Review

The Role of NO in the Amelioration of Heavy Metal Stress in Plants by Individual Application or in Combination with Phytohormones, Especially Auxin

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Abstract: Since the time of the Industrial Revolution, the accumulation of various heavy metals (HMs), such as cadmium (Cd), arsenic (As), lead (Pb), chromium (Cr), mercury (Hg), copper (Cu), zinc (Zn), nickel (Ni), etc., has increased substantially in the soil, causing a real risk to all kinds of consumers in the food chain. Moreover, excess HM accumulation is considered a major factor in decreasing plant growth and productivity. A number of recent studies have exhibited the astonishing impact of nitric oxide (NO), a multifunctional, gaseous signal molecule, on alleviating the destructive effects of HMs. Many reports revealed the noteworthy contribution of NO in reducing HM uptake and toxicity levels. In the present review, focus is given to the contribution of NO to decrease the toxicity levels of different HMs in a variety of plant species and their accumulation in those species. Simultaneously, this review also demonstrates the effects of NO on HM-stressed species, by its use both individually and along with auxin, a plant-growth-promoting phytohormone. Different perspectives about the reaction to the co-application of NO and auxin, as well as the differential role of NO to overcome HM stress, have been expanded.

Keywords: auxins; heavy metal stress; nitric oxide; phytohormones; signaling

1. Introduction

Over many years, plants are repeatedly exposed to certain critical and challenging environmental conditions, such as abiotic factors (drought, cold, heat, salt, metal, etc.), that directly impact the total agricultural productivity [1,2]. Among all the abiotic means, heavy metal (HM) stress is one of the most complicated and emerging issues. HMs, non-biodegradable in nature, are metallic elements possessing relatively higher density than water [3]. Currently, the contamination of soil by HMs has become a very serious concern and a threat to the worldwide agricultural system [4]. The accumulation of these HMs affects the quality of soil, which, in turn, damages the balance of the entire food chain [5]. Among the list of HMs, such as arsenic (As), lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg), copper (Cu), iron (Fe), zinc (Zn), nickel (Ni), cobalt (Co), etc., some play an important part in plant growth and development, as well as in metabolism. However, on the contrary, these HMs, when accumulated to an increased level of concentration, may result in deleterious effects on the physiology and biochemistry of plants [6–10].

As soon as these HMs are deposited on the ground, plants can easily absorb them from the soil and incorporate them into each level of the ecosystem, resulting in an increased risk of toxicity caused by HMs directly for plants and indirectly for both animals and humans [11,12]. The bioaccumulation of these HMs in the food web can be highly dangerous.
Hence, the presence of HMs in the atmosphere, soil, or water, even in trace concentrations, may lead to serious problems for all living organisms [13]. Studies have shown that HMs can cause damage even at the DNA level, along with their carcinogenic effects in higher organisms.

The production of reactive oxygen species (ROS) has great importance in plants in the fight against abiotic stress [14]. HMs may become highly reactive in nature according to their oxidative states [15]. Most of the old theories and findings stated that ROS are potentially active by nature; however, they may be harmful (in high concentration), or sometimes they can even be very useful for the plant cells [16]. On top of that, it is also considered that ROS must not be involved in any sort of reactions with proteins, lipids, or nucleic acids due to the oxidative damage to the cell caused by this [17]. Contrary to such conventional theories, ROS possess a major role in managing a number of physiological processes, for instance, plant development [17–19] and developing stress tolerance [20].

Several methods can be implemented to mitigate the stress caused by HMs, for instance, the replacement method (washing of soil), exchange of ions, metal precipitation, adsorption in water [1,21], or even several natural means like biochar [22], defense mechanisms by tolerance and avoidance [23,24], and phytoremediation [25–27]. Among all of these, the contribution of several natural phytohormones is immense. Phytohormones such as auxin, gibberellins, cytokinin, ethylene, and abscisic acid (ABA) have significant importance in alleviating HM toxicity.

Nitric oxide (NO), which is a versatile, gaseous signal molecule, has great importance in reducing HM toxicity in plants with the help of its antioxidant defense mechanisms to minimize oxidative stress [28,29]. This signaling molecule has displayed involvement in a variety of physiological processes in plant growth and development, and it has also proved its significance under stress conditions [30–32]. The role of NO is a contributing factor towards controlling HM stress in plants. So, in this review, the main focus has been given to the signaling pathways of NO and how it can help plants to overcome HM stressed conditions in the soil, by its use both individually and in combination with auxin.

