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Silicon-mediated heavy-metal tolerance in durum wheat: evidences of combined effects at the plant and soil levels

THESE

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Abstract

Plant growth is seriously limited by heavy metal toxicity. Studies suggest that silicon (Si) can alleviate the metal stress in plants but its bioavailability in soil is not well defined. This study aims at assessing the bioavailability of Si from different soil minerals, the meaning of various extractants in terms of availability in relation with continuous plant uptake, and the specific role of Si on the plant growth and alleviation of cadmium (Cd) and copper (Cu) toxicity in durum wheat. For this purpose a series of experiments were performed both in soil and hydroponic conditions. Firstly, the significance of acid and alkaline methods was assessed in order to determine the bioavailable Si in soil and the applicability of the Na_2CO_3 extraction method was determined by repeated croppings of durum wheat on a variety of soils. Secondly, two hydroponic experiments were carried out to study the physiological response of wheat under Cd and Cu stress treated or not with Si. Thirdly, efficiency of different minerals to release bioavailable silica and its effect on reducing Cd and Cu toxicity was assessed. Finally, the effects of amorphous silica (ASi) application were investigated on metal stressed wheat plant grown in uncontaminated and contaminated soils.

ASi extracted with Na_2CO_3 (ASi_{Na}) was well correlated with the Si in plants shoots in our pot experiment with different soils. We showed that ASi_{Na} can also be used for a good proxy of Si bioavailable to plants. Si present as amorphous Si (diatomite) added to the soil is available for plant uptake. However, in soils with large clay content, clay may be a significant source of Si for plants. Si supplementation suppressed the chlorosis symptoms and increased plant biomass and photosynthetic pigments under metal stress. Si addition decreased Cd and Cu uptake and root-to-shoot translocation by increasing metal adsorption on the roots apoplast and Zn and Mn uptake. ASi application in soil slightly increased the pH of soil, reduced the available Cd in soil and played a significant role in reduction of Cd concentration in wheat shoots. The present results suggest that Si might be useful to enhance Cd and Cu tolerance in durum wheat grown in metal contaminated soils.

Key words: silicon, amorphous silica, heavy metals, uptake, wheat, remediation

+Résumé court

La croissance des plantes est limitée par la toxicité métallique. Des études suggèrent que le stress métallique peut être limité par l'absorption de silicium (Si). Cependant la phytodisponibilité du silicium n'a pas encore été clairement évaluée.

Ainsi dans un premier temps cette étude a pour objectifs d'évaluer la disponibilité de Si issu de différents minéraux du sol et la signification des différents extractants utilisés pour l'extraction de Si du sol en terme de disponibilité lors d'une culture en continu de blé dur. Pour ce faire, des extractants acides et alcalins ont été comparés sur une série de sols non contaminés de nature différente, puis la validité de Na_2CO_3 pour l'estimation de la fraction disponible de Si pour une culture continue de blé dur (*Triticum turgidum* L.) a été testée sur une série de sols contrastés.

Ensuite on a tenté de déterminer le rôle spécifique de Si dans la croissance de plantules de blé dur et la suppression des stress associés à la présence de Cd et Cu dans le milieu de croissance, à la fois en conditions hydroponiques et sur support solide simplifié en comparant un alumino-silicate et une diatomite. Finalement, l'apport de silicium amorphe (ASi) (et disponible) a été testé en pot sur les plantes de blé ayant poussé sur des sols multi-contaminés ou contaminés en Cd.

ASi extrait par Na_2CO_3 (ASi_{Na}) est apparu bien corrélé aux concentrations dans les parties aériennes du blé dur ayant poussé en pot sur différents sols non contaminés et, pour cette raison, pourrait être utilisé pour l'évaluation de la fraction phytodisponible.

L'ajout de Si dans les différentes expériences réduit la chlorose et a augmenté la biomasse et les concentrations en pigments photosynthétiques de plantes sous stress métallique. Il a aussi diminué l'absorption de Cd et Cu ainsi que la translocation vers les parties aériennes en accroissant les concentrations apoplasmiques et l'absorption de Zn et Mn. De plus, l'ajout de ASi sous forme de diatomite dans le sol contribue à une légère augmentation de pH favorisant une diminution de la disponibilité de Cd dans le sol, contribuant ainsi également à la diminution des concentrations en Cd dans les plantes.

Au final, l'ensemble des résultats suggèrent l'existence d'une série de mécanismes concourant à diminuer le stress métallique de plants de blé et nous conduit à proposer l'ajout de silicium pour accroître la tolérance à Cd et Cd du blé dur.

mots-clés: silicium, silice amorphe, cadmium, cuivre, élément trace métallique, abosrption, blé, remédiation

Résumé étendu

Au vu de l'étendue de la contamination des sols par les éléments traces métalliques et du défi posé par la nécessité de remédier et/ou les utiliser pour la culture, il apparaît nécessaire de trouver des solutions alternatives à même de réduire la toxicité métallique et le transfert des métaux le long de la chaîne alimentaire et dans les autres compartiments de l'environnement. Le silicium est un élément présent en grande quantité dans la croûte terrestre et qui peut être accumulé dans les végétaux jusqu'à plus de 10%, surtout dans les monocotylédones, atteignant ainsi des valeurs équivalentes à celles des macronutriments. Bien que le silicium représente environ 28% de la croûte terrestre, la plupart des sources de Si ne sont pas disponibles pour le prélèvement par les plantes. Dans les écosystèmes naturels, les phytolithes (particules de silice amorphe d'origine végétale) sont considérées comme une source de silicium soluble à $pH>4$. Dans les sols cultivés, les phytolithes ne retournent pas au sol si les pailles sont exportées, suggérant un appauvrissement possible en Si soluble. D'autre part, récemment il a été montré que Si pouvait diminuer, voir supprimer, le stress métallique chez plusieurs plantes, bien que les mécanismes qui en sont responsables n'aient pas été entièrement élucidés. Dans une optique de réduction de la toxicité métallique, il est important de s'interroger sur la disponibilité de Si dans les sols ainsi que la manière de la mesurer ainsi que sur les mécanismes à l'origine de cette réduction de toxicité.

Le but de ce travail est d'évaluer la biodisponibilité de Si issu de différents minéraux, de quantifier son appauvrissement suite à une culture continue de blé et de comprendre le rôle de Si sur la croissance et la physiologie du blé dur soumis à un stress métallique (Cd ou Cu). La thèse est divisée en 2 parties principales subdivisées en plusieurs objectifs.

Après une introduction générale (chapitre 1), le second chapitre présente une revue de littérature et identifie les manques dans la compréhension des mécanismes. Il souligne les effets toxiques de Cd et Cu sur la croissance végétale et le manque de connaissances concernant la biodisponibilité de Si. Il identifie également les points nécessitant des développements en ce qui concerne les mécanismes impliqués dans la diminution du stress métallique chez les plantes ou augmentation de la tolérance.

Dans le chapitre 3, nous avons comparé différents extractants acides ou alcalins pour la quantification et comparaison de la fraction extractible de différents sols et évalué la capacité de Na_2CO_3 à prédire l'absorption de Si par le blé. Dans une première partie, deux extractions acides (acide acétique et oxalate d'ammonium) ont été comparés à deux extractions alcalines (carbonate de sodium et Tiron®) afin de caractériser différent sols en termes de quantité de silice amorphe. Ces sols ont été préparés de manière à éviter la formation de nouvelles surfaces réactives sur les argiles pouvant conduire à une surestimation de ASi. Pour ce faire, ils ont été homogénéisés à la main, séchés à 40°C pendant 78h, broyés à la main (et non dans un broyeur) et tamisés à 50 microns. Les résultats montrent que ASi extrait au carbonate de sodium (ASi_{Na}) et ASi extrait à l'oxalate ne représentent pas les mêmes pools de Si, tandis que le Tiron (ASi_{Ti}) extrait des quantités plus importantes de Si et également des quantités appréciables de Al et Fe des Andosols, ce qui est en accord avec les résultats de Sauer et al. (2006).

