



# Bismuth interaction with plants: Uptake and transport, toxic effects, tolerance mechanisms - A review

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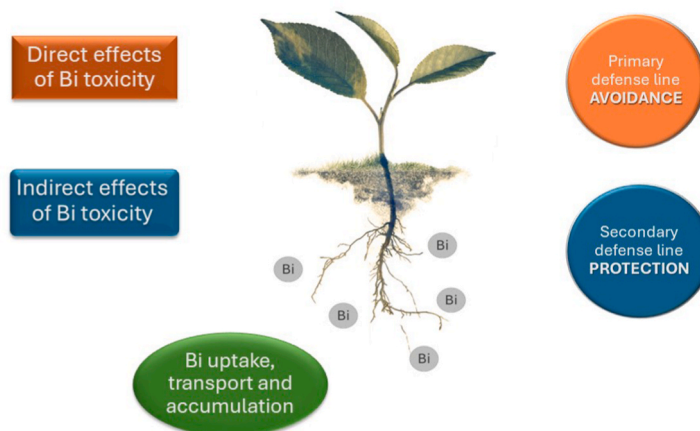
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## HIGHLIGHTS

- Bi presence in the environment is predicted to increase due to its multiple uses.
- Limited literature is focused on the interaction between Bi and plants.
- Bi uptake, accumulation and transport have been observed in plants.
- Toxic effects of Bi on DNA and primary processes in plants are reported.
- Further research is needed to clarify the mechanisms of Bi toxicity in plants.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Bismuth (Bi) is a minor metal whose abundance on Earth is estimated at 0.025 ppm. Known since ancient times for its medical properties, its use in many industrial applications has increased significantly in recent years due to its physical and chemical properties. Considered less toxic than other metals, Bi has been defined as a “green metal” and has been suggested as a replacement for lead in many industrial processes. Although the occurrence of Bi in the environment is predicted to increase, there is still a lack of information on its interaction with biota. Even though it is absorbed by many organisms, Bi has not been directly implicated in the regulation of fundamental metabolic processes. This review summarises the fragmentary knowledge on the interaction between Bi and plants. Toxic effects at the growth, physiological and biochemical levels have been described in Bi-treated plants, with varying degrees and consequences for plant vitality, mostly depending on the chemical formulation of Bi, the concentration of Bi, the growth medium, the time of exposure, and the experimental conditions (laboratory or outdoor conditions). Bismuth has been shown to be readily absorbed and translocated in plants, interfering with plant growth and development, photosynthetic processes, nutrient uptake and accumulation, and metal (especially iron) homeostasis. Like other metals, Bi can induce an oxidative stress state in plant cells, and genotoxic effects have been reported in Bi-treated plants. Tolerance responses to the excess presence of Bi

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have been poorly described and are mostly referred to as the activation of antioxidant defences involving enzymatic and non-enzymatic molecules. The goal of this review is to offer an overview of the present knowledge on the interaction of Bi and plants, highlighting the gaps to be filled to better understand the role of Bi in affecting key physiological processes in plants. This will help to assess the potential harm of this metal in the environment, where its occurrence is predicted to increase due to the growing demand for medicinal and industrial applications.

## 1. Introduction

Bismuth (Bi) is a chemical element (atomic number 83, standard atomic weight 208.98) classified as a metal in the periodic table but has more similarities to semimetals. The name bismuth is derived from the obsolete German “wismuth”, *i.e.* white mass. In ancient times, this element was frequently confused with tin or lead (Pb) due to its similar appearance, despite being one of the first 10 metals to be discovered. Bismuth is categorised among the group of elements conventionally known as “poor elements” due to its rarity. In fact, its abundance in the Earth’s crust is estimated at 0.025 ppm, which is 10-fold less than that of antimony. For this reason, Bi is also known as a “minor” metal.

Although the medicinal properties of Bi have been known since ancient times, interest in its use has increased greatly in recent decades due to its physical and chemical properties (Himeno et al., 2022; Udalova et al., 2008). As one of the few substances other than water that expands during solidification, it is commonly used to produce low-melting alloys that must expand once in the moulds. Moreover, after graphite, it is also one of the most diamagnetic materials known, which is why it is used in the construction of magnetic levitation (Maglev) trains (Mohan, 2010). Bismuth is widely used in cosmetics, laboratories, semiconductors and pharmaceutical production. Due to its lower toxicity compared to Pb, Bi has replaced Pb in many industrial processes (Esquivel-Gaon et al., 2015; Rohr, 2002). In medicine and healthcare, Bi is known to be highly effective in the treatment of burns, intestinal diseases and peptic ulcers, as well as against microorganisms, viruses and malignant tumours (Wang et al., 2019). Recently, the successful use of Bi to inhibit severe acute respiratory syndrome coronavirus (SARS-CoV) has been reported (Wang et al., 2019). In this case, the action of Bi is related to the binding of the SCV NTPase/helicase, a zinc-containing enzyme that has RNA-capping activity and controls virus reproduction. Through this binding, Bi may induce conformational changes in the enzyme, affecting the helicase RNA/DNA unwinding activity and finally resulting in the inhibition of virus proliferation.

Due to its use for rapid relief of heartburn, nausea, indigestion and diarrhoea, Bi subsalicylate is largely used as an over-the-counter pharmaceutical drug, with annual sales of USD 82.6 million in the United States in 2013 (Wang et al., 2019). This medicine is also known to inhibit the growth of *Helicobacter pylori*, a stomach bacteria causing ulcers and digestive issues, through its interaction with gastric acid in the stomach, releasing Bi. In this context, the bactericidal effect of Bi on *H. pylori* is exerted in different ways, ranging from inhibiting several enzymes to inhibiting ATP synthesis (Alkim et al., 2017). Finally, due to its recognised role as a theranostic agent (Badrigilan et al., 2020), it is predicted that the use of Bi in medicine will increase in the near future.

Despite the valuable body of knowledge about the effects of Bi on microorganisms and animals, including humans, as discussed in this review in section 3, there is only limited information in the literature on the interaction of this minor metal with plant organisms. Moreover, to the best of the author’s knowledge, no comprehensive overview of studies investigating the interaction of Bi and plants has been previously reported. This includes studies on the absorption and accumulation of Bi by plants as well as the effects of Bi at different levels on the main physiological processes occurring in plants. A clear report on the present state of the art on this issue can serve as a valuable tool to identify the gaps in knowledge and direct future research efforts to address these gaps.

## 2. Sources and occurrence of Bi in the environment

Native Bi, associated with other Bi minerals, is mostly found in silver and cobalt ore veins as well as cupro-stanniferous deposits, from which the largest amount of Bi is obtained.

In cobalt-silver ore veins, Bi is found in Schneeberg in Saxony and in Joachimsthal in Bohemia. It is also associated with uranium ores. Copper-tin deposits with significant Bi presence are found in Cerro de Chorolque and Tazna in Bolivia, as well as in the stanniferous lodes and alluvium of New South Wales, Tasmania and Queensland. Additionally, deposits of lesser importance can be found in the East Pool and Illogan mines in Cornwall and Meymac in Corrèze, France. A third type of deposit, in which Bi minerals are associated with gold, occurs in Transylvania, Colorado, Georgia, Virginia, and North Carolina.

Bismuth reserves are typically estimated based on the Pb content of resources, as Bi is a by-product of processing other metal ores, including Pb, silver, tin, copper, and zinc. Bismuth is produced only as a major product in the Tasna Mine in Bolivia and a mine in China. World reserves of Bi are estimated to be around 320,000 tonnes. In 2022, approximately 20,000 tonnes of refined Bi were produced worldwide, with China being the leading producer, accounting for approximately 80% of the world’s total production (Merrill, 2023). In nature, although rare, it occurs mainly as a mineral in the form of sulphide (bismuthinite: Bi<sub>2</sub>S<sub>3</sub>) or oxide (bismite: Bi<sub>2</sub>O<sub>3</sub>); the elemental form is rarer.

