Oliveri Conti et al., 2020).

Most researches on this topic uses crop species, such as rice, wheat and maize (Hartmann et al., 2022). The effects observed in crops exposed to MP/NP suggest changes in ionomic and metabolic patterns, reduced photosynthetic rates, deficits in plant growth and development, and uptake, and translocation of plastic particles (Gao et al., 2019; Z. Li et al., 2020c, 2020b; Lian et al., 2020a; Wu et al., 2020; Zhou et al., 2021). These results are important to understand how MP/NP can contribute to decreases in plant mass and health.

Besides most of the experiments have used crops, experiments with native and invasive species are extremely important as well to infer ecological effects of MP/NP on plant communities, since they can transform an entire landscape. Lozano and Rillig (2020) performed an experiment using the species *Festuca brevipila* Tracey (Hard fescue), *Holcus lanatus* L. (Yorkshire fog), *Calamagrostis epigetos* (L.) Roth (Bushgrass); *Achillea millefolium* L. (Yarrow), *Hieracium pilosella* F.W. Schultz & Sch.Bip. (Mouse-ear hawkweed), *Plantago lanceolata* L. (Ribwort plantain), and *Potentilla argentea* L. (Hoary cinquefoil). After exposure to plastic particles, the plant community evidenced an enhancement of growth of drought-tolerant plants and also of the allelopathic ones.

The use of model species such as *Arabidopsis thaliana* (L.) Heynh., *Allium cepa* L., and *Vicia faba* L. facilitates the analyses genomic (and the derives omics) and of geno-cytotoxicity effects due to intrinsic features of these species, but also due to a variety of methods developed and the theoretical background acquired (Maity et al., 2020; Taylor et al., 2020). Experiments performed with these species show that plastic particles can promote cytological disorders, changes in the regulation of genes related to energy production, and in the metabolic routes (Lian et al., 2020a; Zhou et al., 2021). Experimentation with these species is related to the testing the toxic potential of the pollutant, to help to extrapolate the effects occurring in natural and agricultural environments, and to subsidize studies using other species.

The selection of different groups of plants in experiments (models, crops, and natives) is relevant to infer the whole picture of how plastic particles can interfere in individual and ecological level. It is not possible to define the ideal species for carrying out the experiments, and its choice depends on the analysis to be carried out, as explained above.

Which cultivation medium to choose?

Standardization is important to obtain reliable and comparable scientific results. A lack of standardization of MP/NP and plants in both setting up/conducting the experiments and in publications writing is often observed, probably due to the fact that experimental methodologies and techniques are still being developed and established. Considering the novelty of this scientific topic, the absence of essential information concerning methodological details in publications (e.g.: experiment setup, growth conditions, soil/water characteristics, and water amount in irrigation, as well as the containers used, and the methodology for mixing MP/NP in the culture medium) hinders experimental replication. In the same way, different forms of experiment information presentation and results are common, highlighting a lack of particles concentration metrics standardization – sometimes presented as per number of particles, percent in relation to medium quantity, and in weight contamination/quantity of medium.

Germination experiments with seeds exposed to MP were conducted mainly in Petri dishes with water or agar (Bosker et al., 2019; Lian et al., 2020a; Taylor et al., 2020; Q. Zhang et al., 2021). Germination analysis is important to determine the potential risks of a pollutant to the initial life stages of plants. It is known that the delay or malformation of root structures can affect the entire plant development, being decisive for plant growth and ecological competition (Rice and Dyer, 2001). The use of agar as culture medium an interesting way to avoid particles aggregation and to observe the movement of particles towards root (Taylor et al., 2020). Although the methodologies used are well described, some important information are missing.