Dans la seconde partie du chapitre 3, plusieurs paramètres (ASi dans le sol et Si le blé, Si dissous (DSi) dans la solution du sol et le pH) ont été mesurés dans une expérience d'exportation de paille conduite sur 9 rotations de blé dur et 3 sols non contaminés et présentant des teneurs variées en ASi. Un Andosol prélevé sous forêt, un Podzosol et un Calcisol agricoles ont été plantés 2 fois par an entre 2007 et 2012 avec du blé dur, initialement en chambre climatisée puis en serre. Les solutions du sol ont été échantillonnées à partir de la rotation 5 à différents intervalles de temps au cours de la croissance des plantes avec des bougies poreuses de type Rhizon (SMS, Rhizon®) installées après le semis dans chaque pot. Les concentrations en Si dans les plantes ont été comparées aux concentrations en Si mesurées en solution ainsi que l'extrait Na_2CO_3 du sol échantillonné après récolte. Les résultats montrent une bonne adéquation entre ASi_{Na} et les concentrations en Si des plantes et donc que Na_2CO_3 peut estimer de manière satisfaisante la fraction de Si disponible pour les plantes.

Dans la première partie du chapitre 4, une expérience en hydroponie a testé le rôle de Si dans la tolérance Cd de plantules de blé dur soumis à des concentrations croissantes de Cd en solution avec ou sans Si (0, 0,5, 5,0 et 50 µM avec ou sans 1,0 mM Si). Les plantes ont tout d'abord été exposées à 0 ou 1,0 mM Si pendant 10 jours avant de recevoir pendant 11 jours les doses croissantes en Cd citées précédemment. Les solutions nutritives ont été changées tous les 3 jours afin de maintenir une fourniture en nutriments aussi constante que possible. Le pH de la solution était ajusté à 6,5 ± 0.2 avec 1 mM de tampon MES (acide 2-

morpholinoethane sulphonique) si nécessaire. Après récolte, les paramètres suivants ont été mesurés : biomasse, concentrations en chlorophylles et carotenoïdes, concentrations en Cd et nutriments dans les parties aériennes et les racines ainsi que les concentrations en anions inorganiques (Cl, NO_3, SO_4 et PO_4) et organiques (malate, citrate et aconitate) dans le contenu cellulaire des racines et des feuilles. Les phytolithes ont été extraits des feuilles et analysés par microscopie électronique à balayage couplée à un détecteur élémentaire (MEB-EDX). La localisation de Cd, Si et certains éléments majeurs a été également effectuée à la fois par MEB-EDX et en micro fluorescence X sur des échantillons lyophilisés de racines issues du traitement 50 µM Cd + 1mM Si. La localisation dans les feuilles a été faite par µXRF uniquement pour des raisons de limite de détection. Enfin, la surface des racines a été analysée sur une profondeur < 100 Å par spectroscopie photoélectronique X (XPS).

Les résultats montrent que les concentrations utilisées provoquent une diminution des valeurs des paramètres associés à la biomasse, des concentrations en pigments photosynthétiques et des nutriments, à la fois dans les racines et les parties aériennes, et cela de manière croissante avec l'augmentation des concentrations en Cd en solution. Les concentrations en anions organiques augmentent dans les parties aériennes et les racines tandis que les concentrations en anions inorganiques diminuent à l'exception du chlorure, parallèlement à l'augmentation des concentrations en Cd dans la solution. Ces effets sont réduits en présence de Si : l'ajout de Si augmente la longueur racinaire et des pousses, la concentration en pigments photosynthétiques et diminue les concentrations en Cd dans les parties aériennes, tandis que la concentration en Cd apoplasmique augmente (quantité désorbée), et cela par rapport aux plantes sans Si. La concentration racinaire de Zn est également augmentée de même que les concentrations en anions inorganiques dans les parties aériennes et les racines, tandis que les concentrations en anions organiques diminuent dans ces 2 compartiments. Cette production d'acides organiques plus faible en présence de Si, et plus spécifiquement les concentrations en citrate et aconitate pourraient être responsables de la diminution de la translocation de Cd complexé des racines aux parties aériennes. Dans le traitement 50 µM Cd + 1 mM Si, Cd a surtout été observé dans le cortex tandis que Si était surtout localisé dans l'endoderme des racines. La co-précipitation de Cd avec Si n'est pas apparue comme un processus dominant, que ce soit dans les parties aériennes ou les racines. Les analyses par XPS montrent un enrichissement en Si et Cd à la surface des racines (< 100 Å) ayant poussé dans 50 µM Cd + 1 mM Si par rapport à celles issues du traitement 50 µM Cd sans Si. Ceci nous conduit à suggérer l'existence d'un mécanisme de protection des racines par accumulation de Si et Cd à la surface des racines. Le bilan global de Cd et Si montre par ailleurs qu'en présence de Si les quantités totales prélevées de Cd sont moins élevées que lorsqu'il n'y a pas de Si en solution.

Dans la seconde partie du chapitre 4, une expérience en hydroponie, similaire à la précédente, a été conduite avec Cu. De la même manière, les plantes ont été exposées pendant 10 jours à 0 ou 1mM de Si dans la solution nutritive, puis pendant 10 jours supplémentaires à des concentrations croissantes de Cu (0, 0,7, 7,0 et 30 µM). Le reste du protocole est similaire à celui suivi pour l'expérience avec le cadmium. Ainsi, après récolte, les concentrations en pigments photosynthétiques, en anions, Si et micro et macronutriments ont été analysés dans les parties aériennes. Les micro et macronutriments, de même que Cu, ont également été mesurés dans les racines avant et après désorption à l'acide chlorhydrique dilué tandis que les concentrations en anions et Si ont été mesurées dans les racines avant désorption. La localisation de Si, Cu, Zn etc…a été effectuée par µXRF par le biais de transects effectués d'épiderme à épiderme en passant par le cylindre central, et cela sur des coupes transversales de racines.

Les résultats montrent que des concentrations croissantes en Cu diminuent les concentrations en Si dans les parties aériennes tandis qu'elles accroissent les concentrations dans les racines. L'ajout de Si réduit de manière significative la toxicité cuprique dans le cas des concentrations en Cu les plus élevées. L'ajout de Si compense partiellement la réduction de taille des parties aériennes et des racines associée à la présence de Cu et ce, de manière concomitante à une réduction des quantités de Cu prélevées et de la translocation racine-feuilles, et une augmentation des concentrations en pigments photosynthétiques, macronutriments et anions organiques (malate, citrate et aconitate) dans les racines. L'ajout de Si augmente aussi l'adsorption de Cu à la surface des racines tandis qu'il diminue l'adsorption de Zn et Mn. De plus, les analyses par µXRF montrent qu'en présence de Si, Cu et Mn sont préférentiellement localisés dans l'épiderme et Si se trouve dans l'endoderme, tandis qu'ils sont localisés dans le cylindre central lorsqu'il n'y a pas de Si en solution. Cette étude montre donc que la tolérance accrue à Cu induite par l'ajout de Si au milieu de culture est essentiellement due à une réduction de l'absorption et de la translocation de Cu en excès et une fixation plus importante de Cu dans l'épiderme des racines. L'addition de Si régule également les concentrations de complexants potentiels de Cu comme le malate, le citrate ou l'aconitate, en réponse à une diminution probable des concentrations de Cu libre. Ces résultats suggèrent que Si pourrait donc être intéressant pour accroître la tolérance au cuivre du blé dur, en particulier dans les sols contaminés au cuivre.

Dans la dernière partie du chapitre 4, une expérience en pot a été conduite afin d'évaluer la capacité de différents minéraux à fournir du silicium disponible pour les plantes et leur effet sur des plantes soumises à des stress métalliques (Cd et Cu). Pour cela, nous avons utilisé du quartz (Q), une argile (vermiculite, C) et une source de silice amorphe d'origine naturelle, à savoir une diatomite, D, issue d'une mine (poudre de diatomées fossiles commercialisée sous le nom de Clarcel 78® par la CECA, groupe ARKEMA) à Saint-Bauzile (France), et contenant 87% de SiO_2, à savoir ASi sous une forme très concentrée. Les 13% restant comprennent les éléments suivants : Al, Fe, Ca, Mg et K. C'est donc un minéral de type opale A dont il a été montré qu'il se dissout facilement (Fraysse et al., 2009). Ces minéraux ont été mélangés en proportions variables à hauteur d'un total de 300 g de substrat par pot. Les mélanges ont été constitués de la manière suivante (% matière sèche, MS) : 95%Q+5%D/C, 85%Q+15%D/C et 75%Q+25%D/C. Du blé dur a été mis à germer et pousser pendant 60 jours sur ces substrats additionnés d'une solution nutritive et de 0 ou 2 mg Cd kg^{-1} apporté sous forme de $Cd(NO_3)_2.4H_2O$ ou de 0 ou 20 mg Cu kg^{-1} apporté sous forme de $CuSO_4.5H_2O$. Après récolte, le pH_{eau} a été mesuré et les concentrations en Cu et Cd ont été mesurées dans les extraits DTPA-TEA des mélanges, tandis que les concentrations en Si, Cd ou Cu ont été analysées dans les parties aériennes et les racines.