In the environment, Bi concentrations in natural soil are reported as 0.13–40 µg g<sup>-1</sup> D.W., with 1 µg g<sup>-1</sup> D.W. as the most represented value (Das et al., 2006; Fahey et al., 2008; Karagatzides et al., 2008), while in marine/river/lake water and sediments as 0.03–2.3 µg g<sup>-1</sup> D.W. (Das et al., 2006; Filella, 2010). The presence of Bi in higher concentrations compared to the natural background has been detected in areas close to smelters and metal mines in England (Li and Thornton, 1993), as Bi is commonly found as a waste product from Pb, silver, tin, copper, and zinc mines (Mohan, 2010). In this regard, Jung et al. (2002) conducted a survey on soil near a copper-tungsten mine and found Bi values ranging from 42 to 1510 mg kg<sup>-1</sup>. Similarly, Wei et al. (2011) reported a Bi concentration in soil reaching 1672 mg kg<sup>-1</sup> in a mining and smelting area. Extremely high values were also reported by Elekes and Busuioac (2010) for a forest soil close to a highway (Bi concentrations ranged 930–1891 mg kg<sup>-1</sup>). The presence of Bi above the natural background has also been detected in both agricultural and industrial areas in southern Italy because of anthropogenic activities (Polemio et al., 1982). The occurrence of Bi at relatively high concentrations has been reported by analysing PM<sub>10</sub> emission sources in the Netherlands and ascribed to the burning of fireworks (Massimi et al., 2021). Additionally, the presence of Bi in the alpine ice located near the Mont Blanc summit was recently reported by Legrand et al. (2023) and attributed to the military activities of the Second World War, given the use of Bi for low-melting point alloys for shells, thin-walled aluminium alloy, aircraft oil and munitions. In this context, Bi in shotgun shells, approved as a non-toxic alternative to Pb shots, has been shown to be released into wetland soils in Canada (Fahey et al., 2008).

The predicted ever-increasing use of Bi in multiple industrial activities is a cause for concern, as concentrations in terrestrial and aquatic ecosystems can increase rapidly and knowledge about its ecotoxicity is very limited (Wang et al., 2019). In this context, Amneklev et al. (2016) measured a 300% increase in Bi in Stockholm city wastewater in just one year and attributed this increase to the growing use of Bi salts in

cosmetic products. This suggests that the use of sewage sludge as agricultural fertiliser may enhance the presence of Bi in soils in the near future, with unpredictable environmental consequences due to a lack of information on interactions with biota. The use of phosphate fertilisers is also considered a potential source of Bi contamination in soils (Eriksson, 2001), given that, in Brazil the presence of Bi in fertilisers has been reported (Machado et al., 2017). Additionally, it has been claimed that Bi can enter the environment through ash and dust from the incineration of industrial and hospital waste (Xiong et al., 2015).

### 3. Biological action of Bi

The interaction between Bi and biota has been poorly studied thus far, and the physiological implications of Bi presence in biological organisms still need to be clarified. Currently, no metabolic process has been directly associated with the presence of Bi, suggesting that this metal does not have any structural or regulatory functions. However, Bi absorption and accumulation have been reported for various biological organisms, such as microbes (Murata, 2006), macroalgae (Kearns and Turner, 2016), wild mushrooms (Elekes and Busuioc, 2010), earthworms (Omouri et al., 2018), plants (Wei et al., 2011), zebrafish (He et al., 2013), freshwater snails (Al-Abdan et al., 2021), mice (Larsen et al., 2003), waterfowl and game birds (Fahey and Tsuji, 2006), and humans (Slikkerveer and de Wolff, 2023). Although the toxicity of Bi and Bi-based nanomaterials is reported to be lower than that of other metals, the adverse effects of Bi on many organisms have been described (Badrigilan et al., 2020; Omouri et al., 2018; Pietrini et al., 2023), mostly related to its concentration and formulation. The toxicity of Bi to microbes has been attributed to its ability to disrupt multiple biological pathways by binding to key proteins. This property has been exploited for antimicrobial therapy in humans (Wang et al., 2019). Bismuth toxicity in earthworms has been associated by Omouri et al. (2018) with the inhibition of reproduction assessed in all reproduction parameters investigated (number of juveniles, number of hatched cocoons and total number of cocoons). Bismuth nanoparticles were used by He et al. (2013) to evaluate the toxicity of the metal on zebrafish embryos, showing smaller head sizes, shorter body lengths, and pericardial oedema in Bi-treated embryos. In this study, the authors underlined that the severity and occurrence of the resulting phenotype were concentration-dependent. Oxidative stress and DNA fragmentation were shown by Al-Abdan et al. (2021) on *Lymnaea luteola* treated with bismuth oxide nanoparticles, highlighting the ecotoxicity and genotoxicity of this metal on aquatic biota.

Bismuth has also been reported to have toxic effects on humans, such as osteoarthropathy, gingivitis, stomatitis, colitis (Slikkerveer and de Wolff, 2023), nephrotoxicity (Pelepenko et al., 2022), and encephalopathy (Déchy, 2023). In this regard, Slikkerveer and de Wolff (2023) reported that Bi in human blood is normally at a concentration between 1 and 15 µg/L, but concentration can rise following absorption from oral administration, with the kidney as the organ with the highest Bi accumulation and retention. In the kidney, Bi is bound to a bismuth-metal binding protein, synthesised upon induction by the metal itself. Among biomolecules able to carry metals in the plasma, transferrin, metallothionein and glutathione have been proposed as candidates for binding Bi. In particular, Sun et al. (2001) reported the binding of Bi<sup>3+</sup> to the Fe<sup>3+</sup>-binding site of transferrin, while Naganuma et al. (1987) showed that Bi can induce metallothionein and that the binding affinity for Bi to these compounds is higher than those for cadmium or zinc. The ability of Bi to bind glutathione has been reported (Sadler et al., 1996). The role of glutathione in the metabolism and detoxification of Bi in mammalian cells was also reviewed by Wang et al. (2019), underlining how, after the passive absorption of Bi ions, their conjugation to glutathione and transport into vesicles may occur to sequester the metal and protect cells. The toxicity of Bi on plants is specifically addressed in section 5.

### 4. Bismuth uptake, translocation and accumulation in plants

As many metals are required by plants for the functionality of their fundamental processes, plants have developed regulatory mechanisms for the uptake, transport and storage of metals in their tissues (Greger, 2004). Among the metals, copper, zinc, manganese, iron, nickel and molybdenum are widely recognised as essential micronutrients for plants. In general, plants can interact with metals by entering in contact through the root tip and successively absorbing and translocating them to the vascular system via the symplast or apoplast route, depending on their chemical nature (Clemens and Ma, 2016). Long-distance metal transport can occur via xylem and phloem vessels, depending on the plant species, growth stage, and type of metal. In this regard, water transpiration is reported to be the driving force for metal transport from roots to shoots via the xylem (Shen and Ma, 2001), while phloem activity is more involved in metal storage in fruit and seeds and metal transport within the shoot (Page and Feller, 2015). The mobility of metals within plants varies between plant species and metals, with some metals, such as nickel and zinc, being highly mobile in phloem vessels and then highly mobile in leaves, while others, such as iron and manganese, have reduced phloem mobility and are thus retained in older leaves (Page and Feller, 2015).

In terrestrial vascular plants, the background Bi level is reported as less than 20 µg kg<sup>-1</sup> (Kabata-Pendias, 2001). However, there is a dearth of literature on the exposure of plants to Bi, both in laboratory and field trials. In fact, few studies have focused on the ability of plants to absorb and accumulate Bi in different organs (Table 1). In this regard, a notable Bi uptake was observed in experiments in controlled conditions with Bi administration in liquid and agar-solidified solution and in soil. A Bi concentration-dependent accumulation in garden cress (*Lepidium sativum* L.) plantlets, obtained by germinating seeds in aqueous solution in the darkness for 72 h with 30, 60, 121, 242 and 485 mg L<sup>-1</sup> of Bi nitrate, was reported by Passatore et al. (2022), evidencing a linear relationship (R<sup>2</sup> = 0.94) between Bi content in plantlets and Bi nitrate concentrations in the solutions. In this study, the Bi concentration found in plantlets exposed to the highest Bi concentration assayed was greater than 1200 mg kg<sup>-1</sup>, evidencing that the accumulation of Bi in plants was not saturated, even with 485 mg L<sup>-1</sup> of Bi nitrate in the medium. In an agar-solidified Murashige & Skoog (MS) medium with 0.1, 1, 2, 3, 4, 5, 6, 7, 8 or 9 µM Bi nitrate, Nagata and Kimoto (2020) cultivated tomato (*Solanum lycopersicum* L.) plantlets for 14 days under laboratory conditions and observed that Bi accumulation in the shoot was related to its concentration in the medium, while in roots the Bi concentration was saturated at >3 µM Bi treatment. The results of this work showed that higher metal accumulation in roots and reduced translocation in the shoots occurred at lower concentrations of Bi nitrate. At higher Bi concentrations in the medium, root accumulation of Bi did not increase compared to that observed at lower Bi concentrations, whereas higher metal translocation to shoots was detected, suggesting the occurrence of a mechanism regulating Bi uptake and translocation in tomato plants. In a previous study by Nagata (2015), *Arabidopsis thaliana* seeds were germinated on an agar-solidified MS medium with different concentrations of Bi nitrate under laboratory conditions, showing that, while no Bi accumulation occurred in 0.1 or 1 µM Bi-treated plants, plants exposed to 2 µM Bi absorbed the metal and accumulated it preferentially in the roots (7-fold higher root Bi accumulation compared to the shoot). Bismuth uptake and tissue accumulation were also observed in an aquatic plant by Pietrini et al. (2022), exposing *Lemna minor* L. to Bi nitrate added to Hoagland solution. Dose-dependent Bi accumulation in the fronds to metal concentration in the nutrient solution was also observed in this trial, with extremely high Bi concentrations (over 5000 mg kg<sup>-1</sup>) detected in plants treated with 242 mg L<sup>-1</sup> of Bi nitrate. The bio-concentration of Bi in *L. minor* plants increased up to 60 mg L<sup>-1</sup> Bi nitrate in the solution to remain significantly unchanged at 121 and 242 mg L<sup>-1</sup>, evidencing the ability of this plant species to bioaccumulate the metal even at relatively high Bi concentration in the medium (Zacchini