Soil experiments are important to observe and infer the effects of contaminants in the environment (de Lima et al., 2021) . Plastic particles can change soil structure, such as bulk density, granulometry, water retention, nutrient cycling, and biota, directly influencing plant development. Soil experiments also allow the detection of the abrasive effects related to plant organ and plastic particle contact (de Souza Machado et al., 2019). Different methodologies and analyses were conducted in experiments exposing plants to MP/NP in soil, and many of them also analyzed in a more integrative way changes in soil structure and microorganisms, allowing a more ecological view (de Souza Machado et al., 2019; Lozano and Rillig, 2020; Qi et al., 2018). Some important points to be considered while setting up a soil experiment is that the physical and chemical structure of the soil selected affect the relationship of plastic particles with the environment, which can generate bias if not analyzed correctly. The technique used for irrigation is also important; if water is supplied through the soil surface, it can lead to the leaching of MP/NP to the bottom of the recipient - thus an alternative to this is perform capillary irrigation, which means that the water source is provided on the bottom of plant recipient and reaches the plant through the capillarity of the soil (Semananda et al., 2018). In addition, soil experiments enable to measure effects in microbiota community and metabolism, and their effects in plant development. It is also worth mentioning the lack of information regarding the chemical composition and granulometry of soil particles, which would be crucial to understanding the effects observed on plants and to relating them to the chemical and physical conditions of the soil.

The use of hydroponic system is another option for MP experimentation with plants. It facilitates the observation of several morphological and physiological changes in roots and allows for a more controlled environment (Conn et al., 2013). Until 2021, most experiments about the effects of plastic particles in plant development used this cultivation medium (Hartmann et al., 2022). Hypothetically, the analysis can be facilitated due to easier contact of plastic particles with plant parts (mainly roots), making plant organs more prone to damage caused by the contaminants. In addition, the roots remain clean due to the absence of soil particles in the medium, facilitating the acquisition of images, and chemical and molecular analyses, for example. However, some points must be analyzed carefully. Plastic particles are hydrophobic particles and can aggregate and float on the surface if not in constant motion or without the addition of products such as detergents to reduce hydrophobicity (Lian et al., 2020a).

Due to the wide distribution of plastic particles in the environment, including water bodies, soil, and air (Allen et al., 2019; Prata et al., 2020), experiments conducted in open air places may be susceptible to external contamination by these sources. For this reason, experiments aiming effects of particles at very low and precise concentrations must be done in controlled environments to avoid cross-contamination. In this case, we recommend experiments to be performed in closed rooms, using glass pots, and ultrapure water for irrigation and the use of soil medium is not recommended.

Case study

To illustrate the troubles faced during germination experiments in Petri dishes, we describe an experiment performed by our group to verify the effects of MP on rice (*Oryza sativa* L.) seeds. The experiment was carried out in late 2019, when only seven publications concerning MP on plants were available, and only three dealt with the germination effects (Hartmann et al., 2022), so few experimentation methods were available at that time. The problems faced during the experiment, some methods to avoid biases, as well as strategies to avoid mistakes that we made are the focus of the discussion.

Material and methods: We used IRGA 424 IR rice seeds, a genotype commonly cultivated in Rio Grande do Sul, Brazil. The seeds were sterilized by immersion in a 49.5% NaOH, 49.5% H2O, and 1% neutral detergent solution for 1 minute. After sterilization, the seeds were rinsed and immersed in distilled water for 5 minutes. The rinsing process was repeated four times; at the end, the seeds were placed in a beaker with distilled water, remaining immersed for 24 hours to break the dormancy. The plastic source in this experiment was PVC microparticles of random sizes and shapes (kindly provided by Prof. Dr. Álvaro Meneguzzi - Materials Department - UFRGS). The plastic particles were measured with the ImageJ software (Abramoff et al., 2004) using photographs taken with a camera attached to a stereomicroscope to analyze their shape (called circularity - how circular the particles are, where 1 is a perfect circle and 0 is a line) and size. Two treatments were defined: control (distilled water only) and 10 mg L^{-1} (PVC in water). Fifteen rice seeds were put in Petri dishes, each treatment was replicated five times. Each plate was identified and closed with a glass lid and film to prevent water loss. Petri dishes were placed in a bowl, preferably in the place where the experiment was being carried out, avoiding transporting the plates and moving the analyzed material. The tray used to place the plates was closed with aluminum foil and kept in a dark and warm place (~25 °C). After seven days, the seeds and roots were photographed to analyze length, diameter, surface area and volume in SmartRoot (ImageJ extension; Lobet et al., 2011; Fig. 2).