Les résultats montrent que Si apporté sous forme de diatomite accroît la biomasse et la concentration en Si des parties aériennes et des racines quand les plantes sont exposées à Cd ou Cu tandis qu'on n'observe aucun changement de ces paramètres pour les plantes ayant poussé sur vermiculite et quartz. Il apparaît cependant qu'une partie du silicium apporté par la vermiculite est disponible pour les plantes, ce qui démontre que les argiles peuvent également être une source de Si disponible pour les plantes. Il est donc possible de conclure que des quantités importantes de Si disponible, conjointement à une immobilisation partielle de Cd et Cu dans les mélanges testés et une séquestration des métaux dans les racines, semblent réduire la toxicité métallique dans le blé dur. La réduction dépendra des quantités de ASi présentes. De plus, une augmentation de pH dans la modalité Q+C semble contribuer à immobiliser Cd et Cu dans le substrat.

Dans le chapitre 5, une expérience en pot avec des sols contaminés a été menée afin d'évaluer les effets bénéfiques de ASi sur des plants de blé dur en conditions plus réalistes et afin de vérifier les résultats obtenus dans les chapitres précédents. Dans une première partie un sol historiquement multi-contaminé a été testé, tandis que dans la seconde partie on a procédé au dopage d'un sol agricole par ajout de Cd. Les principaux objectifs de ce travail étaient de nouveau d'étudier l'effet d'application de ASi au sol sur des plantes sous stress métallique et de mettre en évidence l'effet de cette application sur les pools de métaux disponibles dans le sol et sur le transfert au blé avec un focus particulier sur Cd.

On a utilisé un Luvisol limono-sableux pollué il y a 20 ans par un panel de métaux (Cd, Cu, Zn et Pb) suite à l'application régulière de boues d'épuration municipales et industrielles auquel ont été ajoutées des doses croissantes de la même source de ASi naturelle (diatomite) que celle utilisée dans le chapitre 4. ASi a été ajouté à des doses croissantes de 0, 1, 10 et 15 tonnes ASi ha^{-1} et mélangé manuellement avant répartition dans 3 pots pour les contrôles sans plantes et 4 pots pour les modalités avec plantes. Les plantes ont été récoltées après 71 jours, juste avant épiaison. Les parties aériennes et les racines ont été séparées avant rinçage à l'eau distillée, ensuite rinçage à l'EDTA pour les racines suivi de 3 rinçages supplémentaires à l'eau distillée et séchage à respectivement 70 et 80°C pour les parties aériennes et les racines jusqu'à poids constant. La solution du sol a été prélevée par Rhizon à différents intervalles de temps (47, 57, 64 et 71 jours après semis). Le pH a été mesuré puis les solutions ont été acidifiées avant analyse des concentrations en Si, Cu, Zn Cd et Pb. Les concentrations en métaux ont été également mesurées dans les extraits DTPE-TEA des sols selon la procédure modifiée de Lindsay and Norvell (1978) ainsi que dans les parties aériennes et les racines des plantes.

Les résultats montrent que la longueur des pousses, ainsi que le poids sec des parties aériennes et des racines augmentent avec les doses croissantes de Si ajouté. De même, les concentrations en Si des parties aériennes et des racines et les quantités totales de Si prélevé augmentent avec les doses croissantes de Si. A l'inverse, les concentrations en Si dans les parties aériennes diminuent tandis que celles des racines augmentent avec les doses croissantes de Si. Les concentrations de Zn dans les 2 compartiments des plantes augmentent tandis que celles de Cu et Pb ne montrent pas de variations significatives. Le pH des sols des pots avec plantes est plus élevé que celui des pots sans plantes et diminue légèrement dans les traitements 10 et 15 t ASi ha^{-1} de ces derniers, mais ne présente pas de variation en présence de plantes. Les concentrations en DTPA-extractible Cd diminuent légèrement dans tous les traitements avec ASi. Il en est de même pour Zn, mais aucun changement n'intervient pour Cu et Pb. Le pH est plus élevé dans la solution du sol collectée dans les pots avec plantes et augmente encore avec les apports croissants de ASi. Les concentrations en Si sont plus élevées dans les solutions de sol prélevées dans les pots sans plantes que dans celles des pots avec plantes et augmentent progressivement avec le temps. De même, les concentrations en Cd sont plus faibles dans les pots plantés après 57 jours mais il n'y a pas de différence entre les différentes modalités de traitement avec ASi.

Nous en concluons que la présence de Si disponible pour les plantes diminue les concentrations en Cd dans les parties aériennes de plants de blé dur et pourrait être utilisée en soutien des différentes techniques ou approches utilisées pour limiter les concentrations en Cd dans les plantes. Les doses d'apport utilisées semblent suffisantes pour couvrir les besoins de la plante, mais la diatomite est rapidement épuisée (solubilisée) et cette approche nécessite donc probablement des apports réguliers pour maintenir une certaine efficacité.

Dans la seconde partie de ce chapitre 5, l'expérience de dopage avec Cd d'un sol initialement non contaminé a été conduite avec l'addition de 10 mg kg^{-1} de Cd sous forme de $Cd(NO_3)_2.4H_2O$. L'objectif était d'une part de pouvoir comparer dans un même sol l'effet de ASi sur des plantes non stressées ou stressées par la présence de cadmium, et d'autre part, de mettre en évidence l'effet d'un apport de ASi à un sol fraîchement contaminé sur le pool de métal disponible ainsi que sur son transfert aux plantes. Afin d'éviter une distribution hétérogène de Cd ajouté à une dose relativement faible, Cd a été apporté sous forme soluble. L'ensemble du protocole est ensuite similaire à celui suivi précédemment. Les résultats sont sensiblement différents de ceux précédemment exposés, puisque visuellement il n'apparaît pas de différences sur la longueur des parties aériennes quel que soit l'apport de ASi sur les 2 modalités de traitement (avec ou sans Cd). Dans le sol sans Cd, l'apport de ASi n'induit pas d'augmentation de biomasse. Cependant l'ajout de Cd diminue le poids sec des parties aériennes dans tous les traitements ASi, sans pour autant modifier celui des racines. Par contre les concentrations en Si dans les plantes sont plus importantes avec ajouts de ASi et sont toujours plus importantes dans les parties aériennes que dans les racines avec ou sans Cd. En ce qui concerne les concentrations en Cd, celles-ci diminuent significativement dans les parties aériennes avec les ajouts croissants de ASi tandis qu'elles ont tendance à augmenter dans les racines. L'apport de ASi n'a pas d'incidence sur les concentrations en Zn et Cu dans les plantes sans Cd, tandis qu'il les augmente dans les parties aériennes et les diminue dans les racines des plantes ayant poussé dans le sol dopé avec Cd. Enfin le pH du sol et de la solution du sol, de même que les concentrations en métaux extraits par DTPA-TEA ne sont pas affectées par l'ajout de ASi, tandis que les concentrations en Cd dans les solutions du sol diminuent. En conclusion, les résultats montrent que l'effet de ASi sur la tolérance au stress induit par la présence de Cd dans le sol est essentiellement dû à des modifications de la balance de Cd et des micronutriments Zn et Cu. On observe un effet combiné sol-plante : en effet, l'ajout de ASi au sol tend à immobiliser Cd dans le sol, tandis qu'au niveau de la plante, Cd est séquestré de manière plus importante dans les racines, son transfert dans les parties aériennes est réduit et l'absorption de Cu et Zn est accru, dans les plantes bénéficiant d'apport de ASi par comparaison à celles non supplémentées. Cet effet n'est pas observé sur les plantes non soumises au stress métallique.

Chapter 1: General Introduction

Wheat is the World's major crop in terms of food production. Wheat is a nutritious and economical source of food. On dry matter bases the total food produced by the World's top 30 crops, about 23.4% comes from wheat, followed by maize 21.5% and rice 16.5% (Harlan, 1995). Wheat (*Triticum aestivum* L.) is the most produced cereal crop in Europe and the second most produced in the world, with 228 and 686 MT produced in 2009, respectively (FAOSTAT, May 2011). There are different varieties of wheat grown worldwide depending upon climatic conditions of the area and durum wheat (*Triticum turgidum* L.) is one of them. Durum wheat is an important and valuable crop in many parts of the world which is due to its high protein content and gluten strength. It is cultivated in semiarid regions of the world such as North Africa, Mediterranean Europe, the North American Great Plains and the Middle East (Elias, 1995). France is the second largest durum wheat producer in Europe after Germany. However, durum wheat is sensitive to soil contaminants received by different sources. Durum wheat accumulates larger, due to unknown reasons, amount of heavy metals than bread wheat (*Triticum aestivum* L.) plant (Stolt et al., 2003; Koleli et al., 2004). Heavy metals enter the human body by different channels including food, water, air, or arial deposition on skin. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues (Roberts, 1999).

