Role of nitric oxide in plant responses to heavy metal stress: exogenous application vs. endogenous production

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Highlights: In response to heavy metal stress exogenous NO prevents oxidative damage alleviating plant fitness-loss while endogenous NO should be fine-tune regulated and NO-dependent signalling pathways are involved in plant resistance.

Abstract

Anthropogenic activities, such as industrial processes, mining and agriculture, lead to an increase in heavy metal concentrations in soil, water and air. Given their stability in the environment, heavy metals are difficult to eliminate and can even constitute a human health risk by entering the food chain through uptake by crop plants. An excess of heavy metals is toxic for plants, which have different mechanisms to prevent their accumulation. However, once metals enter the plant, oxidative damage sometimes occurs, which can lead to plant death. Initial nitric oxide (NO) production, which may play a role in plant perception, signalling and stress acclimation, has been shown to protect against heavy metals. Very little is known about NO-dependent mechanisms downstream from signalling pathways in plant responses to heavy metal stress. In this review, using bioinformatic techniques, we analyse studies of the involvement of NO in responses to heavy metal stress, its possible role as a cyto-protective molecule and its relationship with reactive oxygen species (ROS). Some conclusions are drawn and future research perspectives are outlined in order to further elucidate the signalling mechanisms underlying the role of NO in plant responses to heavy metal stress.

Key words: arsenic; heavy metals; cadmium; nitric oxide; reactive oxygen species; reactive nitrogen species; signalling

1. Introduction

Heavy metals are metallic elements with relatively high density compared to water (Tchounwou *et al.*, 2012) and, as recommended by Appenroth (2010), can be defined on the basis of the periodic table of the following elements: 1) transition elements; 2) rare earth elements, (lanthanides and actinides); and 3) lead group, which is a heterogeneous group which includes elements that form amphoteric oxides (Al, Ga, In, Tl, Sn, Pb, Sb and Po), the metal Bi and the metalloids Ge, As and Te. Some of these metals are non-essential and may be highly toxic even at low concentrations, such as cadmium (Cd), mercury (Hg), lead (Pb) and arsenic (As; Emsley, 2011; Mustafa and Komatsu, 2016). Many other elements, which have different functions in metabolisms can be toxic when concentrations in plants exceed requirements, are essential for life (Viehweger, 2014; Andresen *et al.*, 2018). Approximately 70 metallic chemical elements are classified as heavy metals, whose concentrations in the earth's crust range from less than 0.1% to less than 0.01% (Appenroth, 2010; Tchounwou *et al.*, 2012; Hurdebise *et al.*, 2015); nevertheless, some of these metals are among the most dangerous pollutants according to the United States Environmental Protection Agency (Chen *et al.*, 2006).

Naturally accumulated metals are insignificant compared to those caused by anthropogenic activity. Agricultural activities (irrigation, limestone amendments, as well as inorganic fertilizers, pesticides and sewage sludge), electricity generated from coal and oil, industrial activities (iron and steel smelting and chemical products), mining (Jaishankar *et al.*, 2014) and household waste are the main causes of heavy metal contamination. Their accumulation leads to a decrease in soil quality and contaminates plants, giving rise to vegetal cover loss and erosion and to the transport of pollutants to subterranean and superficial water and to the trophic food chain (Clemens and Ma, 2016). Plant roots also upload heavy metals, which are translocated to other organs and consequently enter the food chain (Shahid *et al.*, 2016). A deeper understanding of plant responses to these plant-toxic heavy metals should contribute to the development of more heavy metal-tolerant plants with phytoremediation properties (Clemens and Ma, 2016; Sanz-Fernández *et al.*, 2017).

Nitric oxide (NO), which is a gaseous free radical capable of diffusing through membranes, has, over the last twenty years, been found to be involved in regulating numerous physiological and patho-physiological processes in plants including responses to heavy metals (He *et al.*, 2014; Domingos *et al.*, 2015; Sahay and Gupta, 2017). In this review, we discuss these plant responses, with a particular emphasis on the entry and translocation of metals, as

well as the sources and role of NO. The dual function of NO, when exogenously supplied and endogenously produced, will also be discussed. In addition, we have carried out a bioinformatic analysis of several articles published in the last ten years to draw certain conclusions and to highlight future research perspectives to better understand the role played by nitric oxide in plant responses to heavy metals.

2. Entry of heavy metals into plants and their toxicity

Plants use specific transporters to take up nutrient metals, which are used by metals with no known function (Clemens *et al.*, 2013). Thus, it has been suggested, for example, that Cd enters plant cells via cation transporters of minerals such as Fe, Ca and Zn (Thomine *et al.*, 2000; Aravind and Prasad, 2005). High affinity-Ca channels in tobacco have also been shown to interact with Pb and Ni (Maestri and Marmiroli, 2012). The transporter low-affinity cation transporter (LCT1) has the ability to regulate not only the transport of Ca but also of Cd in *Triticum aestivum* (Perfus-Barbeoch *et al.*, 2002; Antosiewicz and Hennig, 2004). In addition, Zn transporter family members, (Zn regulated transporter/iron regulated transporter, ZIP, ZRT/IRT-related protein) are involved in the entry of Fe^{2+} and Zn^{2+} into plants (Fox and Guerinot, 1998), being ZIP2 and ZIP4 Cu-specific (Guerinot, 2000; Wintz *et al.*, 2003). Furthermore, ZRT1, ZRT2, ZRT3 and ZRT4, which have been identified in *Saccharomyces*, *Thlaspi* and *Arabidopsis* plants, are mainly involved in transporting Zn (Zhao and Eide, 1996; Grotz *et al.*, 1998; Pence *et al.*, 2000) and are also able to transport other metals such as Fe (Eide, 1996). These transporters are also involved in the entry of Zn into *Saccharomyces cerevisiae* vacuoles where they are immobilised (MacDiarmid *et al.*, 2000). Members of the copper cation transporter (COPT) and Yellow stripe-like (YSL) transporter families also transport Cu/nicotianamine conjugates (Curie *et al.*, 2009). Although IRT1 is the main entry vehicle for Fe^{2+} in *Arabidopsis* roots (Guerinot, 2000; Vert *et al.*, 2002), it can also transport other metals such as Cu, Mn, Zn and Cd (Eide *et al.*, 1996; Korshunova *et al.*, 1999; Komal *et al.*, 2015). In addition, nodulin-26-like intrinsic protein (NIP) aquaporins are involved in AsIII absorption and translocation (Bienert *et al.*, 2008; Xu *et al.*, 2015; Chen *et al.*, 2016; Farooq *et al.*, 2016; Souri *et al.*, 2017), while high-affinity Pi transport systems have the capacity to absorb AsV (Finnegan and Chen, 2012; Souri *et al.*, 2017). The transcription factor WRKY6 has also been observed to regulate the arsenate-induced expression of phosphate transporter PHT1 (Catarcha *et al.*, 2007; Castrillo *et al.*, 2013; Sánchez-Bermejo *et al.*, 2014).

After entering root cells, metals are immobilized in the vacuole or translocated to the upper side of the xylem through the apoplast and/or symplastic complexes. Most of the transporters involved in Mn translocation are broadly specific to divalent cations such as Fe, Zn, Cu, Cd, Ca, Co and Ni. These transporters include natural resistance associated macrophage protein (NRAMP), YSL, ZIP, cation exchanger (CAX), cationic diffusion facilitator/metal tolerance protein (CDF/MTP), P-type ATPase and vacuolar iron transporters (VITs; Socha and Guerinot, 2014). While VIT1 is involved in transporting Fe, Cd and Mn into the vacuole, NRAMP3 and NRAMP4 are mainly involved in their removal (Thomine *et al.*, 2000; Lanquar *et al.*, 2005). On the other hand, the P-type ATPase superfamily plays a role in the transport of a wide range of cations across cell membranes (Axelsen and Palmgren, 2001; Mills *et al.*, 2012). ATP-binding cassette (ABC) transporters are a superfamily of transmembrane proteins involved in a wide variety of transport functions (Kang *et al.*, 2011; Theodoulou and Kerr, 2015). In plants, 13 subfamilies of this superfamily have been identified, including multidrug resistance-associated protein, peroxisomal membrane protein, pleiotropic drug resistance, and multiple drug resistance (MRP, PMP, PDR and MDR) (Kang *et al.*, 2011).

Once accumulated in plant cells, it has been suggested that heavy metal toxicity is manifested in four main ways: a) similarity to nutrient cations, resulting in competition for absorption at the root; b) direct interaction with the sulfhydryl protein group (-SH), which disrupts their structure and function; c) displacement of essential cations from specific binding sites which inhibits protein function; and d) generation of reactive oxygen species (ROS), which damage macromolecules (Luo *et al.*, 2016; Singh *et al.*, 2016). Thus, the functions of non-essential metals may replace those of essential metals due to their affinity for different ligands, which leads to protein function loss (Sharma and Dubey, 2005; Schiavon *et al.*, 2012). Therefore, while Mn, Zn, Fe, Ni, Cd, Pb and Cu have a preference for S- or N-bond ligands (SH, -SS-, -NH₂, =NH, etc.), K, Ca, Na, Mg, Al, and Cs favour O-donor ligands, all of which bind through oxygen (COOH, -H₂PO₄, -OH, -CHO, among others; Gupta and Sandalio, 2012). All these mechanisms lead, in most cases, to a macro- and micro-nutrient imbalance, inactivation of enzymes involved in the Calvin cycle, carbohydrate and phosphorus metabolism and CO₂ fixation; this lead to an inhibition of seed germination, plant growth and yields and sometimes to genotoxicity and plant death (Kalaivanan and Ganeshamurthy, 2016; Mustafa and Komatsu, 2016; Ayangbenro and Babalola, 2017; Tiwari and Lata, 2018).

One of the principal effects of heavy metal toxicity in plants is oxidative stress due to an excess of ROS and changes in the antioxidant system (Cuypers *et al.*, 2016; Romero-Puertas *et al.*, 2018). ROS is produced by Fenton and Haber-Weiss reactions, in which $O_2^{\cdot-}$ and H_2O_2 require the presence of metals, such as Cu and Fe, whose redox state can change, resulting in the production of $HO^{\cdot}$, one of the most powerful known oxidants (Halliwell and Gutteridge, 2015). However, although certain metals such as Cd and Pb, which do not undergo oxide reduction, are not directly involved in ROS generation, they can act as pro-oxidants and decrease antioxidant availability (Rodríguez-Serrano *et al.*, 2009; Singh and Shah, 2015; Gupta *et al.*, 2017; Loix *et al.*, 2017). These metals may also boost ROS generation by diverting electrons from electronic transport mainly in mitochondria and chloroplast (Heyno *et al.*, 2008; Keunen *et al.*, 2011; Sourì *et al.*, 2017).

3. Mechanisms enabling plants to cope with heavy metal toxicity

Plants respond to heavy metal toxicity through a variety of mechanisms. To prevent metals from entering cells, as a first line of defence, plants alter the permeability of the plasma membrane to restrict the transport of metal ions to the apoplast (Manara, 2012) and induce metal ion transporters involved in the exit flux such as NRAMPs, ATPases, ZIPs and CDFs (Clemens *et al.*, 2013). The generation of cellular exudates, resulting in an external coating around the root, also favours the formation of complexes with heavy metals including Cd, Cu and Pb (Clemens, 2006; Shah *et al.*, 2010).

Plants can modulate the absorption of specific metals by using, for example, citrate and histidine to prevent Ni uptake (Mustafa and Komatsu, 2016). In addition, they can bind metals to the cell wall through electrostatic interactions to keep them within the cellulose and lignin structures (Van Belleghem *et al.*, 2007; Memon and Schröder, 2009; Loix *et al.*, 2017). Recently, the *Arabidopsis* mutant MRC-32 has been shown to arrest development in the Cd-affected zone and to induce a number of lateral roots located in the Cd-free zone (Watanabe *et al.*, 2010). Interestingly, mycorrhized plants are more resistant to heavy metals, as fungi can retain metals in roots and thus prevent their translocation to aerial parts, although microbial antioxidant defences may also be responsible for their higher tolerance to heavy metals (Göhre and Paszkowski, 2006; Azcón *et al.*, 2010; Ferrol *et al.*, 2016). Furthermore, plant-microorganism interactions with bacterial strains and mycorrhizal fungi present in the rhizosphere of the hyperaccumulator plant *A. halleri* are able to reduce Cd concentrations in shoots (Farinati *et al.*, 2009).

When these options are exhausted, metals already in the cell are countered through a range of storage and detoxification strategies, including immobilization, synthesis of specific heavy metal transporters, chelation, trafficking and heavy metal sequestration by particular ligands. Therefore, the best characterized plant response to Cd and, to a lesser extent, to Ag, As, and Hg, is the synthesis of phytochelatins (PCs), a family of Cys-rich peptides produced for purposes of heavy metal chelation and sequestration in the vacuole (Cobbett and Meagher, 2002; Mendoza-Cózatl *et al.*, 2010; Mustafa and Komatsu, 2016). Metallothioneins, which are ubiquitous Cys-rich proteins with unique structural characteristics that facilitate metal binding, play an essential role as Zn donors for several essential metalloproteins comprising matrix metalloproteinases and zinc fingers (Ryvolova *et al.*, 2011). On the other hand, amino acids and organic acids can react with metal ions through S, N and O atoms, thereby enabling oxalate and malate to transport metals through the xylem and to keep them in the vacuole (Kumar *et al.*, 2015). Biological transformations can also reduce heavy metal damage by converting toxic compounds into more polar components through the addition of polar groups such as amino, hydroxyl, and carboxyl groups which are easily removed (Yin *et al.*, 2019). When all the strategies described above fail, plants activate defence responses consisting of heat shock proteins, proline, polyamines, antioxidant systems, signalling molecules and hormones (Kalaivanan and Ganeshamurthy, 2016; Romero-Puertas *et al.*, 2018). MicroRNAs (miRNAs), which regulate different transcription factors (TFs), as well as stress response-related genes at the post-transcriptional level have also recently been reported to be involved in heavy metal plant tolerance mechanisms.

4. NO metabolism in plant responses to heavy metals

The highly reactive molecule nitric oxide (NO) is a gaseous free radical, which is involved in virtually all plant physiological and patho-physiological processes such as development, senescence and responses to environmental cues (Sanz *et al.*, 2015; Astier *et al.*, 2017; Umbreen *et al.*, 2018; Costa-Broseta *et al.*, 2018). However, more research into the complex NO metabolism in plants is required, especially in areas such as the sources of NO, as well as the proteins/molecules that regulate NO in the cell (Baudouin and Hancock, 2013).

Thus, unlike animals, in which nitric oxide production is mainly dependent on the nitric oxide synthase (NOS) family of enzymes (Forstermann and Sessa, 2012), up to eleven different mechanisms are capable of producing nitric oxide in plants (Mur *et al.*, 2013; Astier *et al.*, 2017). Two main pathways are broadly involved in all these mechanisms: a reductive nitrate-dependent pathway and an oxidative arginine-dependent and hydroxylamine-

dependent pathway (Fröhlich and Durner, 2011; Gupta *et al.*, 2011; Mur *et al.*, 2013). In the reductive pathway, nitrate reductase (NR), with its nitrite:NO reductase activity, is able to produce NO in plants, although specific environments, such as anoxic and acidic conditions, are necessary for this reaction to take place (Rockel, 2002; Meyer *et al.*, 2005; Chamizo-Ampudia *et al.*, 2017; Astier *et al.*, 2017). Non-enzymatic reduction of nitrite, xanthine oxidoreductase, mitochondrial nitrite reduction via cytochrome c oxidase/reductase and Nitrite:NO reductase in root apoplasts are also involved in reductive pathways (Godber *et al.*, 2000; Stöhr *et al.*, 2001; Stoimenova *et al.*, 2007; Wang *et al.*, 2010). More recently, nitric oxide-forming nitrite reductase (NOFNiR) in *Chlamydomonas reinhardtii* algae has been reported to reduce nitrite to NO via NR-dependent electrons (Chamizo-Ampudia *et al.*, 2017). Apart from reductive NO-production pathways, several studies have demonstrated the existence of an oxidative route, similar to that in animals, although no homologous NOS sequences have been found in approximately 1000 transcriptomes in the land plants analysed, with similar sequences only found in green algae (Jeandroz *et al.*, 2016). However, biochemical and molecular data show that a link exists between arginine and NO in plants (Astier *et al.*, 2017).