Results: The MP had a diameter varying from 1.6 to 216.5 um (76.3 ± 42.4) - it is important to note the non-uniform size and shape of particles. Circularity ranged from 0.13 – 1 (0.55±0.17) (Fig. 1) The formation of particles aggregates was also observed.

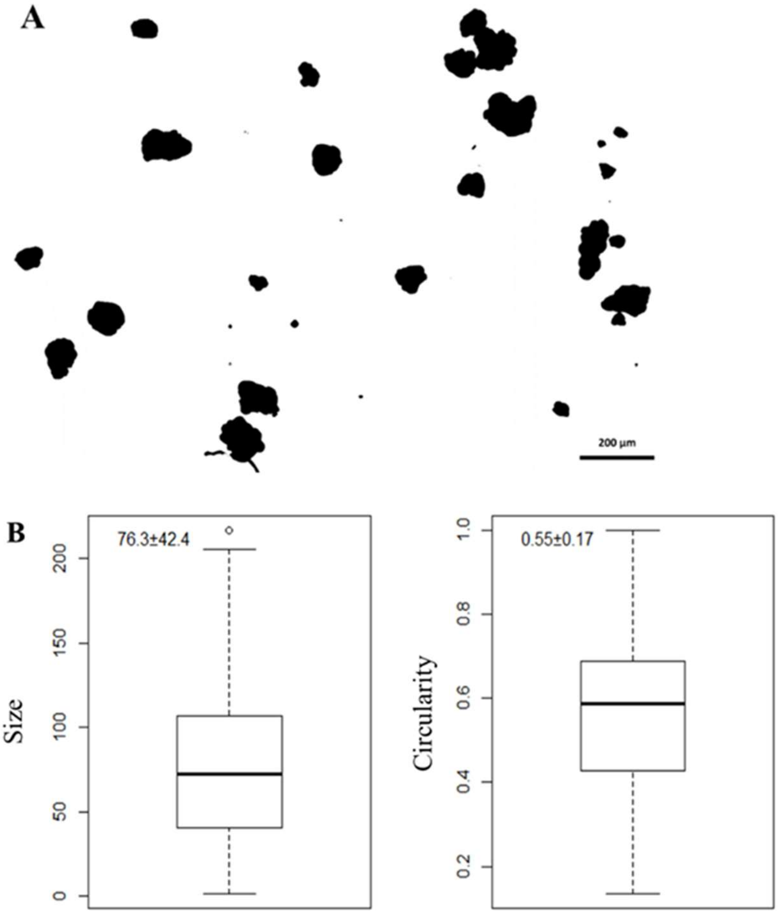

Figure 1. Morphology of PVC particles. **A)** image of plastic particles forming aggregates; **B)** metrics of particles used, graphics correspond to particles size (µm) and form (circularity).

Exposure to MP did not show significant effects in the germination of the rice seeds, although a slightly lower germination rate in seed treated with PVC was detected (Fig 2, Table 1). Roots of rice seeds treated with 10 mg L^{-1} of PVC presented an increased root length (control: 4.53 ± 2.41 cm; PVC: 5.72 ± 2.01 cm), surface (control: 0.82 ± 0.48 cm^2; PVC: 0.94 ± 0.33 cm^2), and volume (control: 0.0122 ± 0.0081 cm^3; PVC: 0.0128 ± 0.0049 cm^3) (Fig. 3). On the other hand, roots presented a reduction in mean diameter (control: 0.0549 ± 0.0061 cm; PVC: 0.0515 ± 0.0053 cm).