Soil contamination is indeed considered one of the main threats to soil as identified in the EU soil communication (CEC, 2002). This contamination originates from natural sources by means of mineral dissociation, weathering of parent material and atmospheric deposition as well as anthropogenic sources related to mining, industrial emissions, disposal or leakage of industrial wastes, application of sewage sludge to agricultural soils, fertilizer and pesticide use. Heavy metals like Cd, Pb, Mn and Zn contribute the major share of soil contamination. Due to the potential toxicity and high persistence of heavy metals, soils polluted with such elements create an environmental problem that threats the plant, animal and human health (do Nascimento and Xing, 2006).

Among heavy metals cadmium (Cd) and copper (Cu) are most important as compared to other metals due to large number of reasons such as Cd has no known biological role while Cu slightly larger than the required concentrations is extremely toxic to plants (Kabata-Pendias and Pendias, 2001). Copper is an essential micronutrient that is required in small quantity to constituent protein and for the functioning of many enzymes, especially those participating in electron flow (Epstein and Bloom, 2005; Yruela, 2005). Copper creates immunity against plant diseases caused by different organisms so that higher concentration of copper is normally used in fungicides, bactericides and pesticides for agricultural use especially in vineyards and greenhouse farming to control plant diseases (Scheck and Pscheidt, 1998; Zheng et al., 2004; Michaud et al., 2007). A concentration of Cu exceeding 10 µM in soil solution is a potential hazard for plants (Yruela, 2009; Marschner, 2002) and acts as a phyto-toxin.

On the other hand, Cd has no known biological function in plants and animals and is highly toxic to both plants and animals (Das et al., 1998; Wagner, 1993). It has been widely reported that accumulation of Cd in plants may cause many biochemical, structural and physiological changes (Das et al., 1998; di Toppi and Gabrielli, 1999; Benavides, 2005). Plant growth and photosynthesis is negatively affected by Cd (Sandalio et al., 2001). In plants, most visible symptom of Cd is the reduction in root length (Guo and Marschner, 1995). However, the mechanisms behind these toxicities are not yet fully explored. In addition, Cd also affects the absorption and translocation of essential micro- (Zn, Cu and Mn) and macronutrients (N, P, K, Ca and Mg) by plants (Jalil et al., 1994; Cheng, 2003; Sarwar et al., 2010). One of the main path ways for heavy metals to enter the plants is through the roots because root is a primary organ that participates primarily in the heavy metal uptake due to the direct contact with the soil solution, containing these metals (Lasat 2002; Lux et al., 2011).

Different technologies exist for *in situ* remediation of these toxic heavy metals in soil and sediments and to reduce metal uptake by plants (Mulligan et al., 2001; Keller et al., 2005; Roy et al., 2005; Wuana and Okieimen, 2011). These technologies include immobilization, phytoextraction, phytostabilization, physical separation and extraction etc. However, these traditional methods are expensive, time consuming and have negative impact on the surrounding ecosystems (McGrath et al., 2001). In the present scenario we need to remediate the soils from these toxic metals by the use of quick, inexpensive and environment friendly manner.

Silicon is a very important part of the earth's crust and it consists about 28% of the earth's crust (Sommer et al., 2006). However, Si is only 0.03% of the biosphere (Fauteux et al., 2005). In fact, silicon is accumulated in many plants up to 10% by weight (Hodson et al., 2005) that is greater than some macronutrients (Epstein, 1994). Silicon has been shown to alleviate the deleterious effects of heavy metals in plants grown on contaminated soils (Chen et al., 2000). Experiments have demonstrated that certain crops benefit significantly from Si application (Takahashi et al., 1990). Various beneficial effects of Si have been identified in plants (Epstein, 1994, 1999). Silicon is known to play a significant role in alleviating the toxic effects of metals in plants by several mechanisms such as: modifying metal uptake and translocation, metal binding to cell walls and also co-precipitation with Si, etc… (Corrales et al., 1997; Rogalla and Romheld, 2002; da Cunha and do Nascimento, 2009). It has been hypothesized that silicates are able to convert the soluble and exchangeable fractions of metals in the soil into stable chemical forms by a number of reactions such as silicate-induced pH rise, adsorbed with Fe-Mn oxides (Chen et al., 2000; Liang et al., 2005). In this way silicate decreases the metal bioavailability (Sommer et al., 2006) and alleviates the deleterious effects of heavy metals in plants (Neumann and Zur Nieden, 2001). In plants, Si may alter translocation and distribution of metals in different plant parts that can help the plants to survive under higher metal stress (Shi et al., 2005b, Zhang et al., 2008). Hence the use of Si to enhance plant growth and alleviate heavy metals toxicity is predicted to become an emerging trend in the agriculture in near future.

The most causal survey of the literature on Si in crop plants revealed that rice dominates the discussion (Ma and Takahashi, 2002; Shi et al., 2005; Zhang et al., 2008). This emphasis might give rise to the impression that this crop is unique in absorption and utilization of silicon. However, Rafi and Epstein (1999) reported that Si is rapidly absorbed by wheat (*Triticum aestivum* L.) plants from solution culture containing Si at 0.5 mM. In fact wheat is also a Si accumulator plant (Mayland et al., 1991) but has received little attention compared to rice and sugarcane. The present work is based on the promise that there is nothing unique in rice or sugarcane in response to silicon.

Keeping in view the importance of silicon in alleviating the deleterious effects of heavy metals in plants, an understanding of Si bioavailability and its mechanisms in the reduction of metal toxicity in durum wheat is required. This dissertation forms the basis for the research described hereafter, with the following specific objectives.

- To improve the methodology for the characterization of the different pools of silica easily available to plants.
- To evaluate the effect of silicon on growth and physiology of durum wheat seedlings under Cd and Cu stress separately to identify and better understand the possible mechanisms of Si-mediated alleviation of Cd and Cu toxicity in wheat plant.
- To evaluate the efficiency of different minerals to release the plant available silicon and its effect on Cd and Cu toxicity in durum wheat plant both in soil and plant.
- To document the effect of silicon amendment in heavy metal tolerance in wheat plants grown on a variety of soils so that successful field experiments can be conducted.

Chapter 2: Review of literature

Rapid growth in population and global changes especially during the 20th century has resulted in the rise of pollution worldwide. The production of metals such as mercury, lead, cadmium (Cd), copper (Cu) and arsenic has resulted due to both natural and anthropogenic activities such as weathering of parent material, mining, the burning of fossil fuels and during the manufacturing of certain products like paints and batteries (Camill, 2010). Some of these metals like Cu, Mn and Zn are essential for normal plant growth and become toxic at higher concentrations regardless of their essentiality (Kabata Pendias and Pendias, 2001) while the second category of metals like Pb and Cd are non essential and toxic at very low concentrations to both plants and animals. Soils act as a natural sink for toxic metals coming from different sources such as: industrial wastes, sewage sludge application and waste water irrigation for growing crops (Nagajyoti et al., 2010; Wuana and Okieimen, 2011).

Bioavailable part of these metals which is directly concerned with ecosystem is more important than the total metal contents present in the soil. However, it is difficult to assess bioavailable portion from soils due to its dependence on a large number of soil and plants factors (Kabata-Pendias and Pendias, 2001). Cadmium and Cu concentration in soils comes from parent materials, mining processes, use of pesticides, fungicides and fertilizers (Marschner 1995; Wuana and Okieimen, 2011). In general, Cd is toxic to both plants and humans whereas Cu is especially toxic to plants at higher concentrations. Cadmium readily accumulates in plants due to its high mobility and solubility in soil solution. Excess Cd is toxic to plants and negatively affects plant growth and mineral uptake (Das et al., 1997; di Toppi and Gabrielli, 1999; Benavides, 2005).

Copper is the third most used metal in the world and is an essential micronutrient required for both plants and animals (Wuana and Okieimen, 2011). The Cu is essential for normal plant growth and development being constituent of proteins and required for the functioning of more than 30 enzymes, especially those participating in electron flow (Epstein and Bloom, 2005; Yruela, 2005). For example, in banana plant application of 1.0 µM Cu stimulated root induction, elongation and shoot growth compared to 0.1 µM Cu (Deo and Nayak, 2011).