2. Origin and Sources of NO

It is important to focus on some major aspects of the origin and source of NO and its signaling pathway before discussing its contribution to HM stress. Probable sites of NO generation in plants have been found in the peroxisome, mitochondria, and chloroplast. The biosynthesis of NO may occur by two types of pathways, enzymatic and non-enzymatic [30].

Under the enzymatic pathway, the L-Arginine (L-Arg)-dependent pathway has a major role in producing NO as it produces a huge amount of end product [33]. This pathway includes NADPH-mediated oxidation of L-Arg with the help of an enzyme, NO synthase [34,35]. There is another pathway called the nitrite-dependent NO biosynthesis pathway, which is regulated by nitrite reductase enzyme [33,36]. However, this nitrite-mediated pathway is a non-enzymatic pathway that occurs under some specific conditions, as in the case of low pH [37]. Besides the reduction of nitrite to NO, the production of NO can also be mediated by the mitochondrial electron transport chain [38,39]. The nitrite reductase enzyme catalyzes the reduction process of nitrate to nitrite. During this step, NADH acts as an electron donor [33]. Along with NADPH, molybdoprotein, iron or heme, and FAD are required to participate as co-factors [40]. The significance of NO production in the presence of nitrite reductase is huge in the field of plant physiology when applying both the aspects of pharmacology and genetics [41,42]. Apart from NR, there are also some other potential sources of NO synthesis in plants, for example, cytochrome 450 and copper amino oxidase-1 [33]. NO can also be synthesized in the peroxisome by Xanthine oxidoreductase (XOR) enzyme [43].

In other ways, NO can be generated by oxidative or reductive pathways [30]. The production pathways of NO from L-arginine, hydroxylamine, and polyamine are included among the oxidative pathways, whereas plasma-membrane- or mitochondrion-associated
NO generation pathways using the previously described nitrate reductase (NR) enzyme and nitrite (NO$_2^-$) as a substrate (commonly called the NR:NiNOR system) are included in the reductive pathways [44]. Oxidative pathways are well established in plants, as many studies have reported from stems, leaves, and roots of various model systems like pea, soybean, chili, tomato, etc. [30,43,45,46]. From our previous communications and others also, the generation of NO can be prohibited by the application of NOS inhibitor (L-NAME) in various model plants [47–50]. According to some works, oxidative and reductive pathways are interdependent and do not work discretely [51].

After synthesis, the main role of NO is acting as a signaling molecule in various pathways in the plant system [52]. It has been observed that NO preferentially reacts with metal-containing proteins of plants and forms complexes [53]. A strong correlation between leghemoglobin and NO in the form of Nitrosyl-leghemoglobin complex (LbFeINO) has been well established [53]. Besides hemoglobin cytosolic and mitochondrial aconitase, catalase, lipoxygenase, ascorbate peroxidase, and cytochrome c oxidase are documented as the preferred targets of NO. Further, reactions between derivatives of NO (highly reactive ONOO$^-$) and tyrosine residues of target proteins have also confirmed NO signaling in plants [54]. Not only that, but S-nitrosylation is depicted as one of the most significant post-translational modifications of various proteins, which is mostly driven by NO in biological organisms, including plants. S-nitrosothioglutathione (GSNO) developed by the interaction of NO and reduced glutathione (GSH) plays an important role in various key processes in the development of plants [55]. The production and probable signaling roles of NO are depicted in Figure 1.

![Figure 1](image-url)

Figure 1. Schematic diagram showing the enzymatic and non-enzymatic pathways of NO production, transport and signaling, and functions in various cell organelles like the peroxisome, mitochondria, and chloroplast. Arg: Arginine, Hb: Hemoglobin, GSNO: S-nitrosothioglutathione, ONOO$^-$: Peroxynitrite, NO$_2^-$: Nitrogen dioxide, SNO: S-Nitrosothiol, TF: Transcription factor, NO$_2^-$: Nitrite.