**Table 1**  
Bismuth concentrations detected in plants in both laboratory experiments and field surveys.

Plant species	Bi concentration level in the substrate	Substrate	Plant organ	Bi concentration in plant	Reference
<i>Arabidopsis thaliana</i> L.	0,0.1,1,2,3,4,5,6,7,8,9 $\mu\text{M}$	MS-agar	Root Shoot	70 $\text{nmol g}^{-1}$ FW <sup>a</sup> 10 $\text{nmol g}^{-1}$ FW <sup>a</sup>	Nagata (2015)
<i>Solanum lycopersicum</i> L.	0, 0.15, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5, 15 $\mu\text{M}$	MS-agar	Root Shoot	20 $\text{nmol g}^{-1}$ FW 25 $\text{nmol g}^{-1}$ FW <sup>a</sup>	Nagata and Kimoto (2020)
<i>Arabidopsis thaliana</i> L.	0, 0.1, 1, 2 $\mu\text{M}$	MS-agar	Root Shoot	140 $\text{nmol g}^{-1}$ FW <sup>a</sup> 13 $\text{nmol g}^{-1}$ FW <sup>a</sup>	Nishimura and Nagata (2021)
<i>Lepidium sativum</i> L.	0, 30, 60, 121, 242, 485 $\text{mg L}^{-1}$	Liquid	Seedlings	20–1230 $\text{mg/kg DW}$	Passatore et al. (2022)
<i>Triticum aestivum</i> L.	500 $\text{mg kg}^{-1}$	Soil	Root Shoot	580 $\text{mg/kg DW}$ <sup>a</sup> 220 $\text{mg/kg DW}$ <sup>a</sup>	Mohammed et al. (2023)
<i>Phaseolus vulgaris</i> L.		Soil	Root Shoot	700 $\text{mg/kg DW}$ <sup>a</sup> 300 $\text{mg/kg DW}$ <sup>a</sup>	
<i>Lepidium sativum</i> L.	0, 30, 121, 485 $\text{mg/kg}$	Soil	Root Shoot	0.6–4.9 $\text{mg/kg DW}$ 4.9–81.2 $\text{mg/kg DW}$	Pietrini et al. (2023)
<i>Carex lacustris</i> Willd.	0.42–6.40 $\text{mg/kg}$	Soil	Aboveground tissues	0.057–0.095 $\text{mg/kg DW}$	Fahey et al. (2008)
<i>Agrostis scabra</i> Willd.				DW	
<i>Buddleja davidii</i> Franch.	n.d.-1672 $\text{mg/kg}$	Mine soil	Root and aboveground tissues	2.87 $\text{mg/kg DW}$	Wei et al. (2011)
<i>Hippochaete ramosissima</i>			Root and aboveground tissues	0.08 $\text{mg/kg DW}$	
<i>Myriophyllum spicatum</i> L.	n.d.	Freshwater	Total plant	0.27 $\text{mg/kg DW}$	Kovacs et al. (1984)
<i>Potamogeton perfoliatus</i> L.	n.d.			0.34 $\text{mg/kg DW}$	
<i>Ceratophyllum submersum</i> L.	n.d.			0.41 $\text{mg/kg DW}$	
<i>Zea mays</i> L.	42 - 1510 $\text{mg kg}^{-1}$	Mine soil	Grain	0.01–0.02 $\text{mg/kg DW}$	Jung et al. (2002)
<i>Capsicum annuum</i> L.			Fruit	0.01–0.02 $\text{mg/kg DW}$	
<i>Glycine max</i> L.			Leaves	0.03–0.13 $\text{mg/kg DW}$	
<i>Allium cepa</i> L.			Leaves	0.05–0.42 $\text{mg/kg DW}$	
<i>Zizyphus jujuba</i> Mill.			Grain	0.01–0.04 $\text{mg/kg DW}$	
<i>Perilla frutescens</i> L.			Leaves	0.04–0.11 $\text{mg/kg DW}$	
<i>Potentilla acervata</i> Sojak	5–159.5 $\text{mg kg}^{-1}$	Mine soil	Flowers, seeds, leaves stems, roots	0.01–1.58 $\text{mg/kg DW}$	Yurgenson and Gorban (2020)

<sup>a</sup> These values are approximations calculated from the figure presented in the paper. n.d. not detected.

et al., unpublished data).

Bismuth uptake and accumulation have also been reported for plants grown in soil both in growth chamber and outdoor conditions. In a recent study, Mohammed et al. (2023) added 500  $\text{mg kg}^{-1}$  Bi nitrate to soil in pots in a growth chamber in which wheat (*Triticum aestivum* L.) and bean (*Phaseolus vulgaris* L.) seeds were sown after mycorrhizal inoculation, and seedlings were grown for 5 weeks. In both plant species, Bi exposure resulted in an accumulation of the metal preferentially in the roots, with Bi concentrations higher than 500  $\text{mg kg}^{-1}$ , while in shoots, Bi concentrations were slightly higher than 200  $\text{mg kg}^{-1}$ . Bean plants showed the highest ability to accumulate metal in both organs. Interestingly, the authors observed that plant mycorrhization drastically reduced the Bi accumulation in both plant organs, especially in bean plants (68.9% in the roots and 71.3% in the shoots compared to non mycorrhized plants). In an outdoor condition experiment, Fahey et al. (2008) added Bi pellets to wetland soil, acidified or not, to evaluate the possible transfer of Bi to plants in soil contaminated by Bi pellet deposition. The analysis of Bi content in the above-ground organs of *Carex lacustris* and *Agrostis scabra* revealed low Bi accumulation by both plants, very similar to global background levels (0.065–0.095  $\text{mg kg}^{-1}$ ), in both acidified and not acidified soil. A similar study conducted by Karagatzides et al. (2008) on soil contaminated by shotshell pellets containing Bi evidenced the presence of Bi in the sapwood of sugar maple (*Acer saccharum* Marsh.), bitternut hickory (*Carya cordiformis* (Wangenh.) K. Koch) and red maple (*Acer rubrum* L.), with the highest levels in acidified soils compared to trees in untreated soils. Notably, given the distribution of Bi in the soil layers, the reported interaction of Bi with bacteria (Murata, 2006; Tsang et al., 1994), and the high release of Bi from litter to organic soil compartments (Tyler, 2005), the authors postulated that soil bacteria may facilitate the transfer of Bi from leaf litter to soil thus increasing the availability of Bi for plant uptake. The study also showed the role of soil pH in Bi availability for plants, as already reported by Li and Thornton (1993). The accumulation of Bi by plants was also studied in a survey conducted near an old antimony mine