Similarly, foliar application of Cu + Mg on wheat plant grown in sandy soil conditions increased the chlorophyll contents, leaf area and dry matter m^{-2} of plant (El-Metwally et al., 2010). Copper is also effective against plant diseases caused by different organisms so that higher concentration of copper is normally used in fungicides, bactericides and pesticides sprays for agricultural use especially in grapevines and greenhouse farming to control plant diseases (Scheck and Pscheidt, 1998; Zheng et al., 2004; Michaud et al., 2007; Mackie et al., 2012). Higher use of these chemicals elevates the Cu concentrations in the soil environment which is toxic to plants causing chlorosis and reduction in plant growth and photosynthesis (Michaud et al., 2007; Bravin et al., 2009; Deo and Nayak, 2011). Plants including vegetables, field crops and fodder etc grown on such sites provide entry to these metals in food chain and as a consequence cause toxicities in humans. Therefore, there is need to reduce toxic metal bioavailability and plant uptake for increasing plant growth and yield and ultimately safe food production.

Currently a number of technologies exist for the remediation of Cd/Cu contaminated soils. These techniques include *in situ* and *ex situ* remediation, isolation, immobilization, phytoextraction, phytostabilization, physical separation and extraction etc. (Wuana and Okieimen, 2011). Among these techniques, phytoremediation is mostly used to clean-up metal contaminated soils. In this technique green plants are used to remove metal and other toxic compounds from contaminated sites. There are a number of different phytoremediation techniques including, phytovolatilization, phytostabilization and phytoextraction. Phytostabilization, also called in-place inactivation, is primarily used to immobilize toxic materials in soils and sediments with the help of plants and amendments (Adriano 2001; Wuana and Okieimen, 2011). Contaminants are absorbed and accumulated in roots or precipitated in the rhizosphere so in this way their mobility and bioavailability is limited (Bes and Mench, 2008; Hong et al., 2010).

Phytostabilization can also take place through different processes such as sorption, complexation and precipitation (Lasat, 2000; Wuana and Okieimen, 2011). This technique is useful for quick stabilization of heavy metals including Cu and Cd and is advantageous because disposal of hazardous material is not required (Mench et al., 2006). However, this technique has several limitations and is restricted to the rooting depth of plants (Mench et al., 2006; Danh et al., 2009). Moreover, it has also problem when there is mixed contamination because some plants are resistant to one metal and may be sensitive to other one (Mench et al., 2006; Sarma, 2011). Therefore, there is still need to develop more efficient, individual, simple and economical remediation technique for better reduction of metal assimilation from contaminated soils by edible plants.

Silicon is not considered to be an essential element for higher plants, but now it has been proved that it has several positive effects on the alleviation of both abiotic and biotic stresses in plants (Ma et al., 2004; Liang et al., 2005). The possible mechanisms involved in minimizing metal accumulation in crops by improving silicon nutrition have been summarised by Sarwar et al., (2010), and it appears that the mechanisms of this mitigation remain unclear and need further investigation.

Based upon the above discussion, the following section of this chapter aims to summarize the current relevant knowledge in the field regarding the methods to assess the bioavailability and toxicity of metals, particularly Cd and Cu for plants, as well as to review the concept of silicon bioavailability and heavy metal detoxification.
Overall this chapter provides justification for the importance of the research detailed herein.

2.1 Cadmium

Cadmium (Cd) is a transition metal with atomic number 48, atomic weight 112.4, density 8.65 g cm^{-3}, melting and boiling points 320.9°C and 765°C respectively (Wuana and Okieimen, 2011). Cd is widely used in Ni/Cd batteries, as pigment and stabilizer for plastics, in alloys and electronic compounds (di Toppi and Gabbrielli, 1999). Moreover, Cd is also produced as a byproduct of Zn and lead refining. However, Cd is non-essential and toxic to both plants and animals. In humans the oral uptake of cadmium via food and drinking water contributes the largest of the total Cd uptake (EC, 2000). For example in Japan, higher Cd concentration in rice was the major source of Cd intake in humans that was known to be responsible for itai-itai disease near the Jinzu river basin in the mid 1950s and 1960s (Yamagata and Shigematsu, 1970). Similarly, in recent years rice is still the major source of Cd intake in Japanese people (Watanabe et al., 2000) which is still a threat for human health (Ueno et al., 2010). Mean tolerable weekly intake (TWI) of Cd for European population was established 2.5 µg kg^{-1} of body weight. However, vegetarians, children, smokers and people living in highly contaminated areas may exceed the TWI by about 2-fold (EFSA, 2009).

2.1.1 Cadmium in soil

Cd occurs naturally in the earth crust and is widely distributed but it is a relatively rare element (0.1-0.2 mg kg^{-1}). Cadmium is mostly present in nature as complex oxides, sulphides and carbonates in zinc, lead, and copper ores (UNEP, 2010). Higher concentrations are found in association with Zn, Pb and Cu ores. Cd content of phosphate fertilizers varies from 2-200 mg kg^{-1}. Sedimentary rocks and marine phosphates contain about 15 mg Cd kg^{-1} (EC, 2000). Phosphate rocks of igneous origin generally contain less than 15 mg Cd per kilogram P_2O_5 (phosphate fertilizer) compared with 20 to 245 mg Cd kg^{-1} in sedimentary counterparts (Çotuk, et al., 2010). World's normal Cd concentrations in the soils are reported to range between 0.02 to 6.2 mg Cd kg^{-1} and the soils containing 5 to 20 mg Cd kg^{-1} are likely required remedial actions as being toxic to the surrounding environment (Adriano, 2001). In France, some highly polluted soils are reported to contain over 100 mg Cd kg^{-1} soil (Baize et al., 1999). In agricultural or horticultural soil, Cd concentrations range from 0.2 to 1.0 mg kg^{-1} in rural and from 0.5 to 1.5 mg kg^{-1} in urban areas (EC, 2000). However, higher Cd concentrations in the soil can occur naturally or through anthropogenic activities (He et al., 2005).

Cd level in agricultural soils depends upon parent material, pedogenic processes, use of phosphate fertilizers, pesticides, sewage sludge and disposal of industrial wastes containing Cd (Figure 1) (Grant et al., 1998; Wuana and Okieimen, 2011). According to French ASPITET programme, Cd in agricultural soils can vary from 0.02 to 6.9 mg kg^{-1} (Baize, 1997; Mench et al., 1997). Cd concentration in soil solution is relatively low and ranges from 0.2 to 6 µg L^{-1}. However, much higher values up to 300 µg L^{-1} have been reported by Itoh and Yumura (1979) that indicates presumably contaminated soil. Moreover, domestic sewage sludge contains higher concentration of Cd as compared to other heavy metals. For example, cigarette butts containing Cd flush down toilets and Cd is given off from rubber when car tires run over streets, and after a rain, the Cd is washed into sewage systems where it collects in the sludge. In addition, composted sludge may contain higher levels of Cd. For example, the composted sludge from Topeka, Kansas, which is applied to crop land, contains up to 4.2 mg Cd kg^{-1} compost (Liphadzi and Kirkham, 2006). Cd is also present as an impurity in several products such as phosphate fertilizers, manures, disposal of industrial and urban wastes and pesticides (Nagajyoti et al., 2010; Wuana and Okieimen, 2011). The application of these agricultural inputs increases the total Cd in soils. However, to limit Cd concentrations in edible parts, Cd concentration lower than 3 mg kg^{-1} dry soil has been recommended for agriculture and horticulture (Lux et al., 2011).