We sourced studies of NO and heavy metals in plants and carried out a deep web search of the relevant literature with the aid of databases (<https://www.ncbi.nlm.nih.gov/pubmed/>; <https://www.scopus.com/search/>; <https://apps.webofknowledge.com/UA>). The search was narrowed down to the last ten years. The articles retrieved were divided into two groups: one dealing with the analysis of endogenous NO production (produced by the plant) as a response to applications of metal (Suppl. Table S1) and the other devoted to supply of exogenous NO before and/or during metal exposure (Suppl. Table S2). We data-mined both groups of papers and then created a database of the plant fitness, oxidative stress damage and general plant state parameters measured in each article. After eliminating studies with less than four variables measured, the databases were curated. 14 of the 68 papers in the endogenous NO group (Suppl. Table S1), and 28 of the 98 papers in the NO exogenous group (Suppl. Table S2) were eliminated. In addition, all variables were categorised in order to avoid the very different measurements used in the studies. All figures were then categorised under the following headings: increase, decrease, no change and not data.

In order to pinpoint groups of papers dealing with similar plant behaviours in response to heavy metals, we performed a hierarchical clustering analysis of the two databases generated. This enabled us to objectively search for groups of articles in an unsupervised way

without specifying the number of clusters to be created. We used H-clustering in the R software package, the k-means and Ward's parameters were used (Mitchell, 1995; Jain *et al.*, 1999) and the previously estimated data clusterability index. We found 10 clusters of papers in the endogenous NO group (Fig. 1) and 2 cluster in the exogenous NO group (Fig. 2). We used a similar approach for studies dealing with As and Cd alone, which were the most numerous studies (Suppl. Fig.S1-S4).

After this analysis, an overall increase in endogenous NO production was observed in plant responses to heavy metals particularly after short-term treatments (Fig. 1). For instance, after Cd treatments more than 80 % of the studies showed an increase of NO production after short treatments while less than 4% showed a decrease. For long treatments (over a one-week period) however, 66 % of the papers showed a decrease of NO production, clustered in groups II, III and VIII (Suppl. Table S1; Fig. 1). Long-term treatments with heavy metals can induce early senescence, excess ROS and ethylene, which may affect NO production (McCarthy *et al.*, 2001; Rodríguez-Serrano *et al.*, 2009; Romero-Puertas *et al.*, 2012). Groups VII and IX, mainly involving long-term treatments, are also affected in both enzymatic and non-enzymatic antioxidants (Fig. 1). However, in groups V to VII, involving different species except for *Arabidopsis*, enzymatic antioxidants are induced.

Most of these studies suggest that NOS-I activity is the main source of NO production in response to heavy metals and focus, in particular, on the use of NOS inhibitors in this type of pathway (Suppl. Table S1; Fig. 1). Following long-term treatment with heavy metals, Ca deficiency may also affect NOS-I activity and consequently NO production (Rodríguez-Serrano *et al.*, 2009). Some reports however, point to a NR-dependent NO burst in response to heavy metals such as Cu, Al and As (Kolbert *et al.*, 2012; Sun *et al.*, 2014; Xue and Yi, 2017; Suppl. Table S1). On the other hand, LeSPL-CNR (an SPL-type transcription factor) has been shown to be negatively involved in NO production by modulating NR expression and activity, which contributes to Cd tolerance suggesting a possible balance and/or crosstalk between the different NO sources, which contributes to NO level in plant response to heavy metals (Chen *et al.*, 2018). Recently, NO homeostasis in respiratory burst oxidase homologues (*rboh*) mutants has been shown to differ from that in WT plants in response to Cd, suggesting also that an unknown ROS-dependent NO regulation mechanism is present under these conditions (Gupta *et al.*, 2017).

NO needs to be highly regulated and/or metabolized in order to control its impact and functioning, with only non-symbiotic haemoglobin1 (nsHb1) and so-called phytooglobin1 from different species being capable of metabolising NO-producing nitrate (Perazzolli *et al.*, 2006;

Gupta *et al.*, 2011). In the green algae *Chlamydomonas*, the truncated haemoglobin (THB1) showed NO-dioxygenase activity associated with NR (Sanz-Luque *et al.*, 2015; Chamizo-Ampudia *et al.*, 2017), although the functioning of this activity in plants has not yet been studied. In addition, nsHb1 regulates NO production under hypoxic and low oxygen stress conditions when an increase in NO caused by nitrate reductase is assumed to protect plants against these stresses (Perazzolli *et al.*, 2004; Gupta *et al.*, 2011). In addition, non-symbiotic haemoglobins appear to play a role in the establishment of symbiosis, as the over-expression of *LjnHb1* and *AfnHb1* in *L. japonicus* induces an increase in the number of nodules with a concomitant decrease in NO production (Shimoda *et al.*, 2009; Hichri *et al.*, 2015; Fukudome *et al.*, 2016). Overexpression of *Nicotiana tabacum NtHb1* in both tobacco and *Arabidopsis* plants has recently been shown to downregulate NO levels in response to Cd and to induce greater Cd tolerance due, in part, to lower metal accumulation (Lee and Hwang, 2015; Bahmani *et al.*, 2019). On the other hand, NO can react with glutathione (GSH) and produce S-nitrosogluthatione (GSNO), which is considered to be a reservoir of NO and less reactive than this molecule (Liu *et al.*, 2001; Sakamoto *et al.*, 2002). The GSNO reductase (GSNOR) enzyme, which metabolizes nitrosothiols and indirectly regulates NO levels, is possibly involved in plant responses to heavy metal stress (Feechan *et al.*, 2005; Frungillo *et al.*, 2014). The *gsnor1-3* mutant, has been used in *Arabidopsis* plants under Cu stress conditions to show that excess NO due to a lack of GSNOR activity, whose role depends on a finely tuned balance between NO and ROS, can increase metal sensitivity or tolerance depending on the mildness or severity of the stress (Peto *et al.*, 2013). Several studies demonstrate that GSNOR activity increases or decreases in response to Cd (Wang *et al.*, 2015; Hu *et al.*, 2019) and As stress (Leterrier *et al.*, 2012; Rodríguez-Ruiz *et al.*, 2018) in different species depending on the strength of the treatment and the level of NO in the cell (Wang *et al.*, 2015; Hu *et al.*, 2019).

Given its half life of just a few seconds, NO can react rapidly with oxygen to produce nitrogen dioxide (NO₂), which leads to the production of nitrite and nitrate in aqueous solutions (Neill *et al.*, 2008). The reaction of NO with O₂⁻ also produces ONOO⁻, a highly pro-oxidant species (Ischiropoulos and al-Mehdi, 1995; Radi, 2018). As will be seen below, the relationship between NO and ROS plays a very important role in the regulation of NO and in NO-dependent functions.

5. NO role in plant responses to heavy metals

Given that the highly reactive nature of NO molecules facilitates their regulatory role, a key feature of their mode of action is an ability to directly alter proteins through covalent post-translational modifications (PTMs), which enable biological processes in the cell to be regulated (Martínez-Ruiz *et al.*, 2011). NO-dependent PTMs facilitate gene regulation, interactions with most phytohormone-dependent signalling pathways and ROS regulation (Simontacchi *et al.*, 2013; Gibbs *et al.*, 2014; Albertos *et al.*, 2015; Romero-Puertas and Sandalio, 2016; Castillo *et al.*, 2018; Cui *et al.*, 2018). The role played by endogenous (produced by the plant) and exogenous NO (a cyto-protective molecule) in response to heavy metals is also analysed.

5.1. Protection by exogenous NO against heavy metals stress

Numerous studies have shown that exogenous applications of NO donors (mainly sodium nitroprusside: SNP) protect plants against heavy metal-induced damage (summarized in Suppl. Table S2). Hierarchical clustering analysis described above produced two groups of profiles, which mainly differ in terms of the antioxidant system response. Exogenous NO correlated negatively with the antioxidants in group I, but correlated positively with antioxidants in group II (Fig. 2). Group I is mainly associated with high metal concentrations and/or long-term treatment with the metal (over a one-week period) and/or high concentrations of a NO donor, suggesting that the plant's antioxidant system is affected by intense/long-term treatments (Suppl. Fig. S1). Although it is difficult to detect a pattern in either group, all AsIII treatments are localized in group I, while group II contains AsV, Ni, Zn, Cu and Al (not heavy metals as such), which we have been also analysed (Fig. 2; Suppl. Table S2).

In general, NO donors applied prior to or at the same time as the treatment with heavy metal, regardless of the metal and species used, showed a positive correlation with biomass, root length and chlorophyll. On the other hand, the presence of the donor negatively correlated with ROS (O_2^- and H_2O_2) production and oxidative damage mainly caused by lipid peroxidation (Suppl. Table S2; Fig. 2). Thus, exogenous NO protects against heavy metal stress and alleviates oxidative stress (Fig. 2). While high concentrations of NO have been shown to have cytotoxic properties (Beligni and Lamattina, 2001), the response of antioxidant system to As in rice appears to be more sensitive to NO donors (SNP), as five out of eight studies show that the antioxidant system is negatively co-regulated (Suppl. Fig. S2).

Interestingly, NO donors correlated negatively with heavy metal uptake except for Cd which showed a positive correlation in over 40% of the studies analysed and in two Cr and Ni

studies (Suppl. Fig. S1). One of the problems associated with the use of NO donors could be the induction of IRT1, which has been shown to be NO-dependent in different species and is repressed by plants under Cd stress conditions to prevent Cd accumulation (Connolly, 2002; Connolly *et al.*, 2003; Graziano and Lamattina, 2007; Jin *et al.*, 2009). This repressive mechanism is enhanced in *Arabidopsis* roots treated with Cd and in the presence of a nitric oxide synthase inhibitor, thus suggesting that NO may promote Cd accumulation (Besson-Bard *et al.*, 2009). NO donors may have a stronger effect on IRT1 during Cd uptake, as this metal, whose main entrance to the cell appears to be IRT1, has no specific transporter. Recently, NO has been shown to modulate metal transporters such as NIP, NRAMP and ABC in rice plants treated with AsIII, as well as proton pumps and antiporters (CAX) in *Trifolium*, *Arabidopsis* and tobacco under Cd stress conditions (Singh *et al.*, 2017b; Liu *et al.*, 2015; Lee and Hwang, 2015; Bahmani *et al.*, 2019). The negative correlation between heavy metal uptake and NO donors observed also points to NO-dependent regulation of not only uptake transporters but probably also of heavy metal translocation from roots to shoots, a topic that needs future research (Fig. 3).

Most of the studies analysed focus on the clear relationship between NO and ROS metabolisms. However, some analyses focus on other protective mechanisms used by NO, such as increases in pectin and hemicellulose content in the root cell wall to prevent Cd accumulation in the soluble fraction of the cell in rice leaves (Xiong *et al.*, 2009) and to maintain the auxin equilibrium (Xu *et al.*, 2010). Hormone levels are also regulated by NO donors in plant responses to heavy metals; gibberellins, indol acetic acid, abscisic acid, cytokinins (GA3, IAA, ABA and CKs) increase in *Vicia faba* plants treated with As (Mohamed *et al.*, 2016); jasmonic acid (JA) increases in *Trifolium* plants subjected to Cd stress, while salicylic acid (SA) and ethylene were shown to decrease (Liu *et al.*, 2015). NO indirectly contributes to plant protection against Cd toxicity, with *Bacillus amyloliquefaciens* acting as a signalling molecule downstream of auxins (Zhou *et al.*, 2017). Recently, NO has been shown to reduce AsIII toxicity by modulating JA biosynthesis (Singh *et al.*, 2017b) and to increase Pb resistance by increasing IAA, CKs and GA3 levels and by decreasing ABA content (Sadeghipour, 2017). In rice plants treated with SA and As, NO production and NR activity were found to increase, with a concomitant reduction in oxidative damage (Singh *et al.*, 2017a) and GA alleviates Cd toxicity by reducing NO accumulation (Zhu *et al.*, 2012), thus suggesting the presence of a feedback loop between NO and hormones. However, further research is needed to unravel the mechanisms underlying other NO-dependent signalling pathways, involved in plant responses to heavy metals.

5.2. Role of endogenous NO in plant responses to heavy metals

Several studies have analysed NO production in response to heavy metal stress as described in the section on metabolism (Fig. 1; Suppl. Table S1). As mentioned above, length of treatment appears to affect NO production (Fig. 1), and a clear relationship exists between oxidative stress and heavy metal toxicity. Information concerning the function of endogenous NO produced in response to heavy metal is still limited, although initial studies have established that NO-dependent PTMs are involved in ROS regulation and cellular metabolic pathways such as photorespiration. In plant responses to Cd stress, CAT S-nitrosylation in pea peroxisomes decreases (Ortega-Galisteo *et al.*, 2012), which may account for the increase in activity described in a previous study (Romero-Puertas *et al.*, 1999), with H₂O₂-producing glycolate oxidase (GOX) S-nitrosylation also changing in response to Cd (Ortega-Galisteo *et al.*, 2012). Furthermore, the S-nitrosylation of PCs increases in *Arabidopsis* culture cells under Cd stress conditions which affects their capacity to chelate the metal (De Michele *et al.*, 2009). Nitration was also observed in soybean responses to Cd stress, and, although no significant changes were detected in the pattern of nitrated proteins, Cd may induce finely tuned regulation rather than major modifications (Pérez-Chaca *et al.*, 2014). A later study showed that half of the nitrated proteins identified were related with proteolysis (Gzyl *et al.*, 2016), a highly important process in plant responses to heavy metal stress.

NO-dependent transcript changes in response to heavy metals have also been analysed, although the underlying mechanisms involved remain unclear. Thus, over forty genes were found to be regulated by NO in *Arabidopsis* plants in response to Cd, which are associated with root growth, iron homeostasis, nitrogen assimilation and metabolic proteolysis (Besson-Bard *et al.*, 2009). Although exogenous NO prevents a decrease in root growth in response to heavy metals, various studies show that endogenous NO is involved in reducing root growth (Groppa *et al.*, 2008; Besson-Bard *et al.*, 2009; Valentovičová *et al.*, 2010); this points to a timely fine-tuned relationship between NO and root growth. Furthermore, NO represses auxin accumulation and signalling in *Arabidopsis* plants in response to Cd stress, which inhibits root meristem growth (Yuan and Huang, 2016). Similarly, metal uptake, especially in response to Cd, is regulated by endogenous NO. Biochemical and genetic techniques have shown that an increase in NO can increase metal uptake, while a decrease reduces uptake (Besson-Bard *et al.*, 2009; Lee and Hwang, 2015; Bahmani *et al.*, 2019). Interactions between Ca and NO also appear to play an important role in plant responses to heavy metals, although the mechanisms involved are unclear. Therefore, Ca prevented the NO synthase-dependent NO production

reduction in response to Cd in pea plants (Rodríguez-Serrano *et al.*, 2009) suggesting a cross-talk between NO and Ca as it has been shown also in *Arabidopsis* plants where NO appears to favor Cd versus Ca uptake and/or Ca extrusion through regulating Cd channels or transporters (Besson-Bard *et al.*, 2009). However, Ca was not found to affect NO production in chamomile plants treated with Cr (IV; Kováčik *et al.*, 2014).

Ethylene biosynthesis, mitogen-activated protein kinase2 (MAPKK2) signalling pathways and the induction of various TFs have been observed in response to Cd in soybeans (Chmielowska-Bak and Deckert, 2013). However, further research is required to better understand the role played by NO in plant responses to heavy metals.

6. Conclusions and future challenges.

In recent years, numerous studies have shown how NO is involved in plant responses to different heavy metal stresses, although the underlying mechanisms remain unclear. The use of NO donors and the cytoprotective role of NO have been extensively analysed. NO, induces antioxidant systems when present in appropriate concentrations, prevents excess ROS and oxidative damage and thus alleviates plant fitness loss. Most of the parameters analysed in these studies relate to ROS metabolism, while information on hormones and other signalling molecule is limited (Fig. 3). Future research therefore needs to be carried out on the protective role of NO in plant responses to heavy metal stress. The mechanisms underlying NO-dependent induction of antioxidant systems also need to be studied in more depth, although some initial data are available on NO-dependent PTMs which regulate ROS production-related enzymes and antioxidant systems. As problems such as the ferricyanide release are associated with SNP, which is by far the most commonly used donor, special care needs to be taken in relation to NO donors and their concentrations, adequate controls are required (Kováčik *et al.*, 2014), and if possible, more than one NO donor should be used.