3. Correlation between ROS and NO under HM Stress in Plants

Both ROS and NO are well-known signaling molecules in plants; they play very crucial roles in fighting against HM stress via several complex mechanisms. Stable production of ROS and NO is an important phenomenon in a plant cell in response to different biotic and abiotic stresses, including HM stress [56]. Extreme ROS generation during excessive oxidative stress has negative impacts on cell membrane lipids and causes huge peroxidation of lipids and electrolytic leakage [50,57]. However, NO production counteracts oxidative
stress and maintains cellular homeostasis by acting as an intracellular signaling molecule for
the construction of enzymatic and non-enzymatic defense administrations in the infected
region of plants [56,58]. Furthermore, NO alleviates Cd stress in plants by acting as an ROS
scavenger. On top of that, reports have revealed that NO impedes ROS-induced cytotoxicity
in Brassica juncea under Cd stress. H2O2 (an important form of ROS) showed up-regulation
of antioxidant enzymes in Cd-stressed plants; on the other hand, NO is considered a factor
responsible for the down-regulation of these antioxidant enzymes. The signaling activities
and the relationship between ROS and NO play an essential role in stress-tolerant gene
expression and in producing long-distance sensing, such as from root to shoot regions [59].

4. The Interplay of NO and Auxin to Reduce Heavy Metal Stress in Plants

Many recent studies have demonstrated that NO can act both upstream [60] and
downstream of auxin [61]. The efficient coordination of plant responses demands auxin to
act along with other phytohormones or plant growth regulators (PGRs) and some signaling
molecules, such as NO [62]. NO synthesis increases in the roots of rice seedlings when it is
applied after the extrinsic application of auxin [63]. Auxin promotes the formation of root
hairs [64] and lateral roots [65] through this greater production of NO. This is an instance
in which NO functions downstream of auxin.

According to [66], the co-application of auxin and NO has shown remarkable effects
in the formation of root nodules (Figure 2). In the case of adventitious root formation, NO
plays a very important role as a second messenger. It has been demonstrated through
experiments [66] that NO not only helps in the intermediate formation of root nodules but
also stimulates the growth of lateral roots, for which they used Sinorhizobium meliloti (a
bacterium) and Rhizobium leguminosarum.

A report showed that NO damages root growth in rice by controlling auxin transport
under the supply of nitrate [67]. On the other hand, if we look into the co-action of auxin and
NO under HM stress, it has shown some contradictory results. For example, in Arabidopsis
thaliana, NO application under Cd-stressed conditions decreased the auxin level in primary
roots, thereby affecting the root meristem severely [68]. However, under Mg deficiency,
NO acted positively on the same plant by controlling AUX1 expression. Another relevant
example was found by [69] in rice (Oryza sativa) seedlings, in which exogenous application
of NO induced auxin transport in the roots under Hg-stressed conditions, resulting in
an improvement of the quality of resistance in rice (Figure 2), but, interestingly, under deficiency of iron (Fe), NO has been noticed to reduce auxin levels, eventually restricting root elongation.

While specifically focusing on the interaction and complex mechanisms between auxin and NO under HM stress, we found diverse reports. By the findings of [70], both As and Cd affect the root system of rice seedlings by altering the biosynthetic pathway of auxin (also known as indole acetic acid or IAA) and its distribution; NO could reduce the Cd-mediated effects in rice roots but was unable to mitigate As stress [71].

5. NO Production in Different Plants under Heavy Metal Toxicity

The chemical background of NO becomes complicated when it is applied to biological systems [72]. The main reason behind this is the rapid reaction of NO with oxygen to form different nitrogen oxides; on the other side, several factors such as the concentration and system redox states of NO, along with the concentrations of its target molecules and metals, regulate its stabilization [73]. Delledonne [74] demonstrated some contradictory effects of HMs on the internal concentration of NO in plants as a result of changes in both the concentration and redox state of NO. In addition to such controversy, some other factors have also been identified and found responsible, such as difficulties in detecting NO, and, moreover, in measuring it [75]. However, NO synthesis in plants by purified mitochondria and peroxisomes and NR have potentially been measured via many different types of methods. Examples include chemiluminescence, DAF-fluorescence, fluorescence imaging, electron paramagnetic resonance spectroscopy (EPR), oxyhemoglobin/methemoglobin, laser photo-acoustics, NO electrodes, and mass spectrometry [76–80].

The content of NO in different parts of different plants may either increase or decrease depending upon the type of HM, as represented in Table 1.