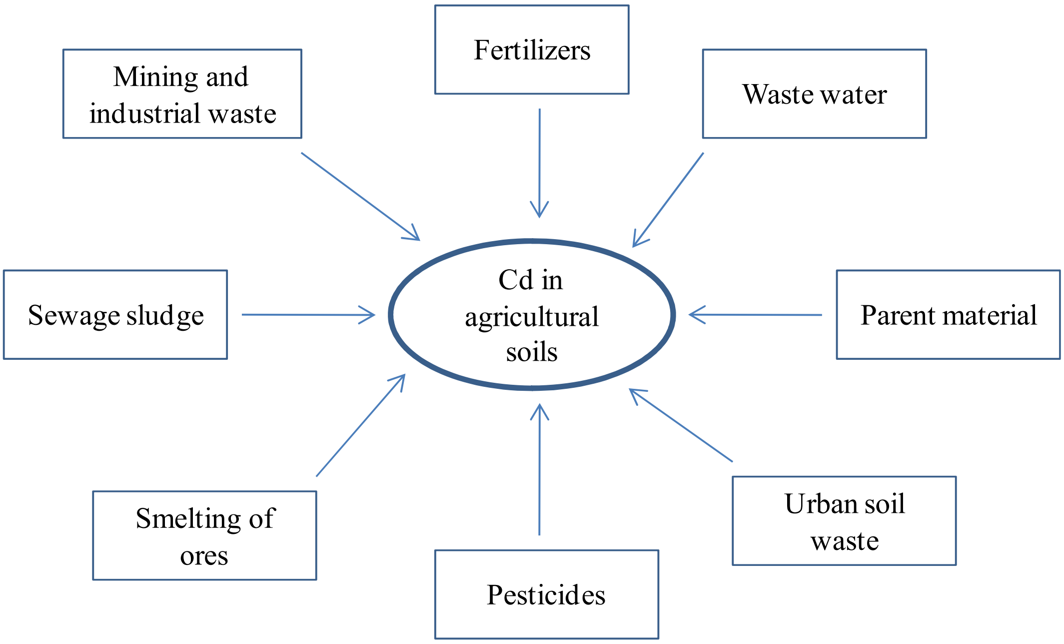

Figure 1. Major Cd sources in agricultural soils

2.1.2 Assessment the bioavailability of heavy metals

Metal bioavailability in soil is most important than the total metal concentration in the soil because it is the part of metal that plant can take up from soil (Kirkham, 2006). In order to assess the metal phyto-availability it is very important to extract and measure the portion of metals that plant can take up from the soil. For this purpose several methods have been developed as an attempt to predict phytoavailability of heavy metals. The choice of extractant is an essential step to evaluate the availability of metals to the plant. The extractant should reflect the same phenomenon as the absorption of heavy metal by the plant. It should only remove bioavailable metals. Bioavailability varies with the soil and plant studied. However, there is no universal extractant but many chemical extractants are often used to approach the bioavailability of metals to plants (Lakanen and Ervio, 1971; Gupta and Aten, 1993). Ideally, the chosen extractant should not affect the solid solution equilibrium of the soil, so it does not change the pH, the form of complexes or participate in exchange reactions of ions (Kennedy et al., 1997). The DTPA-TEA (Diethylenetriamine Pentaacetic Acid Triethanolamine) at pH 7.3, developed by Lindsay and Norvell, (1978) presents the best results for the extractable fraction of heavy metals in soils (Borges, 2002). Similarly, Keller et al., (2005) showed that the DTPA-TEA can be relied upon as extractant for estimating the bioavailability of metals for tobacco plant on the soils studied (including soil Rafz, of our study). It is also used as a standard method in many soil testing laboratories in USA (Kirkham, 2006). Based upon above discussion we selected DTPA-TEA for the extraction of bioavailable metals from the soil.

2.1.3 Cd behaviour in soil

The major pollution problem involving Cd can be correlated with the behavior of Cd in soil. Cd in the soil exists in different forms such as a free metal ion, exchangeable (free ion Cd^{2+}), adsorbed (e.g., Fe-oxides, organic matter, clay particles) and in the form of organic (e.g., amino acids and carboxylic acids) and inorganic (e.g., SO_4^{2-}, Cl^-) complexes (Sammut et al., 2010; Vega et al., 2010). Cd behavior in soil, in the context of species, retention, mobility and bioavailability depend upon these different forms and is largely controlled by complex interactions governed by many biogeochemical factors (Adriano et al., 2004). The bioavailable Cd is more important for plants rather than total Cd concentration in the soil because it is that part of Cd which can be taken up by plants (Ok et al., 2004; Kirkham, 2006). The bioavailability of Cd to plants depends on a number of soil factors and plant species. Among the soil factors are available Cd and total Cd concentrations, pH and organic matter content, (Sauvé et al., 2000a, 2000b; Kirkham, 2006; Jung et al., 2008), speciation (Sammut et al. 2010), cation exchange capacity (CEC) (Lehoczky et al., 2000; Vega et al., 2010), clay content and interactions with other elements such as iron (Krishnamurti et al., 2000) and chloride (Grant et al., 1998; Degryse et al., 2004; Weggler et al., 2004; Kirkham, 2006). Cd solubility and bioavailability is also affected by dissolved organic carbon (DOC) present in the soil solution which is known to complex metals such as Cd, Zn and Cu and affect their solubility as well as plant uptake (Antoniadis and Alloway, 2002; Zhao et al., 2007). Aging is also a factor that effect Cd bioavailability. Bioavailability of metals can decrease with time with little or no reduction in total metal concentration in soil (Kirkham, 2006). Nitrogen fertilizers, soil types, genotypes and management practices also affect Cd bioavailability (Zhang et al., 2009; Perilli et al., 2010; Gao et al., 2010, 2011). Plant nutrient level in soil also affects Cd bioavailability (Kirkham, 2006; Sarwar et al., 2010). Adsorption, complexation and sorption capacity of the soil also affects the amount of Cd in the soil solution. Metal adsorption on the soil solid phase is the major process that control metals present in the soil solution. With increase in adsorption and sorption capacity of soil the Cd in the soil solution decreases (Grant et al., 1998).

Presence of anions also affects the bioavailability of Cd in plants. For example in wheat plant, the presence of Cl^- and SO_4 in soil increased Cd uptake by plants (Zhao et al., 2003). Among all soil factors, pH is a major factor influencing Cd bioavailability (McBride et al., 1997; Grant et al., 1998; Jung, 2008; Zeng et al., 2011). Cd is mobile in acidic soils within pH range of 4.5 to 5.5 but in alkaline soils Cd is rather immobile (Kirkham, 2006). pH can also affect the bioavailability of Cd by altering different processes in soil such as adsorption, complexation, sorption or desorption processes (Naidu et al., 1994, 1997; Bolan et al., 1999). In general, all soil processes controlling Cd behavior in soil are of special importance in the root developing zone of soil.

Cd bioavailability also depends upon plant species (Mench et al., 1989). Dominant form of Cd in the soil solution is Cd^{2+} but also exist as Cd-chelates (Tudoreanu and Phillips, 2004). Specific chelating compounds are also released by some plants called as phytosiderophores and/or root exudates (Mench and Martin, 1991). In general, Phytosiderophores are produced by graminaceous plants such as barley, wheat and rice under Fe deficiency which mobilizes Fe from sparingly soluble forms (Marschner et al., 1986; Reichard et al., 2005). These phytosiderophores can complex metals such as Cd and Cu and affect their bioavailability. Cd bioavailability is strongly affected by root exudates which affect the characteristics of rhizosphere (Hill et al., 2002; Dong et al., 2007). Root exudates may influence the Cd bioavailability and toxicity by modifying the rhizosphere pH and redox potential (Eh), chelating/complexing and depositing with Cd ions. In addition, many plant species can exude organic acids that can form complexes with metals, can change the chemistry of the rhizosphere and alter metal speciation. For example in wheat roots, the uptake of Pb was increased by the presence of acetic and malic acids under hydroponic conditions (Wang et al., 2007). Organic acids also increased the uptake of Cd by solubilization of particulate bound Cd into the soil solution (Cieslinski et al., 1998). Similarly in maize plants, presence of organic acids increased Cd mobilization, plant availability and accumulation (Nigam et al., 2001; Han et al., 2006). Such factors, individually or in combination with each other, may alter the behavior of Cd present in the soil and affect the Cd uptake by the plants, and also alter the community and activities of microbes present in rhizosphere (Shenker et al., 2001; Dong et al., 2007).

Microorganisms also release chelating compounds called siderophores (Neubauer et al., 2000 and the references therein) which may solubilize Cd (Dimkpa et al., 2009). A bacterium can produce many types of siderophores, including hydroxomates and carboxylic acids (Klumpp et al., 2005). These siderophores can desorb Cd at a moderate pH range (Hepinstall et al., 2005). Microorganisms may also decrease Cd solubility by the formation of insoluble metal sulfides and also sequestration of the toxic metal via the cell walls or by proteins and extracellular polymers etc (Francis, 1990; Dong et al., 2007).