The production of endogenous NO during plant responses to heavy metal stress has also been extensively analysed, with NO generally being observed to increase except in the case of long-term Cd/As treatments and the presence of metals in high concentrations. Studies also show that NO-dependent PTMs regulate ROS metabolism, phytochelatins and proteolysis in plant responses to heavy metal stress. Transcriptomic analyses have revealed the presence of signalling pathways such as Ca, hormones, MAPKs and TFs (Fig. 3). Timing and concentrations of endogenous NO however, need to be finely tuned during plant responses to Cd, with further research on other metals possibly producing similar results, as an excess of endogenous NO appears to promote Cd-dependent root-growth inhibition, Cd

uptake and disturbances in the antioxidant system. These functions, regardless of their metal specificity, need to be studied further, although our bioinformatic analysis shows that the entry of Cd into the plant appears to be the main one affected positively by NO. Negative correlation between NO and the entry of other metals also needs to be studied in more depth in order to clarify its association in heavy metal uptake and translocation.

Although the involvement of NO in plant responses to heavy metal stress has been extensively studied, the mechanisms underlying crosstalk between NO and ROS and other signalling components, such as hormones and TFs, are just beginning to be investigated and further research in these areas is needed. Research into the specific functions and general role of NO, such as its involvement in the entry and distribution of heavy metals in different plant species, could facilitate the production of crops with lower heavy metal uptake and enhanced phytoremediation properties in soils normally contaminated by more than one metal.

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Figure Legend

Figure 1: Analysis by bioinformatics when endogenous NO production in response to heavy metal stress is showed. Bioinformatics analysis of the data summarized in Suppl. Table S1 (studies) related to endogenous NO production as a response to metal application and the main variables measured in each article. Categorization of all variables converted all quantitative numbers to the categories: increased (blue), decreased (yellow), no change (light blue) or no data (white), according to the corresponding paper. The hierarchical clustering in an unbiased form showed ten groups: I to X. Code for each study is represented by the metal used and the number on the table associated.

Figure 2: Analysis by bioinformatics when exogenous NO application is used in response to heavy metal stress. Bioinformatics analysis of the data summarized in Suppl. Table S2 (studies) related to exogenous NO supply previously and/or during the metal application and the main variables measured in each article. Categorization of all variables converted all quantitative numbers to the categories: increased (blue), decreased (yellow), no change (light blue) or no data (white), according to the corresponding paper. The hierarchical clustering in an unbiased form showed two groups: I and II. Code for each study is represented by the metal used and the number on the table associated.

Figure 3: Scheme showing the main NO functions in plant response to heavy metal stress. Exogenous NO when supplied in appropriate concentrations, induces antioxidant systems, prevents excess ROS and oxidative damage, which together with a transcriptomic modulation alleviates plant fitness loss in response to heavy metal stress (in purple). Endogenous NO production in response to heavy metal stress (in red) has been involved in regulating ROS metabolism, phytochelatins (PCs) and proteolysis through post-transcriptional modifications (PTMs). Transcriptomic analyses have revealed the NO-dependent signalling pathways involving Ca, hormones, MAPKs and TFs among others. An excess of endogenous NO however, appears to promote Cd-dependent root-growth inhibition and Cd uptake. Main transporters associated with the uptake and translocation of heavy metals in the plant that might be regulated by NO are also shown.

Suppl. Figure S1: Analysis by bioinformatics when exogenous NO application is used in response to Cd stress. Bioinformatics analysis of the data summarized in Suppl. Table S2 (studies) related to exogenous NO supply previously and/or during Cd application and the main variables measured in each article. Categorization of all variables converted all quantitative numbers to the categories: increased (blue), decreased (yellow), no change (light blue) or no data (white), according to the corresponding paper. The hierarchical clustering in an unbiased form showed only one group. Code for each study is represented by the metal used and the number on the table associated.

Suppl. Figure S2: Analysis by bioinformatics when exogenous NO application is used in response to As stress. Bioinformatics analysis of the data summarized in Suppl. Table S2 (studies) related to exogenous NO supply previously and/or during As application and the main variables measured in each article. Categorization of all variables converted all quantitative numbers to the categories: increased (blue), decreased (yellow), no change (light blue) or no data (white), according to the corresponding paper. The hierarchical clustering in an unbiased form showed only one group. Code for each study is represented by the metal used and the number on the table associated.

Suppl. Figure S3: Analysis by bioinformatics when endogenous NO production in response to Cd stress is showed. Bioinformatics analysis of the data summarized in Suppl. Table S1 (studies) related to endogenous NO production as a response to Cd application and the main variables measured in each article. Categorization of all variables converted all quantitative numbers to the categories: increased (blue), decreased (yellow), no change (light blue) or no data (white), according to the corresponding paper. The hierarchical clustering in an unbiased form showed one group. Code for each study is represented by the metal used and the number on the table associated.

Suppl. Figure S4: Analysis by bioinformatics when endogenous NO production in response to As stress is showed. Bioinformatics analysis of the data summarized in Suppl. Table S1 (studies) related to endogenous NO production as a response to As application and the main variables measured in each article. Categorization of all variables converted all quantitative numbers to the categories: increased (blue), decreased (yellow), no change (light blue) or no data (white), according to the corresponding paper. The hierarchical clustering in an unbiased form showed one group. Code for each study is represented by the metal used and the number on the table associated.

Suppl. Table S1: Summary of the studies where endogenous NO production in plants against heavy metal stress has been shown. A literature search in different databases (<https://www.ncbi.nlm.nih.gov/pubmed/>; <https://www.scopus.com/search/>; <https://apps.webofknowledge.com/UA>) related to heavy metals in plants and NO was conducted. The search was narrowed down to the last ten years. In this table studies related to endogenous NO production as a response to metal application are shown. The code of each paper appears in the first column and Fig. 1, Suppl. Fig. S3 and Suppl. Fig. S4. Main conditions used in each paper have been summarized as metal used (Metal); time of the treatment (Timing); Species; age of the plant and tissue used; NO and/or ONOO⁻ detection and method used; main results and the proposed role.

Suppl. Table S2: Summary of the studies where exogenous NO has been applied in plants against heavy metal stress. A literature search in different databases (<https://www.ncbi.nlm.nih.gov/pubmed/>; <https://www.scopus.com/search/>; <https://apps.webofknowledge.com/UA>) related to heavy metals in plants and NO was conducted. The search was narrowed down to the last ten years. In this table studies related to exogenous NO application previously and/or during the metal application are shown. The code of each paper appears in the first column and Fig. 2, Suppl. Fig. S1 and Suppl. Fig. S2. Main conditions used in each paper have been summarized as metal used (Metal); time of the treatment (Timing); NO donor; Species, age of the plant and tissue used; NO and/or ONOO⁻ detection and method used; main results and the proposed role.

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Group I

Group II

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Uptake
Lipid_perox.
H2O2
O2
SOD
CAT
APX
ASC
GSH
GR
GPX
Pro
cell_viability
biomass
root_length
NO
Chl
POD

Studies

Pb_1
As_5
Pb_3
Cd_23
As_2
As_1
Cd_16
Cu_5
Cd_21
Cd_12
As_7
Hg_1
Cd_29
As_10
As_8
As_9
Cd_4

Cu_2
Zn_4
Ni_6
Cd_25
As_11
Cu_6
Zn_6
Zn_2
Ni_3
Pb_5
Cu_4
Zn_3
Zn_5
Cd_32
Cd_33
As_12
Cd_28
Cd_15
Cu_3
Cr_1
Cd_13
Ni_4
Cd_17
Cd_14
Pb_2
Cd_8
Cd_9
Al_2
Cd_6
Al_3
As_3
Cd_11
Al_1
As_6
Cu_1
Zn_1
Ni_2
Cd_1
Cd_2
As_4
Cd_30
Cd_10
Ni_1
Cd_19
Pb_4
Cd_26
Cd_34
Cr_2
Cd_7
Cd_18
Cd_22
Ni_5

- NO-regulated
- Putative NO-regulated
- Exogenous NO
- ROS-regulated

Suppl. Figure S1: Analysis by bioinformatics when exogenous NO application is used in response to Cd stress. Bioinformatics analysis of the data summarized in Suppl. Table S2 (studies) related to exogenous NO supply previously and/or during Cd application and the main variables measured in each article. Categorization of all variables converted all quantitative numbers to the categories: increased (blue), decreased (yellow), no change (light blue) or no data (white), according to the corresponding paper. The hierarchical clustering in an unbiased form showed only one group. Code for each study is represented by the metal used and the number on the table associated.

Suppl. Figure S2: Analysis by bioinformatics when exogenous NO application is used in response to As stress. Bioinformatics analysis of the data summarized in Suppl. Table S2 (studies) related to exogenous NO supply previously and/or during As application and the main variables measured in each article. Categorization of all variables converted all quantitative numbers to the categories: increased (blue), decreased (yellow), no change (light blue) or no data (white), according to the corresponding paper. The hierarchical clustering in an unbiased form showed only one group. Code for each study is represented by the metal used and the number on the table associated.

Suppl. Figure S3: Analysis by bioinformatics when endogenous NO production in response to Cd stress is showed. Bioinformatics analysis of the data summarized in Suppl. Table S1 (studies) related to endogenous NO production as a response to Cd application and the main variables measured in each article. Categorization of all variables converted all quantitative numbers to the categories: increased (blue), decreased (yellow), no change (light blue) or no data (white), according to the corresponding paper. The hierarchical clustering in an unbiased form showed one group. Code for each study is represented by the metal used and the number on the table associated.

Suppl. Figure S4: Analysis by bioinformatics when endogenous NO production in response to As stress is showed. Bioinformatics analysis of the data summarized in Suppl. Table S1 (studies) related to endogenous NO production as a response to As application and the main variables measured in each article. Categorization of all variables converted all quantitative numbers to the categories: increased (blue), decreased (yellow), no change (light blue) or no data (white), according to the corresponding paper. The hierarchical clustering in an unbiased form showed one group. Code for each study is represented by the metal used and the number on the table associated.

Suppl. Figure S1

Suppl. Figure S2

Suppl. Figure S3

Suppl. Figure S4

Suppl. Table S1: Summary of the studies where endogenous NO production in plants against heavy metal stress has been shown. A literature search in different databases (<https://www.ncbi.nlm.nih.gov/pubmed/>; <https://www.scopus.com/search/>; <https://apps.webofknowledge.com/UA>) related to heavy metals in plants and NO was conducted. The search was narrowed down to the last ten years. In this table studies related to endogenous NO production as a response to metal application are shown. The code of each paper appears in the first column and Fig. 1, Suppl. Fig. S3 and Suppl. Fig. S4. Main conditions used in each paper have been summarized as metal used (Metal); time of the treatment (Timing); Species; age of the plant and tissue used; NO and/or ONOO⁻ detection and method used; main results and the proposed role.

Code	Metal	Timing	Species	Age	Tissue	NO and ONOO ⁻ prod. /method/ source	Results	Proposed role	Ref.
Al_1	AlCl ₃ (30 µM)	3-24h	<i>Triticum aestivum</i> cv Yang-5 Jian-864	3d	seedling	+NO (3h Jian 864) +NO (12h Jian 5) (DAF-FM DA) NR	-root length/ cell viability +lipid peroxidation/ carbonyl content +O ₂ ⁻ /H ₂ O ₂ / HO ⁺ +SOD/ CAT/ GR/ POD +LOX +NR/ NOS-I activity +callose	Early NO burst plays an important role in Al resistance of wheat through modulating enhanced antioxidant defence.	(Sun <i>et al.</i> , 2014)
Al_2	AlCl ₃ (30 µM)	3-24h	<i>Triticum aestivum</i> cv. Yang-5 Jian-864	3d	seedling	+NO (DAF-FM DA)	-root length +antiox. capacity +H ₂ O ₂ / O ₂ ⁻ -Pro/ GSH/ DHA/ GSSG +DPPH +ASC/ APX/ GR/ GPX/ GST/ MDHAR/ DHAR =γ-ECS	NO protects wheat root induced oxidative stress, possibly through a regulation of the ASC-GSH cycle.	(Sun <i>et al.</i> , 2015)
Al_3	AlCl ₃ (30 µM)	24h	<i>Triticum aestivum</i> cv. Yang-5	3d	roots	+NO (DAF-FM DA)	-root length/ cell viability +Al uptake +malate efflux = <i>TaALMT1</i> +pectins and hemicelluloses -methylsterification pectin +Al content in pectin +PME	Al-induced enhanced production of NO decreases cell wall pectin methylation, thus increasing the Al-binding capacity of pectin and negatively regulating Al tolerance.	(Sun <i>et al.</i> , 2016)
As_1	Na ₂ HAsO ₄ (25-50 µM)	4-24h	<i>Oryza sativa</i> cv. No. 3	4d	roots	-NO (DAF-2DA)	-root length +As uptake +lipid peroxidation/ root oxidizability +H ₂ O ₂ / O ₂ ⁻ -NO ₂ ⁻ +SOD/ CAT/ APX/ GPX	As stress induces oxidative damage and lipid peroxidation accompanied with a decrease in NO content.	(Singh <i>et al.</i> , 2009)

As_2	KH ₂ AsO ₄ (0.1-1 mM)	7d	<i>A. thaliana</i>	14d	roots	+NO (DAF-FM DA)	-root length/ biomass/ cell viability +lipid peroxidation +O ₂ ⁻ =NADPH oxidase -CAT +GR =GOX/ ICDH/ G6PDH/ 6PGDH +GSNOR activity -GSSG/ GSH -GSNO +protein tyrosine nitration	As stress induces oxidative damage, a rise in NO combined with an associated increase in protein nitration, indicating nitrosative stress.	(Leterrier <i>et al.</i> , 2012)
As_3	Na ₂ HAsO ₄ (25-50 µM)	1-5d	<i>A. thaliana</i> <i>AtrbohC</i>	21d	leaves roots	-NO (DAF-2)	-root length/ biomass +As in leaves after 1d (WT) -As in leaves and roots (<i>AtrbohC</i>) after 5d +H ₂ O ₂ +ASC/ DHA +GSH/ GSSG +lipid peroxidation -CAT +GR/ GOX	NADPH oxidase C regulates uptake and translocation of elements (compromised: As, P, S, Ca, Cu, Zn, and Fe; promoted K).	(Gupta <i>et al.</i> , 2013)
As_4	Na ₂ HAsO ₄ (50 µM)	15d	<i>Pisum sativum</i> cv. Azad P-1	15d	leaves	-NO (Griess reagent)	-biomass +As uptake -Chl/ photosynthesis -DES activity -H ₂ S -NR +Cys +lipid peroxidation +O ₂ ⁻ / H ₂ O ₂ / HO [•] -APX/ GR/ MDHAR/ DHAR -GSH/ ASC +GSSG/ DHA	NO might be involved in reducing the accumulation of As and triggering up-regulation of the ASC–GSH cycle to counterbalance ROS-mediated damage.	(Singh <i>et al.</i> , 2015)
As_5	Na ₂ HAsO ₄ (1.5 mg L ⁻¹)	24h	<i>Pistia stratiotes</i>	Adult	leaves	+NO (DAF-2DA)	+As uptake +H ₂ O ₂ / O ₂ ⁻ -Chl/ photosynthesis/ photorespiratory rate =glucose/ starch -glycerate/ gly/ ser/ sucrose +nocturnal respiration/ mitoch. respiration -trichomes damage in membranes, protoplast and mesophyll cells	NO has a role in the adaptation and integration of physiological processes under As stress, acting as a global mediator and maintaining plant homeostasis and tolerance.	(Farnese <i>et al.</i> , 2017)
As_6	NaAsO ₂ (25 µM)	7d	<i>Oryza sativa</i> cv. Sarjoo52	10d	roots	-NO (DAF-FM DA)	-root length/ biomass -Chl +H ₂ O ₂ +lipid peroxidation +SOD/ CAT/ GPX/ APX/ GR +NR activity -SA +OsIRT1/ OsYSL2	Arsenite stress leads to oxidative stress burst and consequently activity of antioxidant enzymes was increased.	(Singh <i>et al.</i> , 2017a)