Table 1. Variability in the level of NO in different species caused by heavy metal stress.

| Name of Species       | HM Stress | Type of Tissue Exposed to HMs | Duration of Exposure to HMs | Level of NO Content in the Tissue | References |
|-----------------------|-----------|-------------------------------|-----------------------------|----------------------------------|------------|
| Arabidopsis thaliana  | Al        | Root                          | 1 h                         | Fall                             | [81]       |
|                       | Al        | Root                          | 3 days                      | Both rise and fall               | [81]       |
|                       | Cd        | Cell suspension               | 72 h                        | Rise                             | [82]       |
|                       | Cd        | Leaf                          | 96 h                        | Rise                             | [35,83]    |
|                       | Fe        | Cell suspension               | 30 min                      | Rise                             | [84]       |
|                       | Pb        | Seedling                      | 14 days                     | Rise                             | [77]       |
| Brassica juncea       | Cu        | Root                          | 7 days                      | Rise                             | [85]       |
|                       | Zn        | Root                          | 7 days                      | Rise                             | [85]       |
| Glycine max           | Cd        | Cell suspension               | 72 h                        | Rise                             | [86]       |
| Hibiscus moscheutos   | Al        | Root                          | 20 min                      | Fall                             | [87]       |
| Hordeum vulgare       | Cd        | Root                          | 24 h                        | Rise                             | [88]       |
| Medicago truncatula   | Cd        | Root                          | 48 h                        | Fall                             | [89]       |
| Nicotiana tabacum     | Cd        | Cell suspension               | 12 h                        | Rise                             | [90]       |
| Triticum aestivum     | Cd        | Root                          | 5 days                      | Rise                             | [91]       |
|                       | Cd        | Root                          | 3 h                         | Rise                             | [91]       |
| Oryza sativa          | Cd        | Root                          | 24 h                        | Fall                             | [72]       |
|                       | Cd        | Root and shoot                | 7 days                      | Fall                             | [92]       |
| Panax ginseng         | Cu        | Root                          | 24 h                        | Rise                             | [93]       |
Table 1. Cont.

| Name of Species | HM Stress | Type of Tissue Exposed to HMs | Duration of Exposure to HMs | Level of NO Content in the Tissue | References |
|-----------------|-----------|------------------------------|----------------------------|----------------------------------|------------|
| *Pisum sativum* | Cd        | Root                         | 7 days                     | Rise                             | [85]       |
|                 | Cd        | Root                         | 15 days                    | Fall                             | [94]       |
|                 | Cd        | Leaf                         | 14 days                    | Fall                             | [95,96]    |
|                 | Cu        | Root                         | 7 days                     | Rise                             | [85]       |
|                 | Zn        | Root                         | 7 days                     | Rise                             | [85]       |
| *Pogonatherum crinitum* | Pb   | Root                         | 24 h                       | Rise                             | [97]       |
| *Solanum nigrum* | Zn; Zn + Fe | Root                        | 0–10 days                  | First rose up to Day 2–3, then began to fall | [89]       |

The levels of NO have mainly been studied under Cd stress. For instance, wheat roots were kept for 5 days [91] or 3 h [83] under Cd stress. Both cases showed a rise in the NO content. However, in the case of rice, both roots and shoots kept for 7 days [92] and only rice roots kept for 24 h [72] under Cd stress showed a decline in the NO content. Studies with Cd-stressed *Pisum sativum* roots exposed for 7 days showed an increased level of NO [85], while the same set kept for 15 days showed a decrease in the NO content [94], but Cd-stressed leaves of *Pisum sativum* kept for 14 days indicated a decreased amount of NO [95,96].

Both a cell suspension of *Glycine max* [86] and *Nicotiana tabacum* [90] exposed to Cd stress for 72 h and 12 h, respectively, displayed a rise in the NO content. Roots of *Medicago truncatula* were kept two times (48 h) longer than roots of *Hordeum vulgare* (24 h) under Cd-stressed conditions. The NO content increased in the roots of *H. vulgare* [88], while *M. truncatula* roots exhibited a decrease in NO production. Both a cell suspension of *A. thaliana* kept under Cd for 72 h [82] and the same material kept under Fe for 30 min [84] displayed an increased amount of NO. Seedlings of *A. thaliana* also exhibited a rise in NO levels when incubated for 14 days under exposure to Pb [77]. Interestingly, a drop in NO content was observed in the roots of *A. thaliana* kept under Al stress for an hour [81]. However, leaves of *A. thaliana* kept under Cd exposure for 96 h displayed a rise in NO levels [35,83].

Bartha et al. [85] used the roots of *Pisum sativum* and *Brassica juncea* and kept them under Cu stress for 7 days. After the incubation period of 7 days, both sets showed an increase in the NO content. Similar results were found with the same plant root specimens under Zn stress [85]. For experimental purposes, [98] kept the roots of *Solanum nigrum* under two different stressed conditions; the first set contained Zn, while the second set included Fe along with Zn, and these two sets were incubated for 0 to 10 days. The result showed an increase in the NO content for 2–3 days, and then it started to decline.

As many different factors, such as the concentration and treatment time of HMs, variety of plant species, size and shape of the plant at the time of HM treatment, etc., are thought to be associated with changes in endogenous NO content in plants, there has been much debate among researchers about the actual cause of such change. One of the possible reasons for the decrease in the endogenous NO content, mentioned by [99], is that calcium deficiency has been observed in plant leaves under Cd stress, which disturbed NOS-like enzyme activity; as a result of this, the endogenous NO content is reduced. An increase in NR enzymatic activity [100,101] and the genotypes of plants [102,103] have been referred to as potential factors that can cause an increase in NO content in Cd-stressed plants. Apart from that, as NO can readily react with oxygen and form nitrogen oxides, the balance between the binding state and intracellular redox state of some specific smaller molecules to NO can also be considered a responsible factor for the increase or decrease in the endogenous NO content in plants [102,103].
6. The Role of NO in Overcoming Stress Caused by Different Heavy Metals

Over the past few decades, rapid industrialization has contaminated natural resources to some great extent [104]. As a result of such industrial development, HM toxicity [105] has adversely affected plants, animals, microorganisms [106], and, all in all, the entire ecosystem [104].

As a multipurpose gaseous signaling molecule [36,107], NO makes a powerful contribution in inducing plants to stand against the toxic attack of HMs (Table 2) through both exogenous and endogenous application [107].

Table 2. Reports on the effects of NO or NO donors against a variety of heavy metal stresses on different plant species.

| Application of NO or NO Donor Individually or in Combination with Other Phytohormones | Name of Heavy Metal Causing Stress | Plant Species under HM Stress | Role of NO in Alleviating HM Stress | References |
|---|---|---|---|---|
| Indirect application of NO | Al | *Phaseolus vulgaris* | Reducing oxidative stress in the roots | [103] |
| Exogenous NO application | Al | *Secale cereale* and *Triticum aestivum* seedlings | Reducing Al accumulation in the apical zone of roots to promote Al tolerance | [104,108] |
| NO individually | As | *Oryza sativa* | Minimizing the levels of ROS and malondialdehyde (MDA) | [29] |
| NO individually | As | *Oryza sativa* | Modulating regulatory networks involved in JA biosynthesis. | [105,109] |
| NO individually | Cd | *Typha angustifolia* | Improvement in the plant growth and development, total yield of biomass by suppressing Cd stress | [106,110] |
| NO individually | Cd | *Oryza sativa* | Reducing alterations in the root system | [73] |
| NO individually | Cd | *Oryza sativa* | Stopping Cd accumulation by enhancing the pectin and hemicelluloses content in the cell wall of the root system | [72] |
| Indirect application of NO downstream of auxin, in presence of a bacterium, *Bacillus amyloliquefaciens* SAY09 | Cd | *Arabidopsis* sp. | Activating auxin-mediated signaling pathway to bring Cd toxicity under control | [111,112] |
| SNP at low concentrations | Cd | *Oryza sativa* | Promoting cadmium tolerance of rice by increasing pectin and hemicellulose contents in root cell wall | [72] |
| SNP along with glutathione | Cu | *Oryza sativa* | Reducing Cu uptake and oxidative damage | [113] |
Table 2. Cont.

| Application of NO or NO Donor Individually or in Combination with Other Phytohormones | Name of Heavy Metal Causing Stress | Plant Species under HM Stress | Role of NO in Alleviating HM Stress | References |
|---|---|---|---|---|
| Indirect application of NO | Cu | *Panax ginseng* | Reducing oxidative damage in the adventitious roots | [114] |
| NO donor | Cd and Pb | Bamboo species (*Arundinaria pygmaea*) | Increasing antioxidant activity, protein content, photosynthetic properties, plant biomass, and plant limiting metal translocation from roots to shoots, and diminishing metal accumulation in the roots, shoots, and stems | [115] |

A very well-suited example includes exogenous application of NO in both rice and *Vigna radiata* L. seedlings under As stress conditions, in which NO was able to ameliorate the toxic effects of heavy metal As by minimizing the levels of ROS and malondialdehyde (MDA). A similar kind of result of overcoming HM toxicity in rice by NO application was reported in another study against Cu stress [108]. In *Typha angustifolia*, NO demonstrated remarkable improvement in plant growth and development and also in the total biomass yield by suppressing Cd stress [106].