2.1.4. Cd uptake and accumulation in plants

All plants take up Cd by the roots from soil solution. Cd uptake by roots seems to occur via different transporters such as Mn^{2+} and Zn^{2+}, Ca^{2+}, Fe^{2+} transporters (Clemens, 2006). However, Cd uptake and accumulation widely differ among crop species and cultivars (Grant et al., 1998, 2008; Liu et al., 2003; Zheng et al., 2011). Some plants, including maize, pea, oat and wheat, are low heavy metal accumulators while some are higher metal accumulators (Sarwar et al., 2010). There are differences among wheat species and between genotypes of a given species in their ability to accumulate Cd in grains (Hart et al., 1998; Ozturk et al., 2003; Greger and Löfstedt, 2004). It has been reported that durum wheat accumulates Cd in grains to a greater extent than bread wheat (Meyer et al., 1982; Hart et al., 1998, 2002; Greger and Löfstedt, 2004). This larger Cd accumulation in durum wheat cultivars may be related to several physiological processes such as higher uptake, higher translocation from roots to shoots and higher translocation from shoots to grains. Higher Cd accumulation in durum wheat can be decreased by altering the physiological processes such as by lower uptake, by higher root sequestration or decreased xylem loading. In general, Cd accumulation in roots, shoots and grains depends upon three transport processes: 1) root uptake of Cd, 2) xylem loading and translocation to shoots and 3) re-translocation to seeds. Root is the main pathway by which water, nutrient and pollutants including heavy metals enter to the plant body. Cd enters the plant through root uptake from the soil solution and is considered a key process in overall plant Cd accumulation. A part of Cd present in the soil solution is adsorbed onto the roots. Cd uptake by roots increased with increasing exposure periods and Cd concentrations (Hentz et al., 2012).

Uptake of Cd by roots is also affected by the root morphology, root length, root hair length and root surface area (Kubo et al., 2011; Zheng et al., 2011). Similarly, root apices can facilitate root uptake of Cd where root cells are young and have thin cell walls (Seregin et al. 2004). Strong adsorption of Cd on root apoplast in maize plants might act as a driving force to extract the metal from the soil (Redjala et al., 2009). Root endodermis and exodermis play an important role in Cd uptake in maize plants and act as barriers to the solute flow (Redjala et al., 2011) and the presence of high Cd accelerated the maturation of the maize root endodermis (Lux et al., 2011). During plant uptake, Cd ions can compete for the same trans-membrane carriers with the nutrients such as K, P, Ca, Mg, Fe, Mn, Cu, Zn and Ni (Benavides et al., 2005; Zheng et al., 2011). Presence of other elements can inhibit the Cd uptake such as Zn^{2+} in the nutrient solution inhibited the Cd uptake in bread and durum wheat at the root cell plasma membrane (Welch et al., 1999; Hart, et al., 2002).

At the molecular level, the mechanisms by which Cd enters roots are still poorly understood. After adsorption on the root surface, Cd enters in the root cells as Cd^{2+} through ZIP transporters like Zn regulated transporter or Fe regulated transporter like Protein or via cation channels such as calcium channels. Cd can also enter root cells in the form of Cd-chelates through YSL (Yellow-Stripe 1-Like) proteins (Lux et al., 2011). Cd uptake at the root surface has been characterized in a number of species, including wheat (Jalil et al., 1994b) and maize (Florijn and Beusichem, 1993).

Once Cd has been taken up by root system, it may accumulate there or be translocated to aerial plant parts (Kabata-Pendias and Pendias, 2001; Gill et al., 2011). In general, Cd ions are retained in roots and a small portion is transferred to shoots but it depends upon plant species (Abe et al., 2008). After absorption by roots, Cd can reach the xylem through apoplastic and/or symplastic pathways (Salt et al., 1995; Benavides et al., 2005). Cd in roots can also be complexed with several ligands such as organic acids and/or phytochelatins (PCs) and is mainly concentrated in vacuoles and nuclei (Hart et al., 2006; Lux et al., 2011). Xylem loading is an important process for long distance transport of Cd (Clemens et al., 2002). In many plant species Cd xylem loading has been shown to be mediated by the P-type ATPase transporters and its homologues (Verret et al., 2004; Hanikenne et al., 2008). Cd transport into the central cylinder is also regulated by casparian strip and plasmalemma of the endodermis (Seregin et al. 2004).

After absorption by roots, Cd is transported by xylem and phloem to the aerial parts of plants (Tudoreanu and Phillips, 2004). However, Cd translocation to shoots depends on species and genotypes within species (Dunbar et al., 2003). Cd translocation from roots to shoots is driven passively by transpiration in leaves (Salt et al, 1995; Hart et al., 2006) or actively through different transporters such as Fe transporters (Nakanishi et al., 2006). In addition, Uraguchi et al. (2009) also suggested that in rice plant root to shoot Cd translocation via the xylem is the major and common physiological process determining the Cd accumulation in shoots and grains. Recently, it has been suggested that citrate might play an important role in the transportation of Cd in the xylem vessels (Zorrig et al., 2010). Similarly, Van der Vliet et al. (2007) reported that most of the Cd translocation from the root to the shoot in durum wheat plants takes place by symplastic pathway.

There is relatively little information available regarding the movement of Cd into developing seeds. However, studies on Cd translocation showed that Cd translocation in the edible parts of plants depends upon genotypes (Meyer et al., 1982; Cakmak et al., 2000) and can be influenced by genetic manipulation and by soil and crop management practices (Grant et al., 1998; Gao et al., 2011). In addition, Cd accumulation in grains also depends upon variations in the translocation from root to shoot and to Cd concentration in shoots and flag leaf (Greger and Löfstedt, 2004). In grains, Cd accumulation may occur through phloem-mediated Cd transport from leaves and stalks to maturing grains (Hart et al., 1998; Harris and Taylor, 2001; Liu et al., 2007; Yoneyama et al., 2010). Presence of other ions may inhibit or increase phloem loading of Cd (Welch et al., 1999; Cakmak et al., 2000). The level of Cd in wheat grains may be affected by a number of physiological factors, including (1) Cd uptake from the soil solution, (2) xylem translocation from root to shoot, (3) sequestration of Cd with organic complexes (Xu et al., 2010).

2.1.5 Cd toxicity in plants

Cadmium does not appear to be an essential element for plant growth because it has no known biological function (Marschner, 1995). Among the toxic heavy metals, Cd is of more concern than others due to its high toxicity at very low concentration and large solubility in water (Benavides et al., 2005). The higher Cd accumulation in plants induces a series of stress factors in plants. Major toxic effects (direct or indirect) of this metal on plant growth and physiological processes are listed in figure 2.

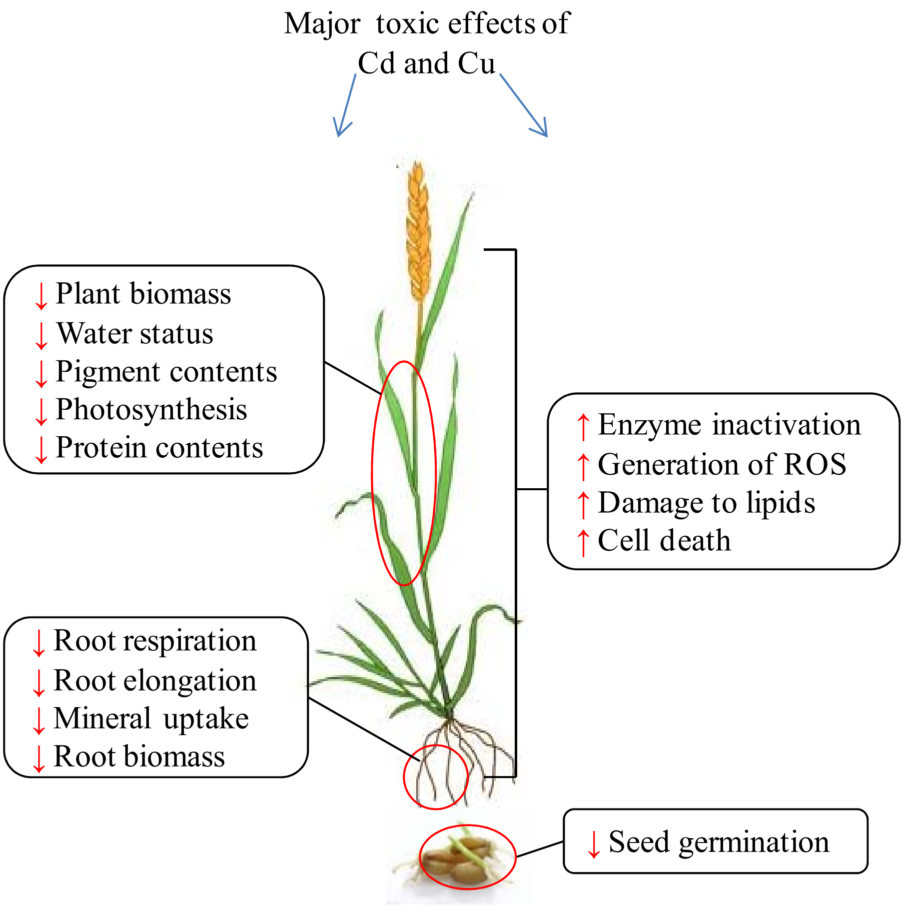

Figure 2. Major toxic effects of Cd and Cu on wheat plants

2.1.5.1 Effects on seed germination

Cd toxicity adversely affects seed germination and plant growth. Cd induced inhibition of seed germination has been reported in many plants such as *Arabidopsis thaliana* (Li et al., 2005), mustard (*Sinapis arvensis* L.) (Heidari and Sarani, 2011), soybean (*Glycine max* L.) (Liu et al., 2011), *Pisum sativum* (Smiri, 2011), cowpea (*Vigna unguiculata* L.) (Vijayaragavan, et al., 2011) and milk thistle (*Silybum marianum* L.) (Khatamipour, et al., 2011). This inhibition of seed germination is likely due to selective uptake of different ions by the intact seeds (Li et al., 2005). In addition, this inhibition of seed germination is related to negative effects of Cd on water uptake and water movement and consequently reduces the availability of water in the embryo axis (Poschenreider et al., 1989; Vijayaragavan et al., 2011).