As_7	NaAsO ₂ (25 µM)	4-12d	<i>Oryza sativa</i> cv. indica IC-115730	12d	roots	+NO (DAF-FM DA)	-root length/ cell viability +As uptake -biomass +H ₂ O ₂ / O ₂ ⁻ +JA +DEGs	NO changes JA content during As stress.	(Singh <i>et al.</i> , 2017b)
As_8	NaAsO ₂ (0.1-10 mg/L)	3h	<i>Vicia faba</i> (Qingpi)	35d	leaves (guard cells)	+NO (DAF-FM DA)	-cell viability +ROS +Ca ²⁺ +NR	Increased levels of NO and ROS activate Ca ²⁺ signaling to control responses to As cytotoxicity and that NR-dependent NO genera- tion contributes to As toxicity in <i>V. faba</i> .	(Xue and Yi, 2017)
As_9	Na ₂ HAsO ₄ (100 µM)	24h	<i>Spirodela interme- dia</i> W. Koch	3d	plant	+NO (DAF-FM DA)	+H ₂ O ₂ / O ₂ ⁻ +As uptake +lipid peroxidation -cell viability +SOD/ CAT/ POX +NR activity	NO play critical roles in alleviat- ing the As-induced oxidative stress possibly by acting as an antioxidant molecule.	(da-Silva <i>et al.</i> , 2018)
As_10	NaAsO ₂ (150 µM)	48h	<i>Oryza sativa</i> var. Pusa Basmati (PB1)	7d	roots shoot	+NO (Griess reagent)	-root length/ biomass/ lateral roots -germination -NO ₂ ⁻ +As uptake and translocation -NiR activity -Chl/ protein content =carotenoids +cys/ Pro +lipid peroxidation +H ₂ O ₂ +SOD +CAT/ APX/ GR -OsPIN1a, 1b, 1c and 1d, OsPIN2, OsPIN5c and OsPIN10b expression +NRT/ AMT/ NiR/ PHT/ KTP	Exogenous NO ameliorated the toxic effect of As by regulating the endogenous NO generation, physiological, stress related pa- rameters and antioxidant en- zymes.	(Praveen and Gupta, 2018)
As_11	KH ₂ AsO ₄ (50 µM)	10d	<i>Pisum sativum</i> cv. Lincoln	20d	roots leaves	-NO (DAF-2) (roots) +NO (leaves) -ONOO ⁻ (APF) (roots)	+As uptake -biomass/ root length +lipid peroxidation/ protein carbonyl groups -GSH/ GSSG/ MDHAR/ DHAR +gly/ glutamic acid/ GABA content/ Pro +endopeptidase activity/ PC2/ PC3 =H ₂ O ₂ =ASC/ HPR -SOD/ APX/ GOX +NADPH oxidase +GR/ CAT -G6PDH =6PGDH +NADP-ICDH/ NADP-ME -GSNOR	AsV causes a differential meta- bolic response in roots and leaves and the biochemical ad- aptation of roots to palliate As effects is more pronounced.	(Rodríguez- Ruiz <i>et al.</i> , 2018)

As_12	NaAsO ₄ (25 µM)	4-8d	<i>Festuca arundinacea</i> cv. Arid3	21d	leaves	+NO (Hb)	-biomass +As uptake +lipid peroxidation +H ₂ O ₂ / O ₂ ⁻ +SOD/ CAT =APX	Tall fescue alleviates damage through raising antioxidant enzymes activities to scavenge ROS.	(Jing Wei <i>et al.</i> , 2010)
As_13	NaAsO ₄ (100 µM)	10d	<i>Oryza sativa</i> cv. Koman	21d	plants	-NO (DAF-2DA)	-root length/ biomass +secondary roots +O ₂ ⁻ / H ₂ O ₂ +As uptake +APX -DHAR +DHA +ASC	A connection of NO-ROS signaling with the redox status of ASC and the cell cycle dynamics, governing formation of NARs and PRBA in rice seedlings under AsV stress.	(Kushwaha <i>et al.</i> , 2019)
As_14	NaAsO ₂ (150 µM)	12d	<i>Oryza sativa</i>	12d	roots leaves	+NO (DAF-FM DA)	+As uptake +SOD/ CAT/ APX/ GR/ GST +H ₂ O ₂ / O ₂ ⁻ -cell viability	An As increase in NO levels, antioxidants and ROS production, causing some damage in rice.	(Pandey and Gupta, 2018)
-	CdCl ₂ (150 µM)	3-12h	<i>Nicotiana tabacum</i> cv. Bright Yellow2	3d	cells	+NO (Hb)	+Cd uptake -cell viability	NO plays a positive role in Cd ²⁺ induced PCD in tobacco BY-2 cells through modulating Cd entry and promoting Cd accumulation.	(Ma <i>et al.</i> , 2010)
-	CdCl ₂ (1 mM)	24h	<i>Hordeum vulgare</i>	72h	root tips	+NO in pericycle, parenchyma and protophloem (DAF-DA)	+NADPH diaphorase activity -root length	Cd stimulates NO functions through the ectopic accelerate differentiation of root tips and reduces root growth.	(Valentovičová <i>et al.</i> , 2010)
-	CdCl ₂ (100 µM)	7d	<i>Oryza sativa</i> cv. Xiushui 11	7d	roots shoot	-NO (DAF-FM DA)	-Cd uptake -root length +cell wall Cd concentration +non protein thiol +protein thiol	Ca may alleviate Cd toxicity via endogenous NO with variation in the levels of NPT, PBT and matrix polysaccharides.	(Zhang <i>et al.</i> , 2012)
-	CdCl ₂ (100 µM)	1-7d	<i>A. thaliana</i>	14d	roots shoot	+NO (DAF-FM DA, Hb)	+caspase -root length/ biomass	The cross talk between NO and MPK6 suggested that maybe there was amplification loop in the NO signalling pathway during Cd induced <i>Arabidopsis</i> PCD.	(Ye <i>et al.</i> , 2013)

-	CdSO ₄ (50 µM)	5d	<i>A. thaliana</i> <i>hy1-100/ hy1-1/</i> <i>ho2/ ho3/ ho4/</i> <i>nia1/nia2</i> <i>atnoa1/ phya/</i> <i>phyb</i> (KO and 35s)	5d	shoot roots	+NO depending on mu- tants (DAF-FM DA)	-root length +putrescine =spermidine -spermine -PAs content -DAO -PAO +Cd uptake	HO1 has been shown to benefit <i>Arabidopsis</i> plants under Cd stress by diminishing NO pro- duction and thus improving Fe homeostasis.	(Han <i>et al.</i> , 2014)
-	CdCl ₂ (5-50 µM)	2-3d	<i>Nicotiana tabacum</i> cv. Yunyan 85	28d	roots shoot	+NO (DAF-FM)	+Cd uptake -lateral roots/ root length	Cd stress enhances NO accu- mulation in tobacco roots tips; NaCl may alleviate Cd toxicity thrown reducing NO accumula- tion and affection roots develop- ment.	(Zhang <i>et al.</i> , 2014a)
-	CdSO ₄ (9 mM)	24-72h	<i>Triticum aestivum</i> cv. Sommez-2001/ Quality	20d	leaves roots	=NO (NO assay kit, ENZO)	+spermine -putrescine/ spermidine +Cd uptake	No clear conclusion.	(Mutlu and Yürekli, 2015)
-	Cd (75-150 µM)	12h	<i>A. thaliana</i>	5d	roots	+NO (DAF-2 DA)	-root length -Aux content -PIN1, 3 and 7 proteins	NO-dependent repression of auxin accumulation and signal- ling under Cd stress has been shown to be involved in inhibit- ing <i>Arabidopsis</i> root meristem growth.	(Yuan and Huang, 2016)
-	CdSO ₄ (0-5 ppm)	14d	<i>Zea mays</i> cv. White/ Yellow	0d	leaves	-NO (Griess reagent)	+Cd uptake -phenols -GSH	High Cd levels provokes signifi- cant alteration of non-enzymatic antioxidant and NO levels.	(Akinyemi <i>et al.</i> , 2017)
-	CdCl ₂ (0-20 µM)	4d	<i>Solanum lycoper- sicum</i> cv. Ailsa craig/ <i>cnr</i>	4d	roots	+NO (DAF-FM DA)	-root length +Cd uptake +NR +S/NR +SBP10, SBP12a, SBP15	A SPL-type transcription factor, LeSPL-CNR, is negatively in- volved in NO production by modulating S/NR expression and nitrate reductase activity, which contributes to Cd toler- ance.	(Chen <i>et al.</i> , 2018)
Cd_1	CdCl ₂ (5 µM)	1-25d	<i>Hordeum vulgare</i> cv. Weisuobuzhi/ Dong 17	10d	leaves roots	+NO (Hb)	-biomass +O ₂ ⁻ / H ₂ O ₂ -Chl/ photosynthetic rate +POD/ APX -CAT/ SOD +POD/ CAT/ APX/ SOD +lipid peroxidation +NR/ NOS-I	Cd induce NO and ROS produc- tion, and increase antioxidant system.	(Chen <i>et al.</i> , 2010)

Cd_2	CdCl ₂ (5-200 µM)	2-7d	<i>Solanum nigrum</i>	5-7d	roots	+NO (DAF 2-DA)	+lateral roots +Cd uptake -biomass/ cell viability +Pro +SOD/ CAT +H ₂ O ₂	Manipulation of the NO level is an effective approach to improve Cd tolerance in plant by modulating the development of lateral and adventitious roots.	(Xu <i>et al.</i> , 2011)
Cd_3	CdCl ₂ (89 µM)	2-24h	<i>Lupinus luteus</i>	3-14d	roots leaves	+NO (DAF-2DA) =ONOO ⁻ (Folic acid method)	+O ₂ ⁻ / H ₂ O ₂ +NADPH oxidase -cell viability +Cd uptake	NO and O ₂ ⁻ production is required for Cd-induced PCD enhanced level of the post-stress signals in leaves, including distal NO crosstalk with H ₂ O ₂ .	(Arasimowicz-Jelonek <i>et al.</i> , 2012)
Cd_4	CdCl ₂ (5-200 µM)	0.5-96h	<i>Brassica juncea</i> cv. Varuna T-59	15d	seedling	+NO (24h) -NO (+24h) (Hb)	+Cd uptake -root length/ biomass/ water content +lipid peroxidation -Chl +Pro +photosynthetic pigments +Non protein thiols -H ₂ O ₂ +SOD/ APX/ CAT	Endogenous NO is able to play important roles in promoting plant tolerance to Cd toxicity.	(Verma <i>et al.</i> , 2013)
Cd_5	CdCl ₂ (60 µM)	2d	<i>Matricaria chamomilla</i>	28d	shoot roots	+NO (DAF-FM DA)	+Cd uptake +GPX/ APX/ GR +GSH =GSSG +ASC/ PCs	Cd induces changes under non lethal conditions such as decrease in PCs and GSH and increases antioxidant enzymes.	(Kováčik <i>et al.</i> , 2014)
Cd_6	CdCl ₂ (15 µM)	1-6h	<i>Hordeum vulgare</i> cv. Slaven	4 cm roots	roots	+NO (DAF-2 DA)	-root length +root swelling +ROS	Short term exposure to Cd revealed that NO production is a very early defence response of barley roots.	(Alemayehu <i>et al.</i> , 2015)

Cd_7	CdCl ₂ (100 µM)	7d	<i>Trifolium repens</i>	14d	seedling	-NO (HbO ₂)	-root length/ biomass -Chl +Cd uptake/ traslocation +H ₂ O ₂ -CAT +lipid peroxidation +SOD (roots) -SOD (shoot) +APX/ GR/ GSH/ NPT +SA/ JA/ ET/ Pro	NO depleted Cd toxicity by eliminating oxidative damage, enhancing minerals absorption, regulating proton pumps, and maintaining hormone equilibrium.	(Liu <i>et al.</i> , 2015)
Cd_8	CdCl ₂ (1 mM)	7d	<i>Cumumis sativus</i> cv. Marketer	28d	protoplast	+NO 4h (DAF-FM DA)	+ROS -cell viabilty -microcallus	NO could act as a crucial mediator of plant cells responses to both short and long term Cd treatment.	(Piterková <i>et al.</i> , 2015)
Cd_9	CdCl ₂ (100 µM)	14d	<i>Solanum lycopersicum</i>	first true leaf	roots	-NO (Griess reagent)	+H ₂ O ₂ / O ₂ ⁻ +SOD/ CAT/ APX/ GR +Cd uptake +SNO -GSH/ ASC +PCs +ERF1/ ERF2/ MYB1/ AIM1/ R2R3-MYB/ AN2 and others	Increases tomato tolerance to Cd stress, promoting the chelation and sequestration of Cd, stimulating NO, SNO and the antioxidant system through a redox-dependent mechanism.	(Hasan <i>et al.</i> , 2016)
Cd_10	CdCl ₂ (1.5 mM)	24h	<i>Vigna radiata</i> L. cv. BARIMung-2	5d	seedling	+NO (Griess reagent)	+Cd uptake/ Cd translocation +GSH/ GSSG/ DHA +PCs +lipid peroxidation +H ₂ O ₂ /O ₂ ⁻ +LOX -biomass/ root length -Pro/ ASC/ Chl -CAT +APX/ SOD/ GST/ GR/ GPX -DHAR/ MDHAR +putrecine/ spermidine/ spermine/ PAs	Exogenous putrescine and/or SNP modulated endogenous polyamines and NO improved glyoxalase system in detoxifying MG and improved physiology and growth. Combined application showed better effects suggesting crosstalk between NO and PAs.	(Nahar <i>et al.</i> , 2016)
Cd_11	CdCl ₂ (10 µM)	12h-7d	<i>Oryza sativa</i> cv. Japonica/ Zhong-hua11	14d	roots shoot	+NO (6h) (DAF-FM DA)	-biomass -Chl/ photosynthesis +APX/ SOD/ GR +H ₂ O ₂ +PAs/ PCs Gene studies	NO signalling is associated with the accumulation of antioxidant enzymes, glutathione and PAs which increases Cd tolerance in rice via the antioxidant defence system.	(Yang <i>et al.</i> , 2016)

Cd_13	CdCl ₂ (0-8 µM)	0-3d	<i>Oryza sativa</i> cv. Nageng 9108	0d	roots	+NO (DAF-2 DA)	-root length/ cell viability/ biomass +H ₂ O ₂ / O ₂ ⁻ +lipid peroxidation +Cd uptake +NR/ NOS-I	Thymol confers plant tolerance by suppressing NO-mediated oxidative injuries, cell death and Cd accumulation.	(Wang <i>et al.</i> , 2017)
Cd_14	CdCl ₂ (5 mg/L)	3d	<i>Boehmeria nivea</i>	30d	roots shoot	=NO (Comercial reagent kit, Jiancheng)	-biomass +H ₂ O ₂ =SNO -GSNOR/ GSH +GSSG -APX/ GR/ SOD +lipid peroxidation	S-nitrosylation is involved in the ameliorating effect SNP against Cd toxicity in a concentration depended way.	(Wang <i>et al.</i> , 2015)
Cd_15	CdCl ₂ (50 µM)	1d	<i>A. thaliana irt1</i>	1cm roots	roots shoot	+NO (DAF-FM DA)	-root length +lipid peroxidation +Cd uptake -IRT1	GA alleviates Cd toxicity by reducing NO accumulation and expression of <i>IRT1</i> in <i>A. thaliana</i> .	(Zhu <i>et al.</i> , 2012)
Cd_16	CdCl ₂ (100 µM)	6-24h	<i>Medicago sativa</i> cv. Victoria	5d	shoot roots	+NO (DAF-FM DA)	-biomass +lipid peroxidation +Cd uptake -SOD/ POD +APX -biomass	NO was involved in the NaHS-induced alleviation of Cd toxicity. Also indicated that there exists a cross-talk between H ₂ S and NO responsible for the increased abiotic stress tolerance.	(Li <i>et al.</i> , 2012)
Cd_17	CdCl ₂ (150 µM)	6d	<i>Triticum aestivum</i> cv. Wenmai	7d	leaves roots	=NO (Hb)	-biomass/ root length +Lipid peroxidation +O ₂ ⁻ / H ₂ O ₂ +SOD/ POD =CAT/ APX -GR -ASC/ GSH/ carotenoids	Microwave pretreatment can enhance Cd tolerance in wheat seedlings by decreasing damage and ROS and increasing antioxidant activity.	(Qiu <i>et al.</i> , 2013)
Cd_18	CdCl ₂ (0-5000 µM)	0-6d	<i>Cynodon dactylon</i>	21d	leaves	+NO (Hb)	+H ₂ O ₂ / O ₂ ⁻ +SOD/ CAT/ POD/ GR -GSH +H ₂ S -biomass/ cell viability +lipid peroxidation	Exogenous NO H ₂ S have a protective role in bermudagrass and the Cd stress modulating ROS accumulation and antioxidant response. NO could activate H ₂ S being essential in the stress response.	(Shi <i>et al.</i> , 2014)
Cd_19	CdCl ₂ (20 µM)	1-6h	<i>Hordeum vulgare</i> cv. Slaven	4cm roots	root tip	+NO (DAF-FM) +ONOO ⁻ (APF)	-H ₂ O ₂ +O ₂ ⁻ -root length -Cd uptake	L-NAME induced enhanced O ₂ ⁻ generation, leading to increased ONOO ⁻ level in the root tips due to the reaction between O ₂ ⁻ and NO.	(Tamás <i>et al.</i> , 2018)

Cd_20	CdCl ₂ (100 µM)	6-72h	<i>Sedum alfredii</i>	21d	roots	+NO (DAF-FM)	+NR/ NOS-I -cell viability +lipid peroxidation +H ₂ O ₂ / O ₂ ⁻ +SOD/ GR -POD/ CAT/ APX +GSH/ ASC +GSNOR +γ-ECS =MDHAR/ DHAR +Cd uptake	NO activated GSH and SOD metabolisms to prevent excess ROS in root tips NO had no effect on Cd uptake and accumulation at the early stage of Cd exposure.	(Hu <i>et al.</i> , 2019)
Cd_21	CdCl ₂ (40 µM)	0-144h	<i>Glycine max</i>	10d	roots	+NO (pick 6h) (DAF-2 DA)	+Cd uptake +H ₂ O ₂ / O ₂ ⁻ (72h) +SOD/ GOX -lipid peroxidation +carbonyl groups (pick 6h)/ N-tyr +CAT/ GST/ APX +APX +G6PDH/ 6PGDH/ ICDH/ NADPH +ME/ DHA/ GSSG (pick 6h) -GSH/ ASC +NR	<i>G. max</i> has an efficiency defence mechanism in response to ROS and NO production imposed by Cd by improving antioxidant defences and activate enzymes that supply reducing power.	(Pérez-Chaca <i>et al.</i> , 2014)
Cd_22	CdCl ₂ (25-100 µM)	1-5d	<i>A. thaliana rboh</i> mutants	21d	leaves roots	-NO (DAF-2 DA)	-biomass +H ₂ O ₂ +lipid peroxidation +ASC/ DHA =GSH +GSSG +CAT/ GOX =GR/ GR1/GR2/ CAT1/ CAT2/CAT3 +Cd uptake	Cd toxicity is associated with the induction of oxidative stress in <i>Arabidopsis</i> leaves by elevated levels of H ₂ O ₂ content, partly induced by NOXs.	(Gupta <i>et al.</i> , 2017)
Co_1	CoCl ₂ (10–20 µM)	1-3d	<i>Oryza sativa</i> cv. Taichung Native 1/Indica	2d	seedling roots	=NO (DAF-FM DA)	+lateral roots -root length +HO activity +OsHO1 =OsHO2 =H ₂ O ₂	HO might be involved in CoCl ₂ -promoted lateral roots formation in rice roots. Neither H ₂ O ₂ nor NO is involved in CoCl ₂ effects in rice.	(Hsu <i>et al.</i> , 2013)
Co_2	Co ₃ O ₄ (0.025-1.0 mg/ml)	2h	<i>Solanum melongena</i> cv. Violetta lunga 2	7d	seedlings	+NO (DAF2-DA)	-root length and absence of root hairs +ROS localization around apical root meristem. +membrane potential +DNA damage -cell cycle	Co ₃ O ₄ -NPs trigger cell death via mitochondrial swelling and NO signaling pathway.	(Faisal <i>et al.</i> , 2016)

-	K ₂ Cr ₂ O ₇ (3-120 µM)	7d	<i>Matricaria chamomilla</i>	49d	shoot roots	+NO (DAF2-DA)	-biomass +Cr uptake +APX/ GPX/ GSH/ GSSG -GR +phenols +ROS +Ca/ Fe/ Zn/ Cu	Protective mechanisms were observed (increase in nitric oxide signal, elevation of phenols and GPX activity) but it was not sufficient to counteract the oxidative damage owing to depletion of GSH, ASC.	(Kováčik <i>et al.</i> , 2014c)
Cu_1	CuSO ₄ (0-50 µM)	7d	<i>A. thaliana nox1</i>	14d	roots	=NO (DAF-FM)	-root length/ biomass/ cell viability -germination +O ₂ ⁻ -H ₂ O ₂	NO can intensify Cu sensitivity or facilitate tolerance depending on the strength of the stress. Cu tolerance is regulated by a fine tuned NO/ROS balance.	(Peto <i>et al.</i> , 2013)
Cu_2	CuSO ₄ (0-25 µM)	7d	<i>A. thaliana nia1nia2atnoa1</i>	7d	seedling	+NO (DAF-FM DA) NR	-root length/ biomass/ cell viability +ROS	Cu induce NO accumulation in <i>nia1nia2noa1-2</i> suggesting the involvement of NR and NOA1 in the NO formation under Cu excess.	(Kolbert <i>et al.</i> , 2015)
Cu_3	CuSO ₄ (0-750 µM)	0-4d	<i>Hordeum vulgare</i>	3d	shoot	+NO (Hb)	-biomass -Chl +GSH/ ASC +H ₂ O ₂ /O ₂ ⁻ +lipid peroxidation +NR/ NOS-I +SOD/ POD/ GR +Cu uptake	Important role of the NR mediated early NO burst involved in the protection process against Cu toxicity in shoot of hulles barley.	(Hu <i>et al.</i> , 2015)
Cu_4	CuSO ₄ (450 µM)	0-48h	<i>Hordeum vulgare</i> cv. Nude	3d	roots	+NO (12h) (DAF-FM DA)	-cell viability/ root length +lipid peroxidation +SOD/ CAT/ POD/ APX +NR +O ₂ ⁻ / H ₂ O ₂	Burst of NO in the roots alleviated Cu stress and could be involved in a cross talk with Ca and ROS.	(Hu, 2016)
Cu_5	CuSO ₄ (5-50 µM)	17d	<i>A. thaliana</i> (WT <i>nia1nia2</i> <i>DR5::GUS</i>)	17d	shoot roots	+NO (root) (WT) -NO (<i>nia1nia2</i>) (DAF-FM DA) NR	-biomass/ root length/ division cell -Aux	In <i>Arabidopsis</i> roots nitrate reductase enzyme may be responsible for Cu-induced NO production.	(Kolbert <i>et al.</i> , 2012)

-	MnCl ₂ (0-1000 µM)	7d	<i>Matricaria chamomilla</i>	49d 7d	shoot roots	+NO (Cell ROX Deep Red reagent) -RNS	+Mn uptake +ROS +lipid peroxidation +O ₂ ⁻ / H ₂ O ₂ =APX +GPX +GR +Mn uptake -biomass -root length +ROS/ H ₂ O ₂ +lipid peroxidation	NO participates in tolerance to Mn excess but negative effects of the highest SNP dose were also observed.	(Kováčik <i>et al.</i> , 2014b)
Ni_1	NiSO ₄ (10-200 µM)	9d	<i>Oryza sativa</i> cv. Yang liang you 6	19d	shoot roots	+NO (NO-2-G kit, Comin)	-root length/ biomass/ cell viability +Ni uptake -Chl/ carotenoids +Pro +H ₂ O ₂ +lipid peroxidation -SOD +CAT/ POD/ ASC/ POD/ APX/ CAT/ GR +GSH -SOD	NO enhanced tolerance to Ni-stress by restricting Ni accumulation, maintaining photosynthetic performance and reducing oxidative damage through improved antioxidant system.	(Rizwan <i>et al.</i> , 2018)
Pb_1	Pb(NO ₃) ₂ (100 µM)	24h	<i>Pogonatherum crinitum</i>	5d	roots cells	+NO (HbO ₂ and Griess reagent)	NO production earlier than Pb accumulation +NR activity +Pb uptake	NO signaling might be upstream of Pb uptake in the cells. NR is involved in Pb-induced NO generation in the cells.	(Yu <i>et al.</i> , 2012)
Pb_2	Pb(NO ₃) ₂ (50-250 µM)	0-8h	<i>Triticum aestivum</i> cv. PBW 502	48h	roots	=NO (DAF-2DA and (Griess reagent)	-root length +lipid peroxidation +O ₂ ⁻ / H ₂ O ₂ / HO -cell viability +CAT/ APX/ GPX/ GR/ SOD	Exogenous NO partially ameliorates Pb toxicity, but could not restore the plant grow on prolonged Pb exposure.	(Kaur <i>et al.</i> , 2015)
Pb_3	Pb(C ₂ H ₃ CO ₂) ₂ (400 µM)	0-72h	<i>Prosopis farcta</i>	21d	shoot	+NO (until 12h) (Griess reagent)	+Pb uptake +ASC +H ₂ O ₂ -biomass -phenolic acids =flavonoids/ polyamines/ ADC +Pro/ aa/ lignin content +CAT/ GPX/ APX/PAL +aconitase activity +PAL	Favoring NO production and reduced H ₂ O ₂ content can improve Pb tolerance via whole-ranging effects on a primary metabolic network.	(Zafari <i>et al.</i> , 2017)
Pb_4	Pb(NO ₃) (150 µM)	14d	<i>A. thaliana</i> CFP-PTS1	14d	seedlings	+NO (DAF-2) +ONOO ⁻ (APF)	-root length -CAT =GOX/ HPR +O ₂ ⁻	Pb affects peroxisomes functioning causing an overall increase in O ₂ ⁻ and NO content with a concomitant production of ONOO ⁻ and a negative effect on CAT.	(Corpas and Barroso, 2017)

-	ZnSO ₄ (0-300 µM)	7d	<i>Brassica juncea</i> cv. Indian mustard <i>B. napus</i> cv. oilseed rape	9d	roots shoot	+NO (DAF-FM) +ONOO ⁻ (APF)	+Zn uptake (<i>B. napus</i> roots). -biomass -lateral roots -O ₂ ⁻ (<i>B. juncea</i>) +O ₂ ⁻ / SOD (<i>B. napus</i>) -H ₂ O ₂ / APX activity (<i>B. juncea</i>) +H ₂ O ₂ and =APX (<i>B. napus</i>)	Efficient translocation in both species and increasing Fe uptake, plants could avoid Fe deficiency in leaves. Zn excess modifies the root system architecture. Zn excess induces protein nitration.	(Feigl <i>et al.</i> , 2015)
-	ZnSO ₄ (200 µM)	4d	<i>Nicotiana tabaco</i> cv. Xanthi	35d	leaves	+NO (DAF2-DA)	+Zn uptake Lesions quite large + <i>NtBI-1</i> , <i>Ntrboh</i> and <i>NtSIPK</i> expression	Zn-related lesions in leaves develop from groups of mesophyll cells in which accumulation of high concentration of Zn contributes to enhancement of the NO levels and to initiation of PCD processes.	(Weremczuk <i>et al.</i> , 2017)
Zn_1	ZnSO ₄ (0.2-0.4 mM)	10d	<i>Solanum nigrum</i>	14d	roots	+NO (Hb)	-cell viability/ root length +Zn uptake -SOD (2-10d) +SOD (2-4d) +FeSOD2 (10d) +CAT1/ CAT2, cAPX, pAPX -SOD (2-4d) +APX/ CAT +NOX activity +O ₂ ⁻ and H ₂ O ₂ (- from 10d) +lipid peroxidation +lateral root +ferric-chelate reductase activity	Zn-induced NO production enhanced O ₂ ⁻ production by mediating NOX activity. NO production is partially related to Zn-induced Fe deficiency.	(Xu <i>et al.</i> , 2010)
Zn_2	ZnO NPs (250 mg/L)	3d	<i>Oryza sativa</i> cv. Jiafuzhan	3d	seedlings	+NO (DAF-2DA)	-root length/ biomass +Zn uptake -Chl +O ₂ ⁻ / H ₂ O ₂ -lipid peroxidation +GSH +Cu/Zn-SOD/ Mn-SOD -CATa, CATb, APX/ POD	ZnO NPs stimulated ROS production and destroyed H ₂ O ₂ scavenging system.	(Chen <i>et al.</i> , 2015)
Zn_3	ZnSO ₄ (10 mg/L)	1-10d	<i>Hydrilla verticillata</i>	14d	leaves	+NO (Hb)	+lipid peroxidation -Chl/ carotenoids +NP-SH =CAT +APX/ POD/ GR/ ASC/ SOD +DHA	Pre-treatment NO may be a useful way to improve the effect of ecological remediation and phytoremediation of Zn polluted water.	(Zhang <i>et al.</i> , 2014b)

Zn_4	ZnSO ₄ (3 mM)	6d	<i>Triticum aestivum</i> cv. Xihan 3	seeds	roots leaves	+NO (DAF-FM DA)	+Zn uptake/ Zn translocation -root length +lipid peroxidation +cell viability +CAT/ APX/ SOD/ GR -POD +NOS activity +H ₂ O ₂ -O ₂ ⁻ -DAO and PAO	PM NADPH oxidase could be associated with the regulation of NO and ROS production as well as NOS, CAT, POD, APX, SOD activities.	(Duan <i>et al.</i> , 2015)
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Suppl. Table S2: Summary of the studies where exogenous NO has been applied in plants against heavy metal stress. A literature search in different databases (<https://www.ncbi.nlm.nih.gov/pubmed/>; <https://www.scopus.com/search/>; <https://apps.webofknowledge.com/UA>) related to heavy metals in plants and NO was conducted. The search was narrowed down to the last ten years. In this table studies related to exogenous NO application previously and/or during the metal application are shown. The code of each paper appears in the first column and Fig. 2, Suppl. Fig. S1 and Suppl. Fig. S2. Main conditions used in each paper have been summarized as metal used (Metal); time of the treatment (Timing); NO donor; Species, age of the plant and tissue used; NO and/or ONOO⁻ detection and method used; main results and the proposed role.