in China. In this work, Wei et al. (2011) sampled seven plant species colonising different sites of the area, observing the highest Bi content in *Buddleja davidii* (mean value of 2.877  $\text{mg kg}^{-1}$ ), while the lowest occurred in *Hippochaete ramosissima* (mean value of 0.088  $\text{mg kg}^{-1}$ ). However, given the different Bi content between the two sites, the ability to accumulate Bi in its organs was much higher for *H. ramosissima*, with a BCF (bioconcentration factor; see Zacchini et al., 2009) value exceeding 4. The ability of plants to concentrate Bi in their organs was previously reported by Kovacs et al. (1984) by detecting Bi in *Myriophyllum spicatum* (0.27  $\text{mg kg}^{-1}$ ), *Potamogeton perfoliatus* (0.34  $\text{mg kg}^{-1}$ ) and *Ceratophyllum submersum* (0.41  $\text{mg kg}^{-1}$ ) in the aquatic medium where Bi was not detected. In this regard, Pietrini et al. (2023) found BCF values for Bi in shoots and roots lower than 0.05 and 0.5, respectively, in *Lepidium sativum* L. plants grown in soil spiked with different Bi nitrate concentrations in the growth chamber. The authors emphasised that the plant species has a low capacity for Bi accumulation in comparison with other metals (Hedayatzadeh et al., 2020). Moreover, in this study, the translocation factor (Tf; see Zacchini et al., 2009) was also calculated, evidencing that the Tf for Bi in their experiment was very similar to that reported for Cr, Ni and Pb by Soriano-Disla et al. (2014) in barley, described as low-mobile elements in plants. In the same work, Pietrini and co-authors reported the dose-dependent accumulation of Bi to the metal concentration in the soil, with Bi concentration in the roots of metal-treated plants being more than 10-fold higher than in the shoots. Bismuth detection in the plant shoots was discussed by the authors, evidencing that at least a part of Bi accumulated in the roots, entering the root cells and reaching the vascular tissues to be transported to the aerial parts, possibly through the transpiration stream. Furthermore, Nagata and Kimoto (2020) reported that in a root-split experiment on *Solanum lycopersicum*, the Bi content was detected in non-treated roots possibly transferred from the Bi-treated roots through the vascular bundles. The mechanisms of Bi transport in plants remain to be clarified, as previously observed by Babula et al. (2010), as no work focusing on this issue has been reported



thus far. Based on the literature, passive Bi ion absorption by plants could be postulated, in line with that reported in mammalian cells (Wang et al., 2019), where, once passively absorbed, Bi is conjugated to glutathione (GSH) to be transported into vesicles to reduce its toxicity. The recycling or de novo biosynthesis of GSH in turn allows further passive uptake of Bi.

## 5. Bismuth toxicity in plants

Excess metal accumulation in plants may result in the induction of toxic mechanisms. These include alterations in nutrient uptake, replacement of other metals in fundamental molecules as enzymes, disturbances to photosynthesis and carbon metabolism, generation of reactive oxygen species (ROS) or reduced quenching, impairment of membranes by lipid peroxidation, and damage to nucleic acids, which ultimately result in reduced growth. These effects have been extensively reviewed (Dal Corso, 2012; Nagajyoti et al., 2010). Fig. 1 provides a schematic illustration of the mechanisms of heavy metal toxicity in plants (adapted from Küpper and Andresen, 2016).

The toxic effects of Bi on various plant species have been reported in the literature (Table 2). In addition to plant species, other factors such as the chemical formulation of Bi, the concentration of Bi, the growth medium, the time of exposure, and other experimental conditions (laboratory or field), are thought to be the main factors influencing the plant responses to the presence of Bi in the substrate. In this context, reviewing the toxicity of heavy metals in plants, Appenroth (2010) included Bi in the “lead-group elements” due to its specific chemical properties and toxicity. A graphical summary of the main effects reported in plants due to exposure to Bi is shown in Fig. 2.

### 5.1. Effects on growth and development

As a common endpoint of the toxicity assessment of metals in plants

(Fig. 1), growth inhibition has been evaluated in almost all works studying the effects of Bi on plants. Overall, the limited literature that has focused on the evaluation of the effects of Bi on plant growth and development is consistent, indicating that the toxicity exerted by Bi is related to various factors mainly affecting the metal availability for plants. As for other better studied metals (Greger, 2004), these factors can be related to the metal concentration, the pH and the organic matter content of the substrate. Most of the studies carried out on the interaction of Bi with plants have shown that root growth is the process mainly affected by the presence of Bi in the substrate. Therefore, according to Omouri et al. (2019), root elongation appeared to be a suitable proxy to assess the toxicity of Bi in plants.

In the literature, aqueous, agar-solidified, growth substrate and natural soil medium were used to dissolve different Bi formulations to obtain various Bi concentrations for the plant treatment. Filter papers in an aqueous medium containing several Bi concentrations, both in the form of nitrate and citrate, were used by Omouri et al. (2019) to assay Bi toxicity in *Lolium perenne* seeds after 5 days of exposure. Overall, Bi citrate was reported as more toxic than Bi nitrate in terms of the inhibition of seed germination (starting at 99 mg L<sup>-1</sup> for Bi-citrate and 485 mg L<sup>-1</sup> for Bi nitrate). Instead, the opposite behaviour was observed for root elongation. Seeds exposed to Bi nitrate showed a dose-dependent effect from 30 mg L<sup>-1</sup>, whereas with Bi citrate, a lesser effect was detected, with significant root growth inhibition observed from 99 mg L<sup>-1</sup> onwards. However, both Bi formulations were reported to dramatically reduce both seed germination and root elongation at the highest Bi concentrations tested. Seed germination and root elongation were also analysed as toxicity endpoints by Passatore et al. (2022) in *Lepidium sativum* seeds treated for 72 h with different concentrations of Bi nitrate, highlighting a slight negative effect on germination at the highest Bi concentrations, while root elongation was inhibited to varying degrees in all Bi treatments. Similarly, Céspedes et al. (2003) showed the toxic effects of three different tertiary bismuthines (i.e. tris(heteroaryl)

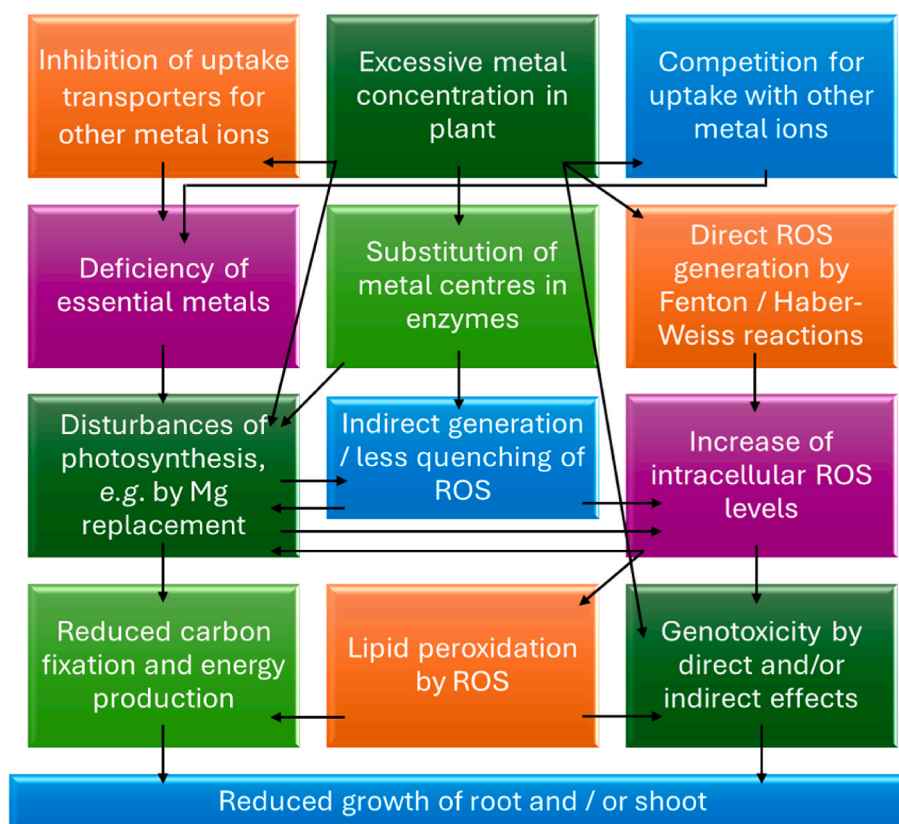


Fig. 1. Mechanisms of heavy metal toxicity in plants (adapted from Küpper and Andresen, 2016).