2.1.5.2 Effects on plant growth and biomass

Cd toxic effects on plant growth and biomass are largely studied. Reduction in root length is one of the most visible symptoms of Cd toxicity in plants (Guo and Marschner, 1995; Lux et al., 2011; Haouari et al., 2012). Higher Cd concentration in the roots accelerated the development of endodermis of roots and also changed the relative size and proportion of root tissues (Seregin et al., 2004; Lux et al., 2011). Moreover, 10 mM Cd concentration in the nutrient solution resulted in the breakdown of root cortex cells of barley (*Hordeum vulgare*) plant (Sridhar et al., 2007).

It is well known that Cd negatively affects the plant growth and biomass. For example in pea plants grown hydroponically with 50 μM Cd for 28 days significantly decreased plant growth and biomass (Sandalio et al., 2001). More recently Haouari et al., (2012) reported that in tomato plants grown hydroponically with increasing Cd (10, 50 and 100 μM) concentrations negatively affected the root and shoot fresh and dry biomass. Wójcik and Tukiendorf, (2005) reported that maize plants grown hydroponically with increasing Cd applied from 5 to 300 μM resulted in Cd accumulation almost linearly both in roots and in shoots with increasing Cd concentration in nutrient solution. Increasing Cd concentrations also increased the inhibition of root elongation (from 12.3 to 91 %), root fresh mass (from 12.7 to 98.8 %) and shoot fresh mass (from 11.5 to 78.2 %), compared with control plants. Moreover, in *Thlaspi caerulescens* plants accumulation of 319 mg Cd kg^{-1} (±12) dry weight of shoots resulted in visual toxicity symptoms such as internerve chlorosis of younger leaves and sometimes necrosis of older ones (Wójcik et al., 2005). Similarly, high Cd (100 mg Cd kg^{-1} soil) perturbed the growth of garden cress (*Lepidium sativum* L.) plants (Gill et al., 2012). In cowpea plants increasing Cd (10, 30 and 50 mg Cd kg^{-1} soil) concentrations also decreased the growth parameters such as, root and shoot length, fresh and dry weight of roots and shoots, total leaf area and the toxic effect of Cd on these parameters increased with increasing Cd concentrations in soil (Vijayaragavan et al., 2011).

Number of previous studies have reported that Cd supply in durum wheat cultivars reduced the shoot and root dry matter, root length, leaf area (Jalil et al., 1994a, 1994b; Hart et al., 1998; Harris and Taylor, 2004). Higher Cd concentration in plants resulted in leaf chlorosis, wilting and leaf abscission in plants (Bavi et al., 2011). Similarly, Shi et al., (2010) showed that Cd exposure depressed peanut plant growth. This decrease in growth and biomass under Cd stress is related to decrease in photosynthesis, inhibition of the metabolic enzymes production and decrease in uptake and translocation of macro- and micronutrient contents in plants (Sandalio et al., 2001; Gonçalves et al., 2009; Feng et al., 2010). However, the toxic effects of Cd on plant growth and biomass depend upon dose and time of exposure to Cd stress (Das et al., 1998; Di Toppi and Gabrielli, 1999). Similarly, chlorosis symptoms and necrotic spots appeared in leaves of tomato (*Lycopersicon esculentum*) plants at 10 and 100 µM Cd in the nutrient solution respectively (López-Millán et al., 2009). Moreover, the toxic effects of Cd varies with plant species i.e., hyperaccumulators tolerate higher Cd toxicity than non accumulators sensitive plants (Das et al., 1998; Di Toppi and Gabrielli 1999; Benavides et al., 2005; Cosio et al., 2005, 2006; Abe et al., 2008).

2.1.5.3 Effects on mineral nutrients

Cd interactions with mineral nutrients are of public concern because decrease in mineral nutrition by Cd is directly related to growth and yield of plants. Numerous studies regarding Cd effects on mineral nutrients have provided contradicting results. Toxic effects of Cd on mineral nutrients uptake and accumulation in plants have been widely reported (Ouzounidou et al., 1997; Sandalio et al., 2001; Wang et al., 2008; Gonçalves et al., 2009). Toxic effects of Cd on mineral nutrients depend upon time and intensity of Cd stress imposed (Hernandez et al., 1998, Ramros et al., 2002). Plant size plays an important role in element accumulation and distribution. For example, in wild garlic the application of 2 and 5 mg L^{-1} Cd in the nutrient solution decreased the leaf Cu, Fe, Mo and Zn contents in small and medium-sized plants but had no effect on the micronutrients in large-sized plants (Street et al., 2010). Toxic effects of Cd on mineral nutrients uptake and translocation vary with plant and nutrient types. For example, external Cd increased the Mn uptake and translocation in the shoots of lettuce plants in contrast to other essential micronutrients (Ramos et al., 2002). However, reduction of Mn uptake and transport in the presence of Cd has been also reported by many authors (Hernandez et al., 1998; Dong et al., 2006). Effect of Cd on Zn uptake and accumulation in plants are not consistent. For example, 0.2 µM Cd concentrations in the nutrient solution decreased Zn uptake in durum wheat when Zn concentration was lower (1.0 µM) in the nutrient solution while at higher Zn (10 and 19 µM) concentrations this effect was synergistic (Welch et al., 1999). Contrarily, in spring wheat with 20 µM Cd in the nutrient solution decreased Zn concentration and increased Cd concentrations in roots and shoots when Zn was lower (1~200 µM) in the nutrient medium while at higher (>200 µM) Zn levels Cd concentration significantly reduced in seedlings while Zn concentration increased indicating antagonistic effect on each other (Zhao et al., 2005). In tomato plants, excess Cd (50 and 100 µM) in the nutrient solution decreased the uptake of K^+, Ca^{2+} and Mg^{2+} ions by roots and consequently decreased in shoots (Haouari et al., 2012).

Excess Cd in the medium not only changed uptake of nutrients but also changed the deposition and translocation. However, again, this trend varies according to plant species and Cd stress imposed (Yang et al., 1998; Zhang et al., 2002). For example, in soybean seedlings Cd concentration decreased Cu, Zn and Mn concentrations in roots but was not affected in shoots (Drazic et al., 2004). In contrast, addition of Cd in the growth medium increased P, K and Mn concentrations in wheat roots and inhibited their translocation to shoots (Zhang et al., 2002). Moreover, Jalil et al. (1994) found that Cd application decreased the concentration of K, Zn, and Mn in roots and shoots of durum wheat. Similarly, another expression of Cd interactions with essential elements is the effect of Cd when there is element deficiency in plants. For example, Ca deficiency increased Cd toxicity in rice seedlings (Cho et al., 2012). Moreover, Chou et al. (2011) observed that Cd decreased Fe and Zn contents in Mg-deficient rice seedlings than that in Mg-sufficient seedlings.

2.1.5.4 Effects on photosynthetic pigments

Cd is highly toxic to photosynthetic machinery of plants. Reduction in photosynthesis is a well-known symptom of Cd toxicity in plants. This reduction in photosynthesis takes place by many processes including decrease in net photosynthetic rate, chlorophyll and carotenoid contents (Sandalio et al., 2001; Mobin and Khan, 2007; Vijayaragavan et al., 2011; Haouari et al., 2012). In addition, in cucumber (*Cucumis sativus* L.) plant, Cd treatment decreased photosynthesis which was related to damage or functional lost of the photosynthetic machinery and enzymes inhibition of nitrate metabolