Elevated levels of heavy metals (HMs) in agricultural soils have led to decreased soil fertility and crop yield and also threatened human health by entering through the food chain (McLaughlin et al. 1999). The accumulation of metals in the major food chain may result in serious ecological and health problems. Nickel (Ni) and zinc (Zn) are metals considered as nutrients essential for plant growth and development, where 0.01-5 µg g\(^{-1}\) dry weight for Ni (Seregin and Kozhevnikova 2006) and 8-100 µg g\(^{-1}\) dry weight for Zn (Shaw et al. 2004) have been reported to maintain enzymes activity and physiological and metabolic processes in crop plants. However, the concentrations of these metals are gradually increasing in agricultural soil through both natural processes and anthropogenic activities (Shikazono et al. 2008; Chen et al. 2009), which may become phytotoxic (Gajewska and Sklodowska 2005; Chen et al. 2009; Tsonev and Lidon 2012). Phytotoxicity induced by HMs may be the result of increased production of reactive oxygen species (ROS), replacement of enzyme cofactors and transcription factors, cellular redox imbalance, ionic transport imbalance, DNA damage, protein oxidation, and inhibition of photosynthetic processes (Sytar et al. 2013). In addition, hormonal changes are also associated with metals stress and photosynthetic inhibition (Masood et al. 2012). Metals stress causes photooxidative damage by altering the chloroplast membrane functions and component of photosynthetic electron transport chain, impairing the light phase of photosynthesis (Ventrella et al. 2011). Stomatal and non-stomatal photosynthetic processes are most sensitively inhibited by elevated ROS level under high HM stress (Khudsar et al. 2004; Mobin and Khan 2007; Chen et al. 2011; Masood et al. 2012). Recently, Li et al. (2013) have shown that decrease of photosynthesis with HM stress is related to the inhibition of maximal PS II photochemical efficiency (Fv/Fm), actual PS II photochemical efficiency (\(\Phi_{PS II}\)), photochemical quenching (qP), electron transport rate (ETR), and increase of non-photochemical quenching (NPQ) in *Elsholtzia argyi*. 

Exposure of plants to elevated levels of HMs increases the production of ROS. To protect themselves against ROS, plants employ several defense mechanisms
including antioxidant defense system. It has been now well established that the induction of the cellular antioxidant machinery has an important role in protection against various abiotic stresses including HMs stress (Khan et al. 2010; Nazar et al. 2011; Masood et al. 2012; Khan et al. 2013; Khan and Khan 2014). Plants up-regulate production of non-enzymatic and enzymatic antioxidants under stressful environments that cooperatively manage to remove/neutralize or scavenge excess ROS (Noctor and Foyer 1998; Noctor et al. 2012) and maintain high rates of photosynthesis (Foyer and Shigeoka 2011). The studies deciphering the ways to modulate redox state to regulate enzymes activity and/or influence the production of antioxidant such as reduced glutathione (GSH) may be an important approach for the alleviation of heavy metal stress-accrued anomalies in crop plants.

In addition to the antioxidant system, scavenging of ROS to restore cell redox metabolism, cellular osmotic adjustment, protection of biological membranes, and stabilization of enzymes/proteins and cellular structures are among the multiple protective functions of compatible osmoprotectants during HM stress (Mittler 2002). The accumulation of proline is one of the adaptive mechanisms that plants operate for survival under stress condition. Considering that proline accumulation is associated with stress tolerance, the information on the enzymes that regulate proline biosynthesis and accumulation, glutamyl kinase (GK) and proline oxidase (PROX), is also essential. Proline metabolism involves enzymes, GK, and glutamyl phosphate reductase, regarded as enzyme complex P5C synthetase. The enzyme GK is considered as the key enzyme in proline metabolism. Moreover, PROX also influences proline accumulation as it degrades proline and provides glutamate to enter the pathway. Thus, proline metabolism may be considered as a strategy to increase tolerance of plants to HM and protection of photosynthesis. The investment of greater N to proline backbone and photosynthetic apparatus by increasing photosynthetic-nitrogen use efficiency (NUE) may also contribute to the protection of photosynthesis against HMs stress.

The up-regulation of sulfur (S) assimilation pathway alleviates HM toxicity and improves capacity of plants to survive in a HM polluted environment (Wangeline et al. 2004). An increase in serine acetyltransferase (SAT) activity and GSH content increased tolerance to Ni (Freeman et al. 2005). Similarly, studies have shown that
both ATP-sulfurylase (ATP-S) and SAT play important roles in cadmium (Cd) tolerance in *Triticum aestivum* (Khan et al. 2007), *Brassica juncea* (Wangeline et al. 2004) and *Arabidopsis* (Howarth et al. 2003). Induction of ATP-S activity has been shown necessary for the maintenance of optimal GSH levels required for the proper functioning of Ascorbate (AsA)-GSH cycle in *Brassica juncea* (Khan et al. 2009). Enhanced GSH production through induction of S metabolism by HMs has been shown to involve a coordinated transcriptional regulation of genes for sulfate uptake and its assimilation as GSH and phytochelatins (PCs) biosynthesis (Nocito et al. 2006). Induction of PCs is one of the main detoxification strategies against HMs and preventing its toxicity (Cobbett 2002).