Table 1. Total number of seeds germination, mean, standard deviation and relative seed germination (RSG)

	Seeds germinated		
Day	Control (mean ± sd)	Treatment (mean ± sd)	RSG (%)
1	0	0	0
2	11.5 ± 3.1	10.5 ± 3.1	91.3
3	14.2 ± 1	13.2 ± 0.5	92.9
4	14.2 ± 0.6	13.8 ± 1	94.8

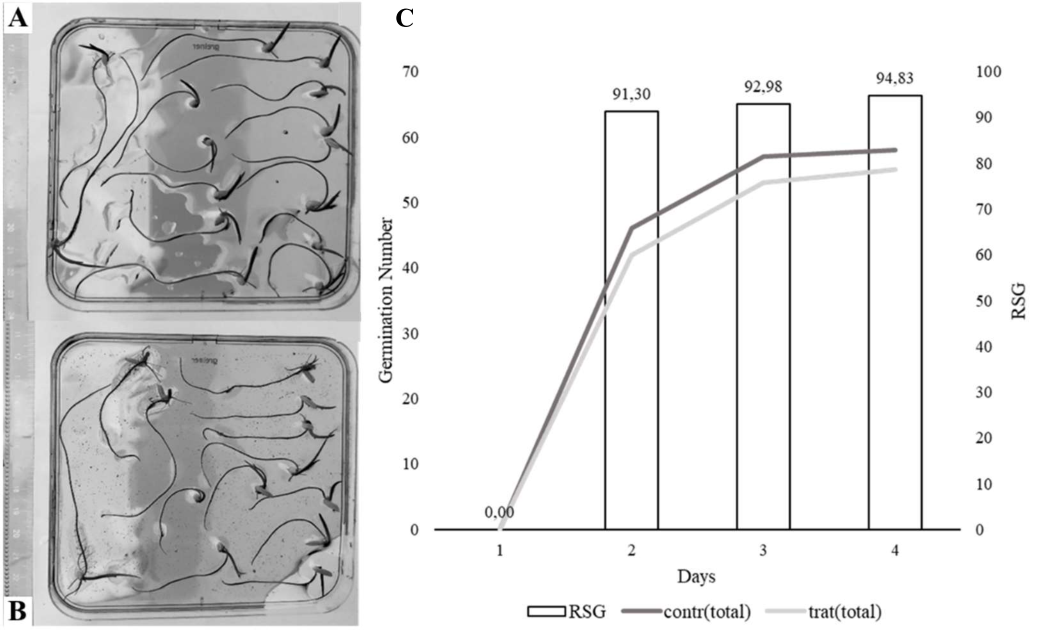

Figure 2. Photos of seeds and roots after seven days of experiment and the results. **A)** Control; **B)** Treatment with microplastics; **C)** On the left of graphic are presented the number of seed germinated and on the right side the relative seed germination (RSG) – also presented in white bars.

These patterns in roots exposed to MP/NP also were observed in other experiments (Bosker et al., 2019; Lian et al., 2020a). Possibly MP promoted a water deficit response in the seedlings, since those symptoms are very similar to those observed in plants submitted to drought, as a way to increase the contact surface for greater water absorption (Khalil et al., 2020). Lian et al., (2020) also propose that stretching may be related to metabolic changes linked to energy production (TCA cycle, starch, sucrose, and galactose metabolism). In this experiment, the authors used NP particles, which can enter the seeds. More research is needed to better understand these patterns.