Experiments were performed on rice seedlings (*Oryza sativa* L.) under Cd and As stress to investigate whether NO could ameliorate both these HMs’ toxicity or not [71]. Their studies revealed that under Cd stress, the endogenous concentration of NO can diminish alterations in the root system, but it is unable to suppress the majority of damage caused by As. Xiong et al. [72] reported that NO enhances the pectin and hemicelluloses content in the cell wall of the root system of rice, which helps to stop the accumulation of Cd in the leaves of rice seedlings. An indirect contribution of NO was observed to alleviate Cd toxicity with the help of *Bacillus amyloliquefaciens* SAY09 acting downstream of auxin by activating the auxin-mediated signaling pathway [107]. Recent studies have reported the ability of NO in decreasing AsIII toxicity by the modulation of jasmonic acid (JA) biosynthesis [88].

Emamverdian et al. [110] proved through their experiments that SNP (sodium nitroprusside, a strong nitric oxide donor) can reduce the accumulation of two specific HMs, Pb and Cd, in the root and shoot system in plants. In addition to that, under HMs like Pb and Cd-stressed conditions, the NO donor showed remarkable contributions in many parameters, including increases in the protein, non-protein, and total thiol contents; protection of the plasma membrane and cell-developing antioxidant-enzyme activities in bamboo plants; elongation of the shoot length; and many more [110]. The application of SNP has shown some incredible results in reducing the effect of Cd-generated ROS production [61] and regulating the metabolic state of antioxidation in some crops, such as mustard [116], wheat [117], rice [104], and peanut [118]. SNP was added to Cd-stressed rice plants at low concentrations, and the result of this experiment showed stimulation of the Cd tolerance of rice by increasing the pectin and hemicellulose contents in the root cell wall [72]. In this context, Correa-Aragunde et al. [118] performed experiments on tomato plants by applying both low and high concentrations of NO individually, in which enhancement of cellulose synthesis was observed in tomato roots, while, on the other hand, application of NO donor in a higher concentration showed reverse results. According to Lombardo et al. [64], NO plays a very significant role as a positive regulator in root hair development. Studies have shown that the exogenous application of NO helps to decrease Al accumulation in the root apical zones of rye and wheat seedlings [104].
7. Conclusions and Future Prospects

The combined results of research studies showed that NO is a very important multifunctional signal molecule in alleviating HM stress in a variety of plants. It plays a defensive role against several heavy metals, such as Cd, Al, Cu, As, Pb, and Hg. Co-application of NO along with auxin promotes the growth and development of root hairs, root nodules, and lateral and adventitious roots, along with an improvement in resistance ability in rice seedlings under Hg stress. The NO content varies from one plant species to another under different HM stresses. So, there is a huge and perpetual conflict regarding this topic.

Reports have also demonstrated that NO plays a significant role as a NO donor (SNP) in reducing oxidative damage and restricting the absorption and accumulation of HMs such as Cd, Cu, and Pb. Exogenous application of NO is functional in significantly reducing metal uptake in Al-stressed seedlings of wheat and rice. Another continual matter of dispute lies between the application of NO individually or NO in combination with auxin. According to some studies, co-application of NO and auxin is more efficient in reducing the detrimental effects caused by HMs, while some other reports demonstrate that NO rather restricts auxin transport, which, in turn, affects the formation and growth of roots in several plants. These controversies may subside in the future with one specific result by extensive studies with the use of different HMs, their different concentrations, different exposure periods, different plant species, and so on.

The application of exogenous NO on plants under different HM stresses downstream of auxin has shown some remarkable results in decreasing HM stress in different plant species. Roychoudhury et al. [119] showed that exogenous application of NO donor (SNP) reduces cadmium-induced oxidative stress in Vigna sp. However, studies are very limited in this particular area. Further investigations will be required to find which of the phytohormones work best along with NO to reduce HM stress in plants.

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