Code	Metal	Timing	NO donor/ scavenger/ mutant	Species	Age	Tissue	NO or ONOO ⁻ production/ method/ source	Results	Proposed role	Ref.
-	AlCl ₃ (30 µM)	24h	cPTIO (30 µM)	<i>Triticum aestivum</i> cv. Yang-5	3d	roots	-NO (DAF-FM DA)	+root length/ cell viability -Al uptake/ accumulation in cell wall =malate efflux =TaALMT1 =hemicelluloses content -pectin methylesterase activity +methylesterification of pectin	Al-induced enhanced production of NO decreases cell wall pectin methylation, thus increasing the Al-binding capacity of pectin and negatively regulating Al tolerance in wheat.	(Sun <i>et al.</i> , 2016)
Al_1	AlCl ₃ (30 µM)	3-24h	SNP (250 µM) cPTIO (100 µM) Tungstate (100 µM)	<i>Triticum aestivum</i> cv. Yang-5/ Jian-864	3d	seedlings	+NO 3h (Jian-864) +NO 12h Yang-5 (DAF-FM DA) NR	+root length -lipid peroxidation/ carbonyl content -H ₂ O ₂ / O ₂ ⁻ / HO ⁻ -LOX/ POD +SOD/ CAT/ GR -callose	Early NO burst plays an important role in Al resistance of wheat through modulating enhanced antioxidant defence.	(Sun <i>et al.</i> , 2014)
Al_2	AlCl ₃ (1 mM)	10d	SNP (2 mM)	<i>Artemisia annua</i>	60d	leaves roots	-	=root length/ biomass +photosynthesis/ stomatal conductance/ internal CO ₂ +Chl/ carbonic anhydrase activity -lipid peroxidation -H ₂ O ₂ / O ₂ ⁻ +NR +CAT/ SOD +artemisinin/ POX	NO application to <i>A. annua</i> plants counteracts the toxicity of Al, inducing the biosynthesis of artemisinin in the presence of excess of Al.	(Aftab <i>et al.</i> , 2012)
Al_3	AlCl ₃ (30 µM)	3-24h	SNP (250 µM) cPTIO (100 µM) Tungstate (100 µM) GSNO (0.5 mM)	<i>Triticum aestivum</i> cv. Yang-5/ Jian-864	3d	seedlings	+NO (DAF-FM DA)	+root length =Pro -O ₂ ⁻ +DPPH scavenging activity +ASC/ GSH/ APX/ DHAR/ MDHAR/ GR/ GPX -DHA/ GSSG +GST =γ-ECS	NO protects wheat root against Al-induced oxidative stress, possibly through its regulation of the ASC-GSH cycle.	(Sun <i>et al.</i> , 2015)

-	NaAsO ₂ (0.1-10 mg/L)	3h	SNP (200 µM)	<i>Vicia faba</i> cv. Qingpi	35d	leaves	+NO (DAF-FM DA) NR	+cell viability	Increased levels of NO and ROS activate Ca ²⁺ signalling to control responses to As cytotoxicity and that NR-dependent NO generation contributes to As toxicity.	(Xue and Yi, 2017)
As_1	NaAs ₂ O ₄ (25 µM)	7d	SNP (25 µM)	<i>Oryza sativa</i> cv. Sarjoo 52	10d	roots	=NO (DAF-FM DA)	+root length/ biomass -As uptake and translocation -H ₂ O ₂ / lipid peroxidation +Chl -NR -SOD/ CAT/ GPX/ APX/ GR +SA -OsLsi1/ OsIRO2/ OsYSL2 +OsLsi2 =OsFRDL 1 and OsNRAMP5	NO decreased the As accumulation in roots probably through the down regulation OsLsi1. NO enhanced the Fe accumulation in shoot and overcame the AsIII mediated chlorosis.	(Singh <i>et al.</i> , 2017a)
As_2	NaAsO ₂ (150 µM)	48h	SNP (100 µM)	<i>Oryza sativa</i> cv. Pusa Basmati (PB 1)	7d	roots shoot	+NO (Griess reagent)	+germination/ root length/ lateral roots/ biomass -As uptake +Chl/ nutrients content =carotenoids -Pro/ cys -lipid peroxidation =H ₂ O ₂ -SOD/ CAT/ APX/ GR +NO ₂ / NiR activity +OsPIN1/ OsPIN2/ OsPIN5c/ OsPIN10b +NRT/ AMT/ NiR/ PHT/ KTP	NO reduced As accumulation, improved root architectural, growth of plant, chlorophyll, protein and mineral nutrients content by reducing the ROS generation. Further, enhanced transcript levels of PIN proteins and mineral nutrition related genes.	(Praveen and Gupta, 2018)
As_3	Na ₂ HAsO ₄ (250-500 µM)	72h	SNP (0.25 mM)	<i>Triticum aestivum</i>	6d	seedlings	-	+Chl/ Pro -lipid peroxidation -H ₂ O ₂ -GSSG +APX/ MDHAR/ DHAR/ GR/ GPX/ CAT +ASC/ GSH	Exogenous application of NO rendered the plants more tolerant to As-induced oxidative damage by enhancing their antioxidant defence and glyoxalase system.	(Hasanuzzaman and Fujita, 2013)
As_4	Na ₂ HAsO ₄ (50 µM)	7d	SNP (100 µM) PTIO (200 µM)	<i>Phaseolus vulgaris</i> cv. VL 63	7d	shoot roots	-	+root length/ biomass -root oxidation/ lipid peroxidation/ cell viability -As uptake +Chl/ carotenoids -SOD -H ₂ O ₂ +APX/ DHAR/ GR/ POX =CAT	NO has remarkable potentials to reverse inhibited growth, changes in anatomical structures, photosynthetic apparatus and antioxidant defence activities induced by As stress.	(Talukdar, 2013)

As_5	Na ₂ HAsO ₄ (25-50 µM)	4-24h	SNP (25-50 µM)	<i>Oryza sativa</i> cv. No. 3	4d	roots	+NO (DAF-2DA)	+root length -As uptake -lipid peroxidation/ root oxidizability -H ₂ O ₂ / O ₂ ⁻ -SOD/ CAT/ APX/ GPX +NO ₂ ⁻	Exogenous NO provides resistance to rice against As-toxicity and has an ameliorating effect against As-induced stress.	(Singh <i>et al.</i> , 2009)
As_6	Na ₃ AsO ₄ (25 µM)	4-8d	SNP (100 µM) PTIO (200 µM)	<i>Festuca arundinacea</i> cv. Arid3	21d	leaves	+NO (Hb)	+biomass -lipid peroxidation -cell viability/ relative ion leakage -H ₂ O ₂ / O ₂ ⁻ +SOD/ CAT/ APX (4d)	Tall fescue alleviates damage through raising antioxidant enzymes activities to scavenge ROS.	(Jing Wei <i>et al.</i> , 2010)
As_7	Na ₂ HAsO ₄ (100 µM)	24h	SNP (50 µM)	<i>Spirodela intermedia</i> W. Koch	3d	plant	+NO (DAF-FM DA) NR	-As uptake/ lipid peroxidation -H ₂ O ₂ / O ₂ ⁻ +cell viability -SOD/ CAT/ POX -NR	The increased production of endogenous NO indicating that NO participates in the protection against As-induced damage besides reduce As uptake, and possibly acts as an antioxidant molecule.	(da-Silva <i>et al.</i> , 2018)
As_8	Na ₃ AsO ₄ (100 µM)	10d	SNP (100 µM) L-NAME (500 µM) cPTIO (200 µM)	<i>Oryza sativa</i> cv. Komal	21d	roots	+NO (DAF-2DA)	+lateral roots/ root length/ biomass -H ₂ O ₂ / O ₂ ⁻ -As uptake -APX +DHAR/ ASC +cell in G1 phase and -cells in G2 phase -NARs and PRBA	A connection of NO-ROS signalling with the redox status of ASC and the cell cycle dynamics, governing formation of NARs and PRBA in rice seedlings under AsV stress.	(Kushwaha <i>et al.</i> , 2019)
As_9	NaAsO ₂ (50 µM)	5d	SNP (75 µM)	<i>Glicine max</i> cv C. JS 335	5d	roots	-	+germination/ root length/ biomass -As uptake -H ₂ O ₂ / O ₂ ⁻ -lipid peroxidation +Pro/ sugar/ PCs	As-induced lesser toxicity in NO-treated samples might be an outcome of lower As accumulation, or oxidative damage, or activation of P5CS gene, or all of these.	(Chandrakar and Keshavkant, 2018)
As_10	NaAsO ₂ (25 µM)	4-12d	SNP (30 µM)	<i>Oryza sativa</i> cv. Indica IC-115730	10d	roots	+NO (DAF-FM DA)	+biomass/ root length -As uptake +cell viability -H ₂ O ₂ +DEGs -JA	Differences in DEGs in the AsIII and AsIII+SNP treated samples indicated an important role of NO in the modulation of gene expression, reducing ROS level, enhancing cell viability and root growth.	(Singh <i>et al.</i> , 2017b)

As_11	Na ₂ HAsO ₄ (1.5 mg/L)	24h	SNP (0.1 mg/L)	<i>Pistia stratiotes</i>	adult	leaves	+NO (DAF-2DA)	=As uptake -H ₂ O ₂ / O ₂ ⁻ =photosynthesis +Chl -sucrose/ glucose +nocturnal and mitochondrial respiration -photorespiratory rate =trichomes normal integrity membrane	NO triggered signalling cascades that altered respiration and photorespiration so as to enable the maintenance of net CO ₂ assimilation and minimize the generation of ROS, reducing the damage.	(Farnese <i>et al.</i> , 2017)
As_12	Na ₂ HAsO ₄ (0-400 µM)	0-45d	SNP (100 µM)	<i>Vicia faba</i>	45d	shoot roots	-	+root length/ biomass/ germination +Chl/ carotenoids -lipid peroxidation/ phenolic compounds +GA/ IAA/ ABA	Exogenous application of NO protected <i>Vicia faba</i> plants against the adverse effect of As.	(Mohamed <i>et al.</i> , 2016)
-	CdCl ₂ (100 µM)	10d	SNP (100 µM)	<i>Cucumis sativus</i>	15d	leaves	-	-O ₂ ⁻ / H ₂ O ₂ iTRAQ: 1691 proteins	Cd-disruptive pathways were markedly reversed by NO treatments, including Cd transport and localization, photosynthesis, chlorophyll metabolism, redox homeostasis, glutathione-mediated Cd detoxification and Ca ²⁺ signaling transduction.	(Gong <i>et al.</i> , 2017)
-	CdCl ₂ (100 µM)	6 -72h	cPTIO (50 µM) Tungstate (100 µM) L-NAME (100 µM)	<i>Sedum alfredii</i>	21d	roots	-NO (DAF-FM DA)	-cell viability +lipid peroxidation +H ₂ O ₂ / O ₂ ⁻ -SOD/ GSH +POD/ CAT/ APX -GR/ GSNOR/ γ-ECS =ASC/ MDHAR/ DHAR =Cd uptake	NO activated GSH and SOD metabolisms to prevent excess ROS in root tips. NO had no effect on Cd uptake and accumulation at the early stage of Cd exposure. Elimination of NO significantly aggravated Cd-induced oxidative stress in roots.	(Hu <i>et al.</i> , 2019)
-	CdCl ₂ (0.2 mM)	50d	SNP (0.05 mM)	<i>Nicotiana tabacum</i>	0d	plant	-	+biomass +Chl +rubisco =rubisco activase	SNP could play a protective role in regulation of plant responses to Cd stress by enhancing rubisco and rubisco activase.	(Khairy <i>et al.</i> , 2016)
-	CdCl ₂ (1 mM)	7d	L-NAME (0.2 mM) cPTIO (0.1 mM) GSNO (0.1 mM)	<i>Cumumis sativus</i> cv. Marketer	28d	protoplasts	=NO (DAF-FM DA)	+cell viability -H ₂ O ₂ +microcallus	NO could act as a crucial mediator of plant cells responses to both short and long term Cd treatment.	(Piterková <i>et al.</i> , 2015)
-	CdCl ₂ (20 µM)	1-6h	L-NAME (1-10 mM)	<i>Hordeum vulgare</i> cv. Slaven	4 cm roots	roots	-NO (DAF-FM) +ONOO ⁻ (APF)	-root length -Cd uptake -H ₂ O ₂ +O ₂ ⁻	L-NAME, induced enhanced O ₂ ⁻ generation, leading to increased ONOO ⁻ level in the root tips due to the reaction between O ₂ ⁻ and NO.	(Tamás <i>et al.</i> , 2018)

-	CdCl ₂ (25 µM)	15d	SNP (500-1000 µM)	<i>Pisum sativum</i> cv. Ran	15d	leaves	-	+biomass/ leaf thickness +intercellular spaces in the mesophyll -length of guard cell/ perimeter of guard cell/ density of stomata -area of pavement cell/ perimeter of pavement cell	NO can effectively facilitate structural adjustment in pea leaves under Cd stress, which could improve stress tolerance at the whole plant leaves.	(Tran <i>et al.</i> , 2013)
-	CdCl ₂ (0-8 µM)	0-3d	Thymol (0-40 µM) SNP (20 µM) cPTIO (20 µM)	<i>Oryza sativa</i>	0d	roots	-NO (DAF-2 DA)	+root length/ cell viability -lipid peroxidation -H ₂ O ₂ +O ₂ ⁻ -Cd uptake -NR	Thymol confers plant tolerance by suppressing NO-mediated oxidative injuries, cell death and Cd accumulation.	(Wang <i>et al.</i> , 2017)
-	CdCl ₂ (5-200 µM)	2-7d	IAA (10 nM) NPA (10 µM) TIBA (10 µM) GSNO (100 µM) cPTIO (200 µM)	<i>A. thaliana</i> <i>Solanum nigrum</i>	5-7d	roots	+NO (DAF-2 DA)	+Cd uptake +biomass/ lateral roots/ cell viability -H ₂ O ₂ +Pro +SOD =CAT	Manipulating NO level is an effective approach to improve Cd tolerance in plant by modulating the development of lateral root and adventitious root, and provide insights into novel strategies for phytomediation.	(Xu <i>et al.</i> , 2011)
-	CdCl ₂ (100 µM)	0-7d	cPTIO (0.5 mM)	<i>A. thaliana</i>	14d	seedlings	+NO (DAF-FM DA) (Hb)	+root length +Caspase 3	NO promote Cd induced <i>Arabidopsis</i> PCD by promoting MPC6 mediated Caspase 3-like activation.	(Ye <i>et al.</i> , 2013)
-	CdCl ₂ (1-50 µM)	3h	SNP (10 µM) L-NAME (0.5 mM) NaCl (10 mM)	<i>Nicotiana tabacum</i>	3 leaf stage	shoot roots	-NO (DAF-FM)	-Cd uptake +lateral roots	NaCl alleviates Cd toxicity contributed to reduction of NO accumulation in plants.	(Zhang <i>et al.</i> , 2014b)
-	CdSO ₄ (9 mM)	24-72h	SNP (100 µM) cPTIO (100 µM)	<i>Triticum aestivum</i> cv. Sommez Quality	20d	leaves roots	+NO (NO assay kit, ENZO)	+PAs -Cd uptake	No clear conclusion.	(Mutlu and Yürekli, 2015)
Cd_1	CdCl ₂ (1.5 mM)	48h	Putrescine (0.2 mM) SNP (1 mM)	<i>Vigna radiata</i> cv. Bari Mung-2	5d	seedlings	+NO (Griess reagent)	+root length/ biomass -Cd uptake -lipid peroxidation -H ₂ O ₂ / O ₂ ⁻ +Chl +SOD/ CAT/ APX/ DHAR/ GR/ ASC -MDHAR/ GPX/ DHA/ GSSG +GSH/ GST/ Pro/ PCs -LOX	Glyoxalase system in detoxifying methylglyoxal, improve physiology and growth. Crosstalk between NO and polyamines have better effects.	(Nahar <i>et al.</i> , 2016)

Cd_2	CdCl ₂ (150 µM)	10d	SNP (100 µM)	<i>Solanum lycopersicum</i>	40d	shoot roots	-	+root length/ biomass/ cell viability +Chl/ carotenoids/ photosynthesis +Pro/ Gly -H ₂ O ₂ -lipid peroxidation +ASC/ GSH/ GSSG/ GST/ DHAR/ MDHAR/ SOD/ CAT/ APX/ GR +flavonoids/ phenols/ mineral nutrients -Cd uptake	Exogenous application of NO allayed the Cd induced damage in seedlings and prevent oxidative damage by upregulating antioxidant enzymes, there by promoting a significant decline in ROS-induced lipid peroxidation and cell death.	(Ahmad <i>et al.</i> , 2018)
Cd_4	CdCl ₂ (200 µM)	8d	SNP (100 µM)	<i>Oryza sativa</i>	21d	shoot roots	-	+root length/ biomass -lipid peroxidation -H ₂ O ₂ -POD/ SOD/ APX/ GR/ ASC +CAT/ GSH +Chl +Cd uptake -Cd translocation	No significantly alleviates Cd-induced oxidative stress in rice.	(Zhao <i>et al.</i> , 2013)
Cd_6	CdCl ₂ (200 µM)	14d	SA (0.1 mM) SNP (0.25 mM)	<i>Arachis hypogaea</i> cv. Huayu 22/ Xiaobaisha	21d	roots shoot	-	+biomass/ root length -lipid peroxidation -O ₂ ⁻ / H ₂ O ₂ +SOD/ POD/ CAT/ ASC +Chl/ photosynthesis +Cd uptake -Cd translocation Hormones studies	Interactive effects of SA+SNP were more effective compared to the separated application.	(Xu <i>et al.</i> , 2015)
Cd_7	Cd (10 ppm)	1-3d	SNP (250 µM) cPTIO (100 µM)	<i>Cicer arietinum</i>	30d	plant	-	+biomass -Cd uptake -lipid peroxidation -H ₂ O ₂ / GSSG +CAT/ POX/ SOD/ ASC/ GSH/ APX/ GR/ DHAR/ MDHAR	NO have a positive effect on seed yield and Cd accumulation protecting chickpea plants from Cd toxicity.	(Kumari <i>et al.</i> , 2010)
Cd_8	CdCl ₂ (100 µM)	14d	SNP (50-400 µM)	<i>Lolium perenne</i>	14d	shoot roots	-	+biomass +Cd uptake -H ₂ O ₂ / O ₂ ⁻ -lipid peroxidation +SOD/ POD/ CAT/ APX +Chl/ carotenoids	Low concentration of NO could significantly alleviate the effects of Cd toxicity and high concentration of NO has no significant effects.	(Wang <i>et al.</i> , 2013a)
Cd_9	CdCl ₂ (100 µM)	14d	SNP (0.1 mM) SA (0.2 mM)	<i>Lolium perenne</i>	21d	roots shoot	-	+biomass +Cd uptake -Pro/ lipid peroxidation -H ₂ O ₂ +SOD/ POD/ CAT/ APX +Chl/ carotenoids/ photosynthesis Hormones studies	Low Cd concentration induces stress in SA+SNP treatment plants due to the inhibition of Cd translocation and sequestration in roots and antioxidants enzymes inhibition.	(Wang <i>et al.</i> , 2013b)

Cd_10	CdCl ₂ (100 µM)	7d	SNP (30 µM) cPTIO (50 µM)	<i>Oryza sativa</i>	7d	roots shoot	-	+germination/ root length/ biomass -Cd uptake +Pro -lipid peroxidation -H ₂ O ₂ +GPX/ SOD/ APX/ CAT	SNP exerted an advantageous effect on alleviating the inhibitory effect of Cd on rice seed germination and seedling growth, which might interact with NO.	(He <i>et al.</i> , 2014)
Cd_11	CdCl ₂ (750 µM)	0-6d	SNP (250 µM) NaHS (500 µM)	<i>Cynodon dactylon</i>	21d	plant	+NO (HbO)	-biomass/ cell viability -H ₂ O ₂ / O ₂ ⁻ +SOD/ CAT/ POD/ GR/ GSH +H ₂ S -lipid peroxidation	NO endogenous and exogenous and H ₂ S have a protective role and speculated that NO-activated H ₂ S and might be essential for Cd stress response.	(Shi <i>et al.</i> , 2014)
Cd_12	Cd(NO ₃) ₂ (50 µM)	7d	SNP (50 µM) cPTIO (50 µM)	<i>Oryza sativa</i>	7d	roots shoot	+NO	+biomass/ cell viability -lipid peroxidation -H ₂ O ₂ / O ₂ ⁻ -CAT/ SOD +Chl -Cd uptake	Exogenous NO diminishes the deleterious effects of Cd in rice plants.	(Singh and Shah, 2014)
Cd_13	CdCl ₂ (50-200 µM)	14d	SNP (250 µM)	<i>Arachis hypogaea</i>	14d	roots shoot	-	+biomass -O ₂ ⁻ -lipid peroxidation +SOD/ POD/ CAT/ ASC/ Pro =Cd uptake -Cd translocation +Chl/ carotenoids/ photosynthesis	NO application can mitigate toxicity and enhance peanut tolerance.	(Dong <i>et al.</i> , 2016)
Cd_14	CdCl ₂ (100-150 µM)	14d	SNP (100 µM)	<i>Lolium perenne</i>	21d	shoot roots	-	+biomass/ root length -H ₂ O ₂ / O ₂ ⁻ +Cd uptake/ Chl -Cd translocation -lipid peroxidation +SOD/ POD -CAT	Exogenous NO could alleviate Cd toxicity through increasing chlorophyll concentration; reducing oxidative stress and improving antioxidant system; regulating mineral nutrient balance in leaves and roots; and decreasing Cd translocation from roots to leaves.	(Chen <i>et al.</i> , 2018)
Cd_15	CdCl ₂ (50-200 µM)	1-4d	SNP (100 µM)	<i>Oriza sativa</i> cv. MSE9	5d	roots shoot	-	+root length/ biomass -H ₂ O ₂ =lipid peroxidation -ASC/ GSH/ CAT +GR/ POX/ SOD	SNP in combination with Cd treatment might possess the way to protect rice seedlings under Cd stress.	(Panda <i>et al.</i> , 2011)
Cd_16	CdCl ₂ (5-200 µM)	6-9h	SNP (0.01-20 mM) cPTIO (100 µM)	<i>Brassica juncea</i> cv. Varuna T-59	15d	roots leaves	+NO (Hb)	+root length/ leaf water content +Chl -H ₂ O ₂ -SOD/ APX/ CAT -Pro/ non protein thiols/ lipid peroxidation	NO strongly counteracts Cd induce ROS mediated cytotoxicity by controlling antioxidant metabolism.	(Verma <i>et al.</i> , 2013)

Cd_17	CdCl ₂ (100 µM)	14d	SNP (100 µM)	<i>Lolium perenne</i>	21d	roots leaves	-	+biomass/ root length -lipid peroxidation -O ₂ ⁻ +SOD/ CAT/ POD/ ASC -Pro +Chl/ carotenoids	NO may acts as one of the potential antioxidant to improve plant resistance to Cd stress.	(Bai <i>et al.</i> , 2015)
Cd_18	CdCl ₂ (50 µM)	10-15d	SNP (100 µM) SO ₄ ²⁻ (1 mM)	<i>Brassica juncea</i>	30d	roots leaves	-	+biomass +photosynthesis/ Chl -Cd uptake/ translocation -H ₂ O ₂ -lipid peroxidation +SOD/ GR/ GSH =APX +rubisco/ thylakoid membrane +ATP-S +Cys	NO counteracts Cd toxicity in <i>B. juncea</i> strongly by regulating S-assimilation and GSH production.	(Per <i>et al.</i> , 2017)
Cd_19	CdCl ₂ (5 µM)	1-25d	SNP (250 µM)	<i>Hordeum vulgare</i> cv. Weisuo- buzh/ Dong 17	10d	leaves roots	+NO (Hb)	+Chl/ photosynthetic rate -ROS/ lipid peroxidation +SOD/ CAT/ APX +POD =NR +NOS-I	Under Cd stress NO as a potent antioxidant, protects barley seedlings against oxidative damage by direct and indirect scavenging ROS and helps to maintain stability and integrity of the sub-cellular structure.	(Chen <i>et al.</i> , 2010)
Cd_21	CdCl ₂ (5 mg/L)	3d	SNP (100 µM) cPTIO (100 µM)	<i>Boehmeria nivea</i>	30d	roots shoot	+NO	+S-NO +Biomass +GSH/ GSNOR -GSSG =GR -lipid peroxidation +H ₂ O ₂ -APX/ SOD	S-nitrosylation is involved in the ameliorating effect SNP against Cd toxicity in a concentration depended way.	(Wang <i>et al.</i> , 2015)
Cd_22	CdCl ₂ (10 µM)	12h-7d	SNAP (30 µM) cPTIO (30 µM)	<i>Oryza sativa</i> japonica cv. Zhonghua11	14d	roots	+NO (DAF-FM DA) (6h)	+biomass -H ₂ O ₂ +GSH/ APX/ SOD/ GR +phosphatidic acid -Cd leakage +photosynthesis/ Chl/ PCs +phosphatidic acid/ phospholipase D	NO and PAs serve a protective role in rice seedlings treated with Cd.	(Yang <i>et al.</i> , 2016)
Cd_23	CdCl ₂ (444.8 µM)	4d	SNP (100 mM)	<i>Typha angustifolia</i>	20d	leaves roots	-	+biomass/ cell viability -lipid peroxidation +Cd uptake -SOD/ CAT/ PCs -NPT +GSH/ ASC	NO alleviated Cd toxicity by enhancing the inherent ability of cell walls to chelate Cd as well as regulating antioxidant metabolism.	(Zhao <i>et al.</i> , 2016)

Cd_24	CdCl ₂ (100 µM)	6-24h	SNP (100 µM) cPTIO (200 µM)	<i>Medicago sativa</i> cv. Victoria	5d	shoot roots	+NO (3h) (DAF-FM DA)	+biomass -lipid peroxidation/ Cd uptake +APX -POD/ SOD	NO was involved in the NaHS-induced alleviation of Cd toxicity in alfalfa seedlings indicating a cross-talk between H ₂ S and NO responsible for the increased abiotic stress tolerance.	(Li <i>et al.</i> , 2012)
Cd_25	CdCl ₂ (60 µM)	2d	PTIO (60 µM) SNP (300 µM) GSNO (300 µM) DEA NONOate (300 µM)	<i>Matricaria chamomilla</i>	28d	shoot roots	+NO (DAF-FM DA)	+Cd uptake =GPX/ GR/ ASC +APX/ GSSG -GSH/ PCs	cPTIO and DEA NONOate revealed the less pronounced side impacts and are recommended as suitable NO scavenger/ donor in plant physiological studies under Cd stress.	(Kováčik <i>et al.</i> , 2014)
Cd_26	CdCl ₂ (400-800 µM)	6d	SNP (200 µM)	<i>Sesamun indicum</i>	0d	seedlings	-	+germination/ biomass/ root length +SOD/ CAT/ APX/ POX	Mitigation of Cd damage using SNP occurs due to increase of the activity of the antioxidant enzymes.	(Pires <i>et al.</i> , 2016)
Cd_28	CdCl ₂ (50 µM)	1d	GSNO (50 µM) GA (5 µM)	<i>A. thaliana irt1</i>	1 cm roots	roots shoot	-NO (DAF-FM DA)	+root length -lipid peroxidation/ Cd uptake -RGL1/ RGL2/ GAI/ RGA -IRT1	GA alleviates Cd toxicity by reducing NO accumulation and expression of <i>IRT1</i> in <i>A. thaliana</i> .	(Zhu <i>et al.</i> , 2012)
Cd_29	CdCl ₂ (100 µM)	7d	Hb (10 µM) cPTIO (200 µM)	<i>Oryza sativa</i> cv. Xiushui 11	7d	seedlings	+NO (DAF-FM DA)	+biomass/ root length -Cd uptake/ PBT/ NPT +CaCl ₂ +hemicellulose =cellulose content +pectin content	Ca may alleviate Cd toxicity via endogenous NO with variation in the levels of NPT, PBT, and matrix polysaccharides.	(Zhang <i>et al.</i> , 2012)
Cd_30	CdCl ₂ (100 µM)	7d	SNP (50-400 µM) cPTIO (50 µM) L-NAME (50 µM)	<i>Trifolium repens</i>	14d	seedlings	+NO (HbO)	+biomass/ root length/ Chl -Cd uptake/ traslocation -H ₂ O ₂ -lipid peroxidation +Pro +APX/ CAT/ SOD/ GR -GSH -SA/ ET +JA	NO depleted Cd toxicity by eliminating oxidative damage, enhancing minerals absorption, regulating proton pumps, and maintaining hormone equilibrium.	(Liu <i>et al.</i> , 2015)
Cd_32	CdSO ₄ (50 µM)	21d	Over-expression of <i>NtHb1 nia1</i> and <i>nia2</i>	<i>Nicotiana tabacum A. thaliana</i>	21d	seedlings roots	-NO (DAF-2DA)	-Cd uptake +biomass +CAX3 +CAX3/ ZIP1/ MTP1A/ NRAMP1/ IRT1/ <i>NtHMA-A</i>	Increase of Hb by <i>NtHb1</i> expression promotes Cd tolerance and reduces Cd accumulation by alleviating the level of NO induced by Cd in transgenic tobacco.	(Lee and Hwang, 2015)

Cd_33	CdCl ₂ (50-500 mg/L)	40d	SNP (100 µM) L-NAME (100 µM)	<i>Festuca arundinacea</i>	40d	plants	-	+biomass +Chl/ PSII =germination -Cd uptake (depend on tissue)/ translocation =Photosynthesis	NO alleviated Cd toxicity on the PSII electron donor side, which may be due to its role in the regulation of the level and toxicity of ROS.	(Zhuo <i>et al.</i> , 2017)
Cd_34	CdSO ₄ (9 mM)	24-72h	SNP (50-200 µl)	<i>Triticum aestivum</i> cv. Sönmez-2001/ Quality	5d	seedlings	-	-lipid peroxidation +CAT/ GST =APX +SOD (Sönmez) -SOD (Quality) +MDA (Quality) -MDA (Sönmez)	NO demonstrated a reduced or counteractive effect on the Cd-induced increase in the activities of some typical antioxidant enzymes.	(Mutlu <i>et al.</i> , 2018)
-	CoCl ₂ (10-20 µM)	1-3d	SNP (500 µM) Hb (0.14 g L ⁻¹)	<i>Oryza sativa</i> cv. Taichung Native 1/ Indica type	2d	seedlings roots	+NO (DAF-FM DA)	-HO activity -OsHO1expression	Neither H ₂ O ₂ nor NO is involved in CoCl ₂ -increased HO activity, CoCl ₂ -increased OsHO1 expression and CoCl ₂ -promoted lateral roots formation.	(Hsu <i>et al.</i> , 2013)
-	CoCl ₂ (0.1-100 µM)	28d	SNP (10-40 µM) BA (2.2 µM)	<i>Sideritis raeseri</i>	ex- plant	shoot	-	+shoot number and callus induction percent- age	Synergistic action between SNP and the cytokinin BA. SNP 40 µM has toxic effects.	(Sarropoulou and Maloupa, 2017)
-	CuCl ₂ (50 µmol/L)	8-21d	SNP (100 µM) Hb (0.1%)	<i>Lycopersicon esculentum</i> cv. Meigui	14d	roots leaves	-	+biomass +Cu translocation/ Cu accumulation +pectates proteins	Hb blocks the alleviating effect of NO. The use of exogenous NO to enhance Cu tolerance in some plants is a promising method for Cu remediation.	(Dong <i>et al.</i> , 2013)
-	CuSO ₄ (200 µM)	50d	SNP (0.05 mM)	<i>Nicotiana tabacum</i>	0d	plants	-	+biomass +Chl +rubisco content +rubisco activity +rubisco activase content	SNP could play a protective role in regulation of plant responses to abiotic stresses Cu by enhancing Rubisco and Rubisco activase.	(Khairy <i>et al.</i> , 2016)
Cu_1	CuSO ₄ (150-750 µM)	3h-4d	cPTIO (150 µM) Tungstate (150 µM) L-NAME (150 µM) SNP (200 µM)	<i>Hordeum vulgare</i> cv. Nude	3d	seedlings	+NO (Hb) NR	+biomass +Chl/ rubisco activity =GSH/ ASC -H ₂ O ₂ -NR =O ₂ ⁻ -NOS-I -lipid peroxidation +SOD/ CAT/ APX/ GR =POD/ GPX =Cu uptake	Important role of the NR mediated early NO burst involved in the protection process against Cu toxicity in shoot of hulled barley.	(Hu <i>et al.</i> , 2015)