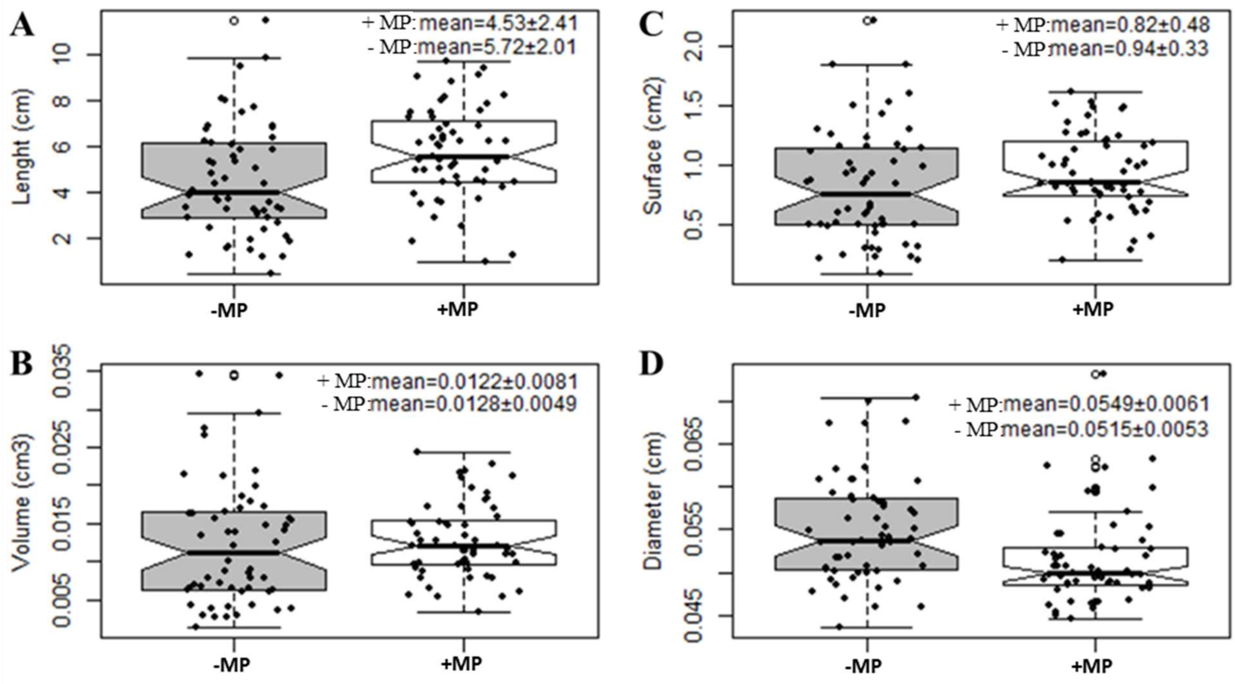

Figure 3. Measurements performed on the collected roots (processed in ImageJ software). Control and treatment with MP (10 mg L $^{-1}$, -MP and +MP, respectively). **A)** length (cm); **B)** Volume (cm^3); **C)** Surface area (cm^2); and **D)** root diameter (cm).

***Discussion*:** Although this experiment did not go as expected, mainly due to methodological problems, it served to understand situations to be improved in future experiments, in addition to calling our attention to the lack of information on the methodologies described in the available bibliography at that time. The formation of particle aggregates scattered in the water was one of the main problems observed, caused by hydrophobicity of these particles (Zhou et al., 2019). To handle this problem, Lian et al. (2020) added sodium dodecyl sulfate (SDS) to the cultivation medium, a detergent with no significant effects to seed germination in the concentration used. On the other hand, Boots et al. (2019) conducted an experiment to measure *Lolium perenne* L. germination and initial growth in soil, where MP/NP interacts directly with soil particles, being incorporated and possible becoming less available to seeds. These aggregates were noticed on the second day onwards. Besides they have decreased the contact between plant organs and MP, it is important to note that growth changes were detected in the roots. Another interesting issue to discuss is the material of recipients used to cultivate. As mentioned earlier, the degradation of plastic products is a major source of MP/NP, and associated chemical components, to the environment (K. Zhang et al., 2021). Therefore, using plastic materials in the experiments can lead to the formation and accumulation of these particles in the culture medium, biasing the experiment. Although the plastic degradation process is slow, handling can fragment and generate these particles. To avoid this, we suggest using glass materials.

Conclusion: Plastic particles affect plant development and productivity, directly interfering with human health. Experiments with plants exposed to MP/NP are necessary to understand and prepare us for the future. Although there is some knowledge about the negative effects generated in plants, there is a great lack of information about the physiological, morphological and ecological effects of MP/NP on the soil and on the exposed plants. Here we highlight the effects on crop species, due to the great contamination in agricultural lands and the dangers that their contamination poses to human health. The development and standardization of methodologies is extremely important for carrying out experiments that are comparable and that clearly show what researchers want to observe (that is, specific or ecological effects), therefore, this chapter seeks to summarize good practices to avoid bias in experiments, including what type and size of plastic particles to use, cultivation medium and good species for certain analyses. We hope that all these information helps to standardize the experiments to create a solid net of research, increasing the knowledge in the area.

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