**Table 2**  
Studies reporting the toxic effects of Bi on various plant species.

Plant species	Bi formulation	Bi concentration level	Substrate	Time of treatment	Parameter analysed	Reference
<i>Lactuca sativa</i> L. <i>Trifolium pratense</i> L. <i>Lolium multiflorum</i> Lam. <i>Physalis ixocarpa</i> Brot. ex Hornem.	Tertiary bismuthines (tris (heteroaryl)Bi compounds)	0, 5.0, 25.0, 50.0, 75.0 $\mu\text{M}$ 0, 0.5, 1, 3, 5, 7.5, 10 $\mu\text{M}$	Liquid	120 h	-Seed germination -Coleoptyle, hypocotyle and root lengths -Seed respiration	Céspedes et al. (2003)
<i>Allium cepa</i> L.	Bismuth (III) oxide bulk and nanoparticles	0, 12.5, 25, 50, 75, and 100 mg $\text{L}^{-1}$	Liquid	4 h	- Root cell mitotic index and chromosomal aberrations -Genotoxicity	Liman (2013)
<i>Arabidopsis thaliana</i> L.	Bismuth nitrate	0,0.1,1,2,3,4,5,6,7,8,9 $\mu\text{M}$	MS-agar	14 days	-Seed germination -Root elongation and shoot growth -Bi accumulation -Gene expression	Nagata (2015)
<i>Lolium perenne</i> L.	Bismuth nitrate	0, 15.1, 30.3, 60.6, 121.2, 242, 485 mg $\text{L}^{-1}$	Liquid	5 days	-Seed germination -Root elongation	Omouri et al. (2019)
<i>Lolium perenne</i> L.	Bismuth citrate	0, 12.4, 24.8, 49.7, 99.5, 199, 398.1 mg $\text{L}^{-1}$	Liquid	5 days	-Seed germination -Root elongation	
<i>Lolium perenne</i> L.	Bismuth nitrate	0, 15.15, 60.62, 242.5, 485 mg $\text{L}^{-1}$	Soil	7 days	-Seed germination -Root elongation -Root mass	
<i>Lolium perenne</i> L.	Bismuth citrate	0, 3.98, 39.8, 398.1 mg $\text{L}^{-1}$	Soil	7 days	-Seed germination -Root elongation -Root mass	
<i>Macrotyloma uniflorum</i> (Lam.) Verdc	Not specified	0, 50, 100, 200, 300, 400 mg $\text{L}^{-1}$	Soil	40 days	-Photosynthesis	Prabhavati et al. (2017)
<i>Solanum lycopersicum</i> L.	Bismuth nitrate	0, 0.15, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, 12, 13.5, 15 $\mu\text{M}$	MS-agar	14 days	-Seed germination -Root elongation -Seedling mass -Bi accumulation	Nagata and Kimoto (2020)
<i>Raphanus sativus</i> L.	Bismuth nitrate	0,0.3,3,30,300 mg $\text{L}^{-1}$	Soil	7 days	-Seed germination -Root elongation	Sudina et al. (2021)
<i>Arabidopsis thaliana</i> L.	Bismuth nitrate	0, 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9 $\mu\text{M}$	MS-agar	14 days	-Seed germination -Root elongation -Seedling mass -Bi accumulation -Gene expression	Nishimura and Nagata (2021)
<i>Lepidium sativum</i> L.	Bismuth nitrate	0, 30, 60, 121, 242 and 485 mg $\text{L}^{-1}$	Liquid	72 h	-Seed germination -Root elongation -Bi accumulation -Genotoxicity	Passatore et al. (2022)
<i>Triticum aestivum</i> L. <i>Phaseolus vulgaris</i> L.	Bismuth nitrate	500 mg $\text{kg}^{-1}$	Soil	5 weeks	-Plant biomass -Pigment content -Photosynthesis -Nutrient uptake and transport -Oxidative stress -Antioxidant response -Bi accumulation	Mohammed et al. (2023)
<i>Lepidium sativum</i> L.	Bismuth nitrate	0, 30, 121, 485 mg/kg	Soil	21 days	-Plant biomass -Pigment content -Photosynthesis -Nutrient uptake and transport -Bi accumulation	Pietrini et al. (2023)

bismuth compounds), as a function of their concentrations, on seed germination and root development evaluated in filter paper toxicity assays in *Lactuca sativa*, *Trifolium pratense*, *Physalis ixocarpa* and *Lolium multiflorum*.

The toxicity of Bi to plant growth was also evaluated by germinating seeds of *Arabidopsis thaliana* (Nagata, 2015) and *Solanum lycopersicum* (Nagata and Kimoto, 2020) in an agar-solidified MS medium supplemented with several concentrations of Bi nitrate for 14 days. Both studies reported an increasing inhibition of seed germination with increasing Bi concentration in the medium, with *Arabidopsis* showing higher sensitivity. Root elongation was also found to be a sensitive endpoint for measuring Bi toxicity in these studies, as this parameter was negatively affected in both plant species at lower Bi concentrations compared to the fresh weights of roots and shoots. In this regard, the root split experiment on *Arabidopsis* by Nagata and Kimoto (2020)

showed increased viability in non-Bi-treated roots compared to Bi-treated roots, suggesting that Bi has local toxicity in the roots. An insight into the mechanisms by which Bi may exert its toxic action on *Arabidopsis* roots was provided by Nishimura and Nagata (2021), who demonstrated that the inhibition of root growth and lateral root development after Bi treatment was accompanied by an alteration in Fe homeostasis, resulting in Fe overaccumulation, which in turn enhanced cell death in the root tip.

Soil experiments confirmed the toxicity of Bi on plant growth and development. Omouri et al. (2019) assayed the toxicity of Bi nitrate and Bi citrate administered at various concentrations to perennial ryegrass plants grown for 7 days in natural sandy and artificial (OECD, 1984) soils. No effect of both Bi formulations on seed germination was observed in either soil types while Bi nitrate was more toxic than Bi citrate in terms of root elongation and dry mass, especially in natural

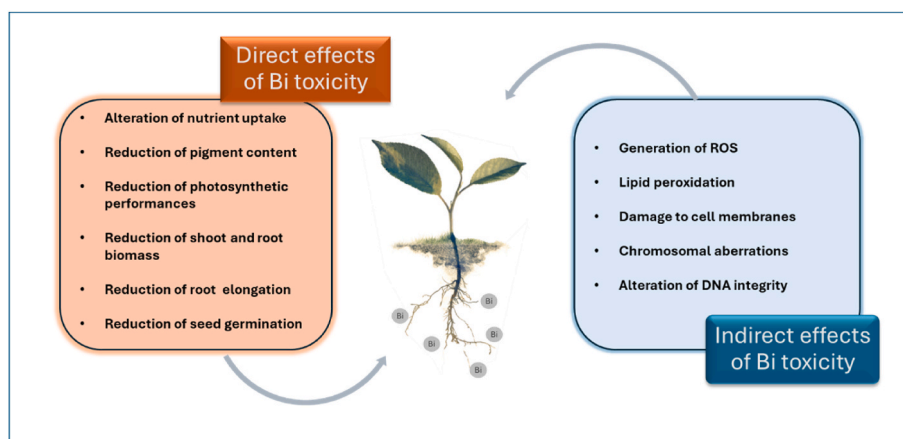


Fig. 2. Direct and indirect toxic effects observed in plants exposed to bismuth.

sandy soils.

In an experiment with three natural soil types sampled from the southern part of Russia (Haplic Chernozem Calcic, Haplic Arenosols Eutric and Haplic Cambisols Eutric, characterised by a heavy loam, sandy loam, and heavy loam, respectively, and different pH and humus content), spiked with 3, 30 and 300 mg kg<sup>-1</sup> Bi nitrate, [Sudina et al. \(2021\)](#) studied the response of radish seeds in terms of germination and seedling root length as indicators of phytotoxicity. The results provided evidence that the lower Bi concentration (3 mg kg<sup>-1</sup> Bi nitrate) stimulated seed germination in all three soil types, while higher Bi concentrations had negative effects on both germination and root elongation, especially at 300 mg kg<sup>-1</sup> Bi nitrate, with the extent of damage being related to the different soil types. In this regard, [Sudina and co-authors](#) discussed the effect of soil type on these results, highlighting that the sandy loam texture of the Haplic Arenosols Eutric soil and the acidic reaction of the Haplic Cambisols Eutric soil (pH = 5.8), as well as the low organic matter content of both soil types (1.8 and 2.3%, respectively), could favour metal mobility in the soil and therefore the high toxicity of Bi to seeds observed in these soil types. Instead, the adverse effects of Bi nitrate on plants not related to its concentration were reported by [Pietrini et al. \(2023\)](#) in a pot study with black peat substrate in a growth chamber, analysing the growth of *Lepidium sativum* plants for 21 days. In this study, a 20–26% reduction in shoot biomass was observed only at the lower Bi concentration tested (30 mg kg<sup>-1</sup>), which was not statistically different from the higher ones (121 and 485 mg kg<sup>-1</sup>), even though Bi accumulation in the shoots increased significantly in relation to the soil Bi concentration. This may indicate that above a threshold of Bi accumulation, plants can activate defence responses to counteract the stress action exerted by the metal (see sections 5.2 and 5.4). Growth inhibition in both the roots and shoots of wheat and bean plants (both as fresh and dry weight) was also reported by [Mohammed et al. \(2023\)](#) when soil in pots was supplied with 500 mg kg<sup>-1</sup> Bi nitrate for five weeks. The susceptibility to Bi differed between plant species, being higher in the roots of bean and in shoots of wheat (on a dry weight basis), although similar Bi accumulation was found in the organs of both plant species.