Ethylene is a gaseous plant hormone that potentially influences many aspects of plant growth and photosynthesis (Abeles et al. 1992; Pierik et al. 2006; Iqbal et al. 2013a; b). It also plays a crucial role in the adaptation to abiotic stress (Masood et al. 2012; Khan et al. 2013). The ethylene-regulated photosynthetic processes depend on the sensitivity of plants to ethylene (Pierik et al. 2006; Iqbal et al. 2012a). Ethephon (2-chloroethyl phosphonic acid) has been used commercially to produce ethylene in crop plants. The ethylene released from ethephon affects several cellular, developmental, and stress response processes related to photosynthesis (Fiorani et al. 2002; Khan et al. 2008a) and the role of ethylene in regulation of physiological process and plant development has been also reported (Khan 2004a; b; Tholen et al. 2007; Khan et al. 2013; Iqbal et al. 2013a; b). Perata and Voesenek (2007) have suggested that ethylene-regulated stress responses are essential for stress tolerance and for the survival of plants. The production of ethylene in plant species subjected to toxic levels of Cd, copper (Cu), iron (Fe), and Zn has been shown (Wise and Naylor 1988; Maksymiec 2007). Moreover, Cd- and Cu-mediated stimulation of ethylene synthesis was found as a result of the increase of 1-aminocyclopropane carboxylic acid (ACC) synthase (ACS) activity (Pell et al. 1997). However, the potential role of ethylene in the regulation of photosynthetic responses through antioxidant metabolism under elevated levels of essential HMs, such as Ni and Zn has not been clearly demonstrated. It was hypothesized that the management of Ni and Zn-induced oxidative stress may become possible through positive influence of ethylene on the plant’s metabolisms, such as antioxidant metabolism, S metabolism, nitrogen (N) metabolism, proline metabolism, and protects photosynthesis and growth under the
metals stress. The integrated knowledge of various metabolisms is necessary to know how complex metabolic networks interact and how they are modulated under stress tolerance processes.

To test the hypothesis experiments on mustard (*Brassica juncea* L.) were conducted with the following objectives.

1. Selection of ethylene-sensitive and ethylene-insensitive mustard cultivars using ethephon as ethylene-source and norbornadiene (NBD) as ethylene action inhibitor, and evaluation of the role of ethylene in augmenting photosynthesis and growth of these cultivars.

2. Study of changes in metabolism of antioxidants, S, N and proline, water relations, ethylene biosynthesis, photosynthesis and growth of ethylene-sensitive mustard type grown with different concentrations of Ni and Zn.

3. To evaluate the role of ethylene in regulation of metabolism, protection of photosynthesis and growth of ethylene-sensitive mustard type grown with Ni and Zn using ethylene modulators, ethephon as ethylene-source and NBD as ethylene action inhibitor.

It is worth mentioning that a part of the present study has been published as Khan and Khan (2014). Briefly, it is reported below:

Khan and Khan (2014) selected ethylene-sensitive cultivar from five mustard (*Brassica juncea* L.) cultivars, namely Type 59, Rayada, Chutki, Trishul and Anmol 555, by treating them with ethylene modulators. An approach of utilizing chemicals to modify ethylene synthesis and action was considered to assess the sensitivity of plants to ethylene. Ethephon and NBD were selected as tool chemicals to modify ethylene synthesis and ethylene action, respectively. The mustard cultivars were treated with 0 (control), 200 µL L⁻¹ ethephon, or 100 µM NBD at 20 days after sowing (DAS). Each of the treatment was given in 50 mL together with 0.5% surfactant teepol. The control group of plants was sprayed with an equal amount of deionized water plus 0.5% teepol. Further, the influence of exogenously sourced ethylene (200 µL L⁻¹ ethephon) in the protection of photosynthesis against 200 mg kg⁻¹ soil each of Ni-and Zn-accrued stress in ethylene-sensitive mustard type was studied. Plants grown with Ni or
Zn but without ethephon exhibited increased activity of ACS, and ethylene with increased oxidative stress measured as H$_2$O$_2$ content and lipid peroxidation compared with the control plants. The oxidative stress in Ni-grown plants was higher than Zn-grown plants. Under metal stress, ethylene protected photosynthetic potential by efficient PS II activity and through increased activity of ribulose-1, 5-bisphosphate carboxylase (Rubisco) and photosynthetic-NUE. Application of 200 µL L$^{-1}$ ethephon to Ni or Zn-grown plants significantly alleviated toxicity and reduced the oxidative stress to a greater extent together with the improved net photosynthesis due to induced activity of ascorbate peroxidase and glutathione reductase, resulting in increased production of reduced GSH. Ethylene formation resulting from ethephon application alleviated Ni and Zn stress by reducing oxidative stress caused by stress ethylene production and maintained increased GSH pool. The involvement of ethylene in reversal of photosynthetic inhibition by Ni and Zn stress was related to the changes in PS II activity, photosynthetic-NUE and antioxidant capacity was confirmed using ethylene action inhibitor, NBD.