Cu_2	CuSO ₄ (100 µM)	3d	SNP (200 µM) GSH (200 µM)	<i>Oryza sativa</i> cv. BR 11	14d	seedlings	-	+lipid peroxidation +Pro +O ₂ ⁻ / H ₂ O ₂ +CAT/ GPX/ DHAR/ GST/ ASC/ SOD/ APX/ MDHAR +PCs -Cu uptake +LOX	Adding SNP and GSH might produce GSNO which could be a source of bioactive NO and may affect many regulatory processes. Combined effect of adding SNP and GSH inhibiting the uptake and translocation of Cu.	(Mostofa <i>et al.</i> , 2015)
Cu_3	CuSO ₄ (450 µM)	3h-1d	SNP (200 µM) c-PTIO (150 µM) Tungstate (150 µM)	<i>Hordeum vulgare</i> cv. Nude	3d	roots	+NO (DAF-FM DA)	+root length/ cell viability -O ₂ ⁻ / H ₂ O ₂ -lipid peroxidation +SOD/ POD/ APX -CAT	NO production is an early response of hulled barley roots and contributes to Cu tolerance possibly by modulating antioxidant defense, reducing oxidative stress and PCD in root tips.	(Hu, 2016)
Cu_4	CuCl ₂ (50 µM)	8d	SNP (100 µM) Hb (0.1%)	<i>Lycopersicon esculentum</i> cv. Meigui	21d	leaves roots	-	-biomass -SOD/ POD/ APX +CAT -H ₂ O ₂ -lipid peroxidation -Cu uptake	NO could effectively assuage Cu toxicity by physiological and biochemical response to maintain normal growth.	(Cui <i>et al.</i> , 2010)
Cu_5	CuSO ₄ (5 50 µM)	14d	SNP (10 µM) cPTIO (50 µM) <i>nox1/ gsnor1-3/ nia1nia2 vtc2-1/ vtc 2-3/ high miox4</i>	<i>A. thaliana</i>	14d	roots shoot	+NO (<i>nox1</i> , <i>gsnor1-3</i>) -NO (WT) (DAF-FM)	+biomass +cell viability in <i>nox1,gsnor</i> (25,50 µM) +cell viability (SNP)	NO improves Cu tolerance via modulation of O ₂ and H ₂ O ₂ levels. It is a well-coordinated interplay between NO and ROS during stress tolerance.	(Peto <i>et al.</i> , 2013)
Cu_6	CuSO ₄ (5-25 µM)	7d	cPTIO (500 µM) SNP (10 µM) <i>nia1nia2noa1-2</i>	<i>A. thaliana</i>	7d	seedlings	+NO (DAF-FM DA) NR	+biomass -cell viability	NO contributes to Cu tolerance and its deficiency favours for ROS production.	(Kolbert <i>et al.</i> , 2015)
Cr_1	K ₂ Cr ₂ O ₇ (1-10 mg/L)	12d	SNP (100 µM) L-NAME (100 µM)	<i>Festuca arundinacea schreb</i>	54d	seedlings	-	+biomass +Cr uptake -O ₂ ⁻ / H ₂ O ₂ -SOD +POD -lipid peroxidation =Pro +photosynthetic act. (Chl, <i>PSII</i> , quinone A) +translocation factors	Improved CrVI tolerance of plants suggest that SNP treatment could be a useful application for Cr phytoremediation.	(Huang <i>et al.</i> , 2018)
Cr_2	K ₂ Cr ₂ O ₇ (200-500 µM)	3-9d	SNP (200-500 µM)	<i>Zea mays</i> cv. Agrister	3-9d	roots cotyledons coleoptiles	-	+root length/ biomass -Cr uptake -lipid peroxidation +CAT/ SOD -GOX -NADP-ICDH / NADPME / G6PDH +6PGDH	NO and H ₂ S act as a defence against the negative effects. There are some different results in cotyledons.	(Kharbech <i>et al.</i> , 2017)

Hg_1	HgCl ₂ (60 µM)	3-7d	SNP (100-200 µM)	<i>Oryza sativa</i> cv. Zhonghua	7d	leaves roots	-	+biomass/ root length/ lateral roots -Hg uptake -O ₂ ⁻ / H ₂ O ₂ -SOD/ CAT/ APX/ POD	NO enhanced resistance promoting the transport of IAA in root, decreasing the absorption of Hg, and eliminating ROS directly.	(Chen <i>et al.</i> , 2015)
-	MnCl ₂ (0-1000 µM)	7d	SNP (100-1000 µM)	<i>Matricaria chamomilla</i>	7d	shoot roots	+RNS	-H ₂ O ₂ +biomass +root length -Mn uptake -Cat/ GPX +APX +ROS =GR +Fe	NO participates in tolerance to Mn excess but negative effects in the highest SNP dose were also observed.	(Kováčik <i>et al.</i> , 2014b)
-	MnCl ₂ (15 mM)	1-2d	SNP (100 µM) cPTIO(100 µM)	<i>Oryza sativa</i> cv. Pant-12	17d	leaves	-	-Mn uptake -lipid peroxidation -H ₂ O ₂ +ASC =GSH/ APX -SOD/ GPX/ CAT/ DHAR/ GR	NO appears to provide a protection to the rice leaves against Mn-induced oxidative stress and that exogenous NO application could be advantageous in combating the deleterious effects of Mn-toxicity in rice plants.	(Srivastava and Dubey, 2012)
Ni_1	NiCl ₂ (0.5 µM)	7d	SNP (0.2 mM)	<i>Eleusine coracana</i>	14d	shoot roots	-	+biomass / root and shoot length +Chl -O ₂ ⁻ / H ₂ O ₂ -MDA/ LOX/ Pro +CAT/ SOD/ APX	The combined effect of SA and SNP on easing the Ni stress was greater than that of either SA or SNP alone.	(Kotapati <i>et al.</i> , 2017)
Ni_2	NiSO ₄ (10-200 µM)	9d	SNP (100-200 µM) cPTIO (200 µM)	<i>Oryza sativa</i> cv. Yangliangyou 6	19d	shoot roots	+NO (NO-2-G kit)	+biomass/ root length/ cell viability -Ni uptake +Chl/ carotenoids/ Pro -O ₂ ⁻ / H ₂ O ₂ -lipid peroxidation/ LOX +ASC/ POD/ CAT +CAT/ POD/ APX/ GR/ SOD	NO enhanced tolerance to Ni-stress by restricting Ni accumulation, maintaining photosynthetic performance and reducing oxidative damage through improved antioxidant system.	(Rizwan <i>et al.</i> , 2018)
Ni_3	NiCl ₂ (0.5 mM)	10d	SNP (0.2 mM)	<i>Brassica napus</i> cv. PF	21d	leaves	-	+biomass -lipid peroxidation/ Pro -Ni translocation -H ₂ O ₂ +GPX/ APX/ CAT +Chl -LOX	NO counteract the negative effects of Ni on canola plants.	(Kazemi <i>et al.</i> , 2010)
Ni_4	NiCl ₂ (1 mM)	30d	SNP (0.5 mM)	<i>Triticum aestivum</i> cv. Samma	30d	plants	-	+biomass +carbonic anhydrase +SOD/ POD =CAT +Pro/ N, P, and K +Chl -lipid peroxidation	NO+SA improved plant growth and development by improving activity of antioxidant enzymes, carbonic anhydrase and balance supply of nutrients.	(Siddiqui <i>et al.</i> , 2013)

Ni_5	NiCl ₂ (100 µM)	4d	SNP (100 µM)	<i>Triticum aestivum</i> cv. Yangmai 158	4d	seedlings	-	+H ₂ O ₂ =lipid peroxidation +POD/ APX/ SOD/ GR/ GST =CAT	NO has a protective role modulation antioxidant enzymes. Non extra protection SNP> 200 µM.	(Wang <i>et al.</i> , 2010)
Ni_6	NiCl ₂ (0.2 µM)	4d	SNP (0.3 mM)	<i>Phaseolus vulgaris</i>	2d	roots leaves	-	+Ni uptake -SOD +CAT/ POD -Pro +Ca and Zn	NO activates Ca and Zn uptake as protective processes and attenuates oxidative stress. Ni effects on ion uptake may not be mediated by NO.	(Mihailovic and Drazic, 2011)
-	Pb (C ₂ H ₃ CO ₂) ₂ (400 µM)	6h -3d	ASC (400 µM)	<i>Prosopis farcta</i>	21d	seedlings	+NO (except at 72h) (Griess reagent)	+flavonoids/ phenolic acids -lignin +ADC and PAL +CAT/ GPX/ APX +aconitase activity (24/48h) +H ₂ O ₂ (6 and 12 h); -H ₂ O ₂ from 24 to 48h and steady-state level 72h.	NO production can improve Pb tolerance via wide-ranging effects on a primary metabolic network. Addition of ASC contributes to the cell-wall barrier. APX reduction (after 48h) is a consequence of APX nitrosylation to reduce H ₂ O ₂ scavenging.	(Zafari <i>et al.</i> , 2017)
-	Pb (NO ₃) ₂ (0-200 mg/kg)	20h	SNP (0.05-1 mM)	<i>Vigna unguiculata</i> cv. Walp.	3 leaf stage	roots leaves seeds	-	+biomass/ germination -Pb uptake and translocation +stomatal conductance +IAA, CKs and GA3 -ABA	NO increased Pb tolerance by lowering Pb uptake and translocation. NO plays the same role as CKs action on betacyanin accumulation.	(Sadeghipour, 2017)
-	Pb(NO ₃) ₂ (150 µM)	14d	DEA NONOate (2 mM) SIN-1 (2 mM)	<i>A. thaliana</i> CFP-PTS1	14d	seedlings	-	-CAT	Catalase activity is affected by NO-mediated posttranslational modifications (nitration and S-nitrosylation).	(Corpas and Barroso, 2017)
-	Pb(NO ₃) ₂ (100 µM)	24h	SNP (5 mM) cPTIO (0.5 mM) L-NAME (0.3 mM) PBITU (0.3 mM) GSNO (3 mM)	<i>Pogonatherum crinitum</i>	5d	roots	-NO (Hb/ Griess reagent)	-Pb accumulation -NR	The suppression of the uptake of Pb can be reversed by application of NO.	(Yu <i>et al.</i> , 2012)
Pb_1	Pb(NO ₃) ₂ (50-250 µM)	0-8h	SNP (100 µM)	<i>Triticum aestivum</i> cv. PBW 502	48h	roots	+NO (DAF-2DA/ Griess reagent)	+root length/ cell viability -lipid peroxidation -conjugated dienes -O ₂ ⁻ / H ₂ O ₂ / HO ⁻ -SOD/ CAT/ APX/ GPX/ GR	Exogenous NO partially ameliorates Pb-toxicity, but could not restore the plant growth under prolonged Pb-exposure.	(Kaur <i>et al.</i> , 2015)

Pb_2	Pb(NO ₃) ₂ (500 µM)	14d	SNP (50-400 µM)	<i>Lolium perenne</i>	21d	shoot roots	-	+biomass/ root length +Chl/ photosynthetic rate -translocation of Pb -O ₂ ⁻ / H ₂ O ₂ -lipid peroxidation +SOD/ APX/ POD -CAT	Increased Pb tolerance by exogenous NO at low concentrations included the regulation of chlorophyll content and photosynthesis, the improvement of antioxidant system and the reduction of Pb translocation.	(Bai <i>et al.</i> , 2015)
Pb_3	Pb(NO ₃) ₂ (100 µM)	7d	SNP (0.5 mM) cPTIO (1 mM)	<i>A. thaliana</i>	0d	seedlings roots	-	+root length =Pb accumulation -H ₂ O ₂ -lipid peroxidation -SOD/ CAT/ GR/ GPX/ POD	Ameliorating effects of SNP pre-treatment were associated with the release of NO because cPTIO reversed these effects and SNP did not trigger an avoidance mechanism.	(Phang <i>et al.</i> , 2011)
Pb_4	Pb(NO ₃) ₂ (200 mg/kg)	20h	SNP (0.05-1 mM)	<i>Vigna unguiculata</i> cv. Walp.	0d	plants	-	+SOD/ CAT/ APX/ GR +Pro +Chl/ photosynthesis -lipid peroxidation	NO pretreatment protects cowpea enhancing antioxidant enzyme activities and Pro accumulation.	(Sadeghipour, 2016)
Pb_5	Pb(NO ₃) ₂ (100-500 µM)	15d	SNP (100-200 µM)	<i>Melissa officinalis</i>	15d	shoot roots	-	+biomass +Chl -lipid peroxidation +APX / CAT/ GPX	NO at low concentration improved photosynthesis and counteracted oxidative damage acts as an efficient membrane stabilizer.	(Jafarnezhad-Moziraji <i>et al.</i> , 2017)
-	ZnCl ₂ (400 µM)	2-10d	PTIO (0.2 mM) L-NAME (0.5 mM)	<i>Solanum nigrum</i>	10d	roots	-NO (Hb)	-root length -Zn accumulation -H ₂ O ₂ / O ₂ ⁻ -MDA (until 4d) +CAT/ APX -SOD -NOX activity	NO is involved in Zn-modulated root system development.	(Xu <i>et al.</i> , 2010)
-	ZnSO ₄ (3 mM)	6d	PTIO (250 µM)	<i>Triticum aestivum</i> cv. Xihan 3	seedlings	roots leaves	-NO (DAF-FM DA)	+cell viability -Zn accumulation -root length -H ₂ O ₂ +O ₂ ⁻ =MDA/ DAO/ POD -CAT/ APX +SOD/ GR/ PAO/ NOS activity	Excessive endogenous NO correlated with the highest NOS activity and the decrease of extracellular ROS production as the result of the inhibition of PM NADPH oxidase, cell wall-bound POD, DAO and PAO.	(Duan <i>et al.</i> , 2015)
-	ZnSO ₄ (0.05 mM)	14d	SNP (0.5 mM)	<i>Triticum aestivum</i> cv. Kazakhstanskaya 10	4d	shoot roots	-	+biomass +photosynthesis +alternative oxidase (shoot) -alternative oxidase (roots) -lipid peroxidation	Similarity between the protective reactions of wheat under the influence of NO through changes in the energy balance (Rtot/ Pnet) and antioxidant balance.	(Gil'vanova <i>et al.</i> , 2012)

-	ZnSO ₄ (100-500 µM)	14d	SNP (100-200 µM)	<i>Plantago major</i>	21d	leaves roots	-	+biomass +Chl -lipid peroxidation =H ₂ O ₂ +peroxidase activity +CAT/ APX/ SOD	Application of low level of SNP could mitigated Zn stress as a defend mechanisms of plants against Zn toxicity.	(Savadkoobi <i>et al.</i> , 2017)
-	ZnSO ₄ (200 µM)	4d	L-NAME (1 mM)	<i>Nicotiana tabacum</i> cv. xanthi	35d	leaves	+NO (DAF-2 DA)	Small and delayed lesions =Zn accumulation in apoplast	Zn-related lesions in leaves develop from groups of mesophyll cells in which accumulation of high Zn level contributes to enhancement of the NO level and to the initiation of PCD processes.	(Weremczuk <i>et al.</i> , 2017)
Zn_1	Zn ONPs (250 mg/L)	3d	SNP (10 µM) cPTIO (100 µM) <i>noa1</i> <i>noe1</i>	<i>Oryza sativa</i> cv. Jiafuzhan	3d	seedlings	+NO (DAF-FM DA)	+root length/ biomass (10 µM) +Chl -Zn accumulation -O ₂ ⁻ / H ₂ O ₂ =lipid peroxidation +GSH -SOD +CAT/ APX/ POD (<i>noe1</i>)	NO could reduce H ₂ O ₂ accumulation and alleviate oxidative damage by regulating GSH level. NO is involved in enhancing Zn tolerance in rice through modulating anti-oxidant gene expression.	(Chen <i>et al.</i> , 2015)
Zn_2	ZnSO ₄ (500 µM)	10d	SNP (100 µM)	<i>Carthamus tinctorius</i> cv. Arak2811	21d	roots shoot	-	+biomass -translocation -lipid peroxidation -H ₂ O ₂ -Pro +CAT/ APX/ GPX/ GSH/ ASC +GSH/GSSG ratio +α-tocopherol	Elevated levels of GSH may be associated with its active role in detoxification of ROS.	(Namdjoyan <i>et al.</i> , 2018)
Zn_3	ZnSO ₄ (2 µM)	10-30d	GSNO (100 µM)	<i>Triticum aestivum</i> cv. Chinese Spring	30d	roots shoot	+NO (DAF-FM DA)	-biomass -Zn uptake =Zn accumulation =GSH =Chl +ASC	GSNO could modulate Zn uptake and root-to-shoot translocation during the transition from deficient to sufficient levels of Zn-supply.	(Buet <i>et al.</i> , 2014)
Zn_4	Zn ²⁺ (10 mg/L)	1-4d	SNP (25-50 µM)	<i>Hydrilla verticillata</i>	10d	leaves	+NO (Hb)	-Zn uptake (4d) +Cu/ Fe/ Mn +lipid peroxidation/ carotenoids -Chl +SOD/ CAT/ APX/ ASC -POD/ DHA	NO application improved the nutrient balance and antioxidant ability in response to Zn stress. Expression of Fe uptake-related genes could be induced by NO.	(Zhang <i>et al.</i> , 2014a)

Zn_5	ZnSO ₄ (0.05-0.5 mM)	25d	SNP (0.1 mM)	<i>Zea mays</i> cv. DK 647 F1	35d	roots	-	+root length/ cell viability +Chl +RWC -Pro	NO is involved in the physiological or metabolic activity in plants grown at Zn toxicity.	(Kaya, 2016)
Zn_6	ZnSO ₄ (500 µM)	10d	SNP (100 µM)	<i>Carthamus tinctorius</i> cv. Arak2811	21d	roots shoot	-	-biomass -Zn uptake -H ₂ O ₂ -Pro/ MDA +Chl +CAT/ APX/ GPX/ ASC +α-tocopherol/ PCs	SA and SNP ameliorate the deleterious effects of Zn toxicity, probably due to stimulation of antioxidative defense mechanisms and PCs biosynthesis.	(Namdjoyan <i>et al.</i> , 2017)

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