### 5.2. Effects on photosynthetic pigments and carbon assimilation

As has been extensively reported for many other metals ([Chandra and Kang, 2016](#); [Pietrini et al., 2015, 2020](#)), the interaction of Bi with plants has also been focused on studying the primary process of photosynthesis. In this regard, pigment (chlorophylls and carotenoids) content, chlorophyll fluorescence parameters, such as maximum quantum yield of PSII photochemistry (Fv/Fm), the quantum efficiency of PSII photochemistry (ΦPSII), and the quantum yield of regulated (ΦNPQ) and non-regulated (ΦNO) energy dissipation in PSII, the electron

transport rate through PSII (ETR) (A), the photochemical (qP) and non-photochemical quenching (NPQ), the photosynthesis rate, and gas exchange have been evaluated in the only two works in the literature devoted to studying the effect of Bi on the photosynthetic machinery of plants. A detailed report on this subject was presented by [Pietrini et al. \(2023\)](#), who analysed the leaves of *L. sativum* plants exposed to Bi for 21 days through soil in pots in a growth chamber using a leaf-clip portable fluorescence sensor, measurements of leaf reflectance spectra, and analysis of the chlorophyll fluorescence in both imaging and data modes. Overall, the study demonstrated that plants exposed to Bi underwent a toxicity state, as evidenced by the reduction of the total chlorophyll index, chlorophyll *a*, chlorophyll *b*, and carotenoid pigment-specific simple ratio (PSSR), obtained by leaf spectra measurements, regardless of the Bi concentration (30, 121 and 485 mg kg<sup>-1</sup>) in the soil. In contrast, Bi-treated plants showed dose-related toxicity for Fv/Fm, ΦPSII and ΦNPQ, while ΦNO had a slightly different trend, showing an impairment of this process only in plants exposed to the highest Bi concentrations. This feature was confirmed by a markedly heterogeneous pattern of light use and photosynthetic activity (ΦPSII, ΦNPQ and ΦNO) across the leaf lamina, as revealed by chlorophyll fluorescence imaging. Interestingly, the different responses observed in regulated (ΦNPQ) and non-regulated (ΦNO) energy dissipation in PSII have been discussed as a photoprotective mechanism activated in plants exposed to Bi to counteract an oxidative attack in the thylakoid membranes (see section 5.4.). As a proxy for photosynthesis, the electron transport rate (ETR) was measured and showed a progressive reduction with increasing Bi presence in the substrate, parallel to the response of ΦPSII in Bi-exposed plants. After evaluating the different extent of the negative impact of Bi on photosynthetic parameters, the authors pointed out that the response of ΦPSII, measured in light-adapted leaves, confirmed the higher sensitivity of this parameter with respect to Fv/Fm, as previously highlighted for other metals ([Pietrini et al., 2010](#)). Similarly, [Prabhavati et al. \(2017\)](#) demonstrated that Bi treatment significantly affected the photosynthetic performance of horse gram (*Macrotyloma uniflorum* (Lam.) Verdc) plants exposed to 50, 100, 200, 300 and 400 mg kg<sup>-1</sup>. Specifically, the values of net photosynthesis, transpiration rate and stomatal conductance showed a decreasing trend with increasing Bi concentration, while the value of intercellular CO<sub>2</sub> concentration showed the opposite trend. An impairment of the photosynthetic processes exerted by Bi was also reported by [Mohammed et al. \(2023\)](#) in wheat and bean plants exposed to 500 mg kg<sup>-1</sup> Bi nitrate in the soil. In this study, the leaves of Bi-treated plants showed a reduction in photosynthetic rate and gas exchange as well as a decrease in Fv/Fm (only in bean plants). Additionally, pigment content exhibited different responses in the two plant species after Bi exposure. In fact, although both plant species showed a significant reduction in chlorophyll *b*, only the leaves of the Bi-treated bean plants exhibited a decrease in

chlorophyll *a* content. Unlike the findings of Pietrini et al. (2023), the addition of Bi to the soil resulted in an increase in carotenoid content in both plant species. The authors discussed how this increase could be related to a defence response against oxidative damage that could possibly occur in the photosynthetic apparatus (see section 5.4.). Root mycorrhizal colonisation was observed to partially mitigate the negative effects of Bi on the photosynthetic parameters, to varying degrees depending on the plant species and parameter analysed. Mohammed et al. (2023) pointed out the positive effects of mycorrhizal colonisation in reducing the Bi availability to plants (resulting in lower Bi accumulation in plants, see section 4) and in restoring, at least partially, the nutrient status of the plants (see section 5.3).

Overall, the limited information available in the literature on the effect of Bi on the primary process of photosynthesis indicates that the exposure of plants to Bi can significantly affect photosynthetic machinery, depending on the Bi concentration and plant species, with the photosynthetic parameters expressing different sensitivities to Bi accumulation in plants.

### 5.3. Effects on the uptake and accumulation of mineral elements

The disturbance of plant nutrition processes is reported as one of the main toxic effects in plants due to the excess presence of metals in the substrate (Fig. 1). This negative action can be realised by the direct competition of heavy metals with macro- or micronutrients for the uptake and transport at root cell levels or indirectly exerted by inhibiting root enzymes involved in nutrient metabolism, including Fe(III) reductase, nitrate reductase, nitrite reductase, glutamine synthetase, and glutamate synthetase (Dal Corso, 2012). As for other metals, among the plant processes affected by the presence of Bi in the growth substrate, the uptake, accumulation and transport of macro- and micronutrients have also been addressed in the limited literature available. In this regard, an ionic study by Pietrini et al. (2023) was carried out on shoots and roots of *L. sativum* plants treated with different concentrations of Bi nitrate in soil in pots, analysing the concentration of 12 elements, including macro- (K, P, Ca, Mg) and micronutrients (Fe, Ni, Cu, Zn, Mn) and other non-essential elements (Na, Co, Li). A different response was observed between plant organs. In roots, the macronutrient concentration was not affected by plant exposure to Bi, whereas micronutrient (Ni, Cu, Zn, Mn) accumulation was significantly reduced in Bi-treated plants, especially at the highest Bi concentration tested ( $485 \text{ mg kg}^{-1}$ ). The iron concentration in the roots was not altered by the presence of Bi in the substrate, in contrast to that observed by Nishimura and Nagata (2021), who indicated an impairment of Fe homeostasis, leading to Fe over-accumulation. This issue was discussed by Pietrini et al. (2023) as likely due to both a plant species specific response and the different experimental conditions, in particular the growth medium (soil vs. agar solidified growth medium) and the Bi concentration. Regarding non-essential elements, except Na, the supply of Bi to the soil caused a reduction in the accumulation of Co and Li in the roots of garden cress. In the shoots of Bi-treated plants, Pietrini and co-authors observed an overall reduction in macro- and micronutrients and non-essential elements (except Na), in most cases in relation to Bi concentrations in the substrate. This feature was discussed by highlighting a prominent effect of Bi on micronutrient uptake and translocation, in line with Nagata (2015), who reported that treatment of *Arabidopsis thaliana* seedlings with Bi resulted in a change in the expression levels of genes involved in the uptake of several ions, including Fe, Cu and Zn. In particular, the expression of 13 metal homeostasis genes was increased 2-fold in Bi-treated plants, and the relative transcription level of the primary  $\text{Fe}^{2+}$  uptake transporter (AtIRT1) in the root was increased 5-fold compared to the control. The induction of AtIRT1 expression in roots by Bi provoked a disturbance in Fe homeostasis, resulting in an increase in root Fe accumulation, which was linked by Nishimura and Nagata (2021) to the toxicity of Bi on *A. thaliana* root tips. An alteration in macro- and micronutrient accumulation following the treatment of plants with Bi

nitrate was also demonstrated by Mohammed et al. (2023) in wheat and bean plants. Indeed, a significant reduction in K, Ca, Mg, P and Mn concentrations was found in both the roots and shoots of Bi-treated plants. In the study, the authors also highlighted how root mycorrhization prevented the reduction in element accumulation compared to control plants, in parallel with the reduction in Bi uptake and translocation in Bi-treated plants. In contrast to Pietrini et al. (2023), Mohammed et al. (2023) observed an increase in Zn concentration after Bi treatment only in bean shoots and roots, highlighting a plant-specific response in the alteration of Zn uptake and accumulation possibly exerted by Bi.

To summarise the limited information available on this subject, it is likely that Bi plays a role in altering metal homeostasis in plants. However, the evidence is still fragmentary and further investigation is needed to gain insight into the processes of mineral uptake, accumulation and transport following Bi treatment in different plant species.

### 5.4. Bismuth-induced oxidative damage and antioxidant system

The increase in ROS in plant cells, both directly through the Fenton/Haber-Weiss reaction or indirectly through a lower quenching activity of ROS (see Fig. 1), is a fundamental aspect of the toxicity mechanisms occurring in plants exposed to excess metals. To counteract the oxidative burst induced by metals, plants have evolved a complex antioxidant system characterised by enzymatic and non-enzymatic molecules with antioxidant activity (Pandhair and Sekhon, 2006).

As previously reported, it has been observed that Bi accumulation in plants interferes with various mechanisms and processes, affecting their primary metabolism and ultimately hindering their growth and development. The mechanism(s) by which Bi exerts its toxic effect is still not fully understood, in view of the limited research efforts made thus far. Among them, Mohammed et al. (2023) reported that treatment of plants with Bi provoked an increase in the levels of  $\text{H}_2\text{O}_2$  and malondialdehyde (MDA) in both shoots and roots of wheat and bean plants, highlighting the onset of oxidative stress conditions. In this study, root mycorrhization reduced Bi accumulation in plants, leading to lower levels of the above-mentioned oxidative stress markers, especially in plant roots. This finding suggests that Bi may play a direct or indirect role in generating an oxidative burst in plants. Indeed, apart from the direct effect of Bi, which may alter membrane lipid peroxidation (increasing MDA levels), Bi may also exert an indirect effect by disrupting the homeostasis of redox-active metals, increasing their levels, as observed by Nagata (2015) and Nishimura and Nagata (2021) for iron, and possibly inducing the overproduction of ROS. As a response to counteracting the toxic effects of Bi in wheat and bean plants, Mohammed et al. (2023) observed an increase in the levels of the detoxification-related enzyme glutathione-S-transferase in both roots and shoots. Conversely, other detoxifying molecules, such as metallothioneins and phytochelatin only increased in bean plants. An induction of the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR), was detected in the roots and shoots of wheat and bean Bi-treated plants, to different extents in relation to both organs and plant species. A less clear response to the oxidative burst caused by Bi accumulation in plants was observed for the non-enzymatic antioxidant molecules in both shoots and roots. In fact, while reduced glutathione (GSH) decreased in both plant species, irrespective of the organ sampled, the levels of reduced ascorbate (ASC), polyphenols, tocopherol and flavonoids varied depending on the plant species and organ analysed. Overall, Mohammed et al. (2023) highlighted the role of Bi in affecting the oxidative status and antioxidant processes in plants, suggesting a species-specific response to counteract the oxidative burst induced by Bi exposure. In garden cress plants exposed to different Bi nitrate concentrations, Pietrini et al. (2023) observed that damage at the growth level was accompanied by a



reduction in the carotenoid content, which, in addition to their role as photosynthetic pigments, are also involved in the antioxidant response in plants, namely by scavenging the oxygen free radicals in chloroplasts and protecting chlorophylls from photooxidative damage (Guidi et al., 2017). In contrast, Bi-treated plants showed a slight increase in the level of flavonoids, whose involvement in the defence against oxidative stress has been well reported (Ferdinando et al., 2012). It was suggested that the Bi-induced damage observed in garden cress plants was possibly caused by an oxidative attack, as also observed by Huang et al. (2022) in the microalga *Chlamydomonas reinhardtii* exposed to nanoscale Bi, which was not sufficiently compensated by an antioxidative response to protect the photosynthetic machinery.

The limited information available suggests that Bi, similar to other metals, may exert its toxic effect directly or indirectly by altering the oxidative status of the plant cell. However, further research is needed to clarify the mechanisms involved in the toxic effect of Bi, both through direct action on cell and thylakoid membranes and through interference with other processes, ultimately leading to an increase in ROS in plants. Although some evidence has been reported, the antioxidant processes activated by plants to counteract the toxic effects of Bi remain to be elucidated.

### 5.5. Effects on nucleic acids and chromosomes

Heavy metals are commonly reported to cause damage to nucleic acids (Fig. 1) as part of their toxic effects in plants (Angulo-Bejarano et al., 2021). In this context, Moura et al. (2012) reviewed the evidence indicating that the oxidative burst produced by increasing ROS formation after metal impairment of the electron transport chains can be extremely deleterious for the integrity of nucleic acids. In fact, ROS attack of DNA may result in altered bases and damaged sugar residues, which can lead to strand breaks. Specifically, the addition of hydroxyl radicals to double bonds and the abstraction of hydrogen from deoxyribose are currently reported as the main damage mechanisms (Roldán-Arjona and Ariza, 2009). This toxic mechanism represents one of the primary causes of DNA decay, ultimately interfering with plant development and affecting crop productivity.

In comparison to other metals, the information available on the interaction of Bi with plant DNA is still fragmentary and needs to be substantiated. In this regard, the first study to evaluate the effects of Bi compounds on nucleic acids and chromosomes in plants was conducted by Liman (2013), who analysed the mitotic index and mitotic phase of the root meristematic cells of *Allium cepa* exposed to different of bismuth (III) oxide concentrations, both in bulk and nanoparticle (BONP) form, for 4 h. The results provided evidence that both forms of bismuth (III) oxide significantly increased the mitotic index, and chromosomal aberrations, such as chromosome laggards, stickiness, and disturbed anaphase-telophase and anaphase bridges in anaphase-telophase cells. Furthermore, exposure of *A. cepa* root meristems to BONPs induced a dose-dependent increase in the level of DNA damage assessed by the alkaline comet assay, possibly due to the release of free radicals by BONPs, as has been demonstrated for other types of nanoparticles (Flower et al., 2012). Indeed, the genotoxicity of Bi oxide, both in bulk and in nanoparticle form, on this plant species was underlined. In a similar experiment on *Lepidium sativum* seedlings treated for 72 h with various Bi nitrate concentrations, Passatore et al. (2022) reported severe genotoxic effects caused by Bi at each concentration tested. Interestingly, the extent of DNA damage assessed by the alkaline comet assay was higher at the lowest Bi concentrations and decreased in plants exposed to 242 and 485 mg L<sup>-1</sup> Bi nitrate. The authors then hypothesised recovery from the damaging effects of Bi at the highest concentrations by activating DNA repair pathways (Nisa et al., 2019).

Bismuth has also been studied by Nagata (2015) to evaluate gene expression by performing microarray analysis in Bi-treated *Arabidopsis thaliana* plants. In terms of molecular function genes, 394 up-regulated and 748 down-regulated genes were analysed, while in terms of

protein class, 493 up-regulated and 952 down-regulated genes were observed. Among the up-regulated genes, more than 40% could be attributed to genes encoding proteins with catalytic activity, while less than 10% referred to genes encoding proteins with transporter activity. The expression level of metal homeostasis genes was also analysed, which showed that Bi induced a marked increase in the genes encoding the Fe transporter IRT1 and zinc transporters 7 and 8 in *A. thaliana* roots. In contrast, the genes encoding the Nramp family, the copper transport family, cation/proton exchangers and metallothionein were not over-expressed in Bi-treated plants, and the expression level of the genes encoding the Fe storage proteins ferritin 1 and 4 even decreased. Considering the Bi accumulation and the induction of Fe transporter gene expression in Bi-treated *A. thaliana* plants, it has been suggested that Bi can be absorbed and transported by the Fe transport mechanism, possibly leading to Fe deficiency. In this regard, Nagata (2015) hypothesised the occurrence of an interplay between Fe transporter IRT1 and the Fe storage protein ferritin to avoid Fe deficiency in Bi-treated plants.

## 6. Mechanisms of Bi tolerance in plants

The protective responses of plants to the damaging actions of metals have been extensively reviewed (Dalvi and Bhalerao, 2013; Moustakas, 2023). The mechanisms activated by plants to counteract the excess presence of metals in their cells have been studied at the physiological, biochemical and molecular levels. Metal avoidance, which is achieved through the immobilisation of metals by mycorrhizal associations and the release of root exudates, is described as a process that reduces the metal uptake in plants. Once absorbed, the toxicity action of metals can be counteracted by activating different processes as a second line of defence. These include metal binding to the cell wall, active pumping at the membrane level to increase metal efflux, binding to organic acids, and inactivation by forming complexes with cysteine-rich molecules, such as phytochelatins and metallothioneins. As previously discussed in section 5.4, tolerance mechanisms to the oxidative stress onset caused by metals include the antioxidant response of cells, both by enzymatic and non-enzymatic molecules. In this regard, the role of hormones in the adaptation to metal stress in plants and the synthesis of stress-related proteins have also been highlighted (Dalvi and Bhalerao, 2013).

The protective mechanisms outlined above are based on findings obtained for the majority of metals that have been studied, including Fe, Cu, Ni, Mn, As and Cd. However, there is a paucity of evidence regarding the activation of these mechanisms for minor metals, such as Bi. The available literature contains only fragmentary information on the matter, graphically summarised in Fig. 3. In a pot study with *L. sativum* grown in Bi-enriched soil, Pietrini et al. (2023) observed that, beyond a threshold of Bi accumulation, the toxic effects on growth due to Bi exposure did not increase. This was interpreted as evidence that metal detoxification mechanisms are activated at a certain Bi concentration in plants. In the same study, the authors proposed that photoprotective mechanisms in thylakoid membranes may protect against oxidative burst potentially generated by Bi. This was discussed in the context of the different responses observed in regulated ( $\Phi$ NPQ) and non-regulated ( $\Phi$ NO) energy dissipation at the PSII level. In bean and wheat plants grown in soil supplied with 500 mg kg<sup>-1</sup> Bi nitrate, Mohammed et al. (2023) attributed a protective response against oxidative attack to photosystems to an increase in the content of carotenoids, a well-known antioxidant molecule that protects photosynthetic machinery from excess light, which generates ROS (Guidi et al., 2017). Furthermore, an increase in flavonoid levels was observed by Pietrini et al. (2023) in plantlets of *L. sativum* exposed to Bi nitrate in soil. This increase was discussed as a defence response to the oxidative pressure exerted by the accumulation of the metal in the leaves, with the involvement of flavonoids in the antioxidant response in plants being widely reported (Ferdinando et al., 2012). The antioxidant response at the enzymatic level in Bi-treated plants was described by Mohammed et al. (2023) as

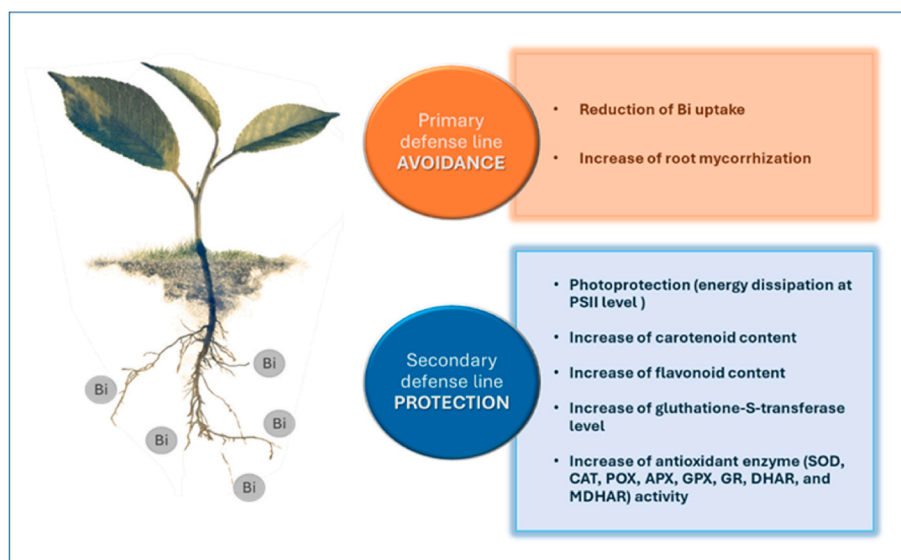


Fig. 3. Mechanisms involved in the primary and secondary lines of defence activated by plants to counteract the excessive presence of bismuth.

an increase in the content of glutathione-S-transferase, in addition to the induction of the activity of the most common antioxidant enzymes, including SOD, CAT, POX, APX, GPX, GR, DHAR and MDHAR. Such a response was observed in roots and shoots of both bean and wheat plants, while a more species- and organ-specific response was reported for non-enzymatic molecules, such as GSH, ASC, polyphenols, tocopherol and flavonoids. A tolerance mechanism to recover from the toxic effects of Bi on nucleic acids, likely involving DNA repair pathways (Nisa et al., 2019), was proposed by Passatore et al. (2022) to explain the decrease in DNA damage occurring in *L. sativum* plants beyond the threshold of Bi accumulation in the tissues. As previously observed for most metals, the reduction in metal uptake represents a primary line of defence against the toxicity status induced by metals in plants. In this regard, the role of mycorrhization in restricting metal uptake in plants has been reported (Dalvi and Bhalerao, 2013). In accordance with this, in plants exposed to Bi, Mohammed et al. (2023) observed that mycorrhizal colonisation was able to mitigate, at least in part, the toxic effects induced by the metal on the photosynthetic apparatus. This positive action was put in relation by the authors to the possible reduction of Bi availability to plants, as lower metal accumulation was found in plants when higher root colonisation occurred. Although some evidence has been presented on the protective mechanisms activated by plants in response to excess Bi, further research is required to elucidate the regulatory processes underlying the activation of specific defence pathways involving various biochemical processes that can counteract the toxic impact of excess Bi on plants.

## 7. Conclusion

Despite the increasing interest in the industrial use of Bi and its compounds and the predicted increase in their occurrence in the environment, the interaction of this metal with the biota is still poorly understood. In particular, there is a lack of literature on the toxicity of Bi in plants, the processes by which it is exerted, and the mechanisms activated by plants to cope with its excess presence. In fact, although Bi is considered a “green metal” and has been proposed as a replacement for the more toxic Pb in many industrial processes, impairment of growth and physiological and biochemical processes in plants have been reported. Seed germination and root elongation have been highlighted as suitable endpoints to assess Bi toxicity on plant growth and development. Furthermore, the photosynthetic machinery has been shown to be clearly affected by Bi exposure, with some parameters, such as pigment content and the quantum efficiency of PSII photochemistry ( $\Phi$ PSII),

being more consistent with the level of toxicity assessed in Bi-treated plants. Bismuth has been shown to alter the macro- and micronutrient uptake and translocation in plants, with a particular effect on metal homeostasis. In this regard, the disturbance of Fe accumulation has been demonstrated as part of the toxicity mechanism in *A. thaliana* roots. Bismuth toxicity has been attributed to an oxidative stress condition, directly or indirectly induced, and an antioxidant defence response involving the main antioxidant enzymes and detoxifying molecules in plants has been reported. In addition, the genotoxic effects of Bi in plants, as evidenced by nucleic acid degradation and chromosomal aberrations, have demonstrated that Bi exposure at levels commonly found in mining and smelting areas and contaminated sites can disrupt the plant genome.

As highlighted in this review, compared to the large amount of information available in the literature on the interaction between heavy metals and plants, less research effort has been devoted to minor metals thus far. In particular, knowledge of the consequences of the predicted increase in the presence of Bi in the environment on plants deserves more attention. Therefore, future research should focus in filling the gap on the mechanisms by which Bi exerts its toxic effect in plants. In particular, the molecular mechanisms involved in Bi uptake, transport and accumulation and in the induction of oxidative stress and antioxidant responses should be targeted. Bismuth toxicity to the photosynthetic process should be clarified by investigating whether the impairment of this process is due to the direct action of Bi on some key molecules of the photosystems (i.e. pigments, cytochromes, redox-active molecules) or an indirect influence through the alteration of related mechanisms, such as the uptake of essential nutrients for pigment biosynthesis and efficiency of the electron chains. In this respect, interference with carbohydrate metabolism can lead to changes in a wide range of physiological processes in plants, the most obvious of which is growth. The advancement of knowledge regarding the impact of Bi on plants will pave the way for a better assessment of the impact on the ecosystem health of potential increases in the levels of this metal in the environment.

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### CRedit authorship contribution statement

**Massimo Zacchini:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Conceptualization.

### 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.

### Data availability

No data was used for the research described in the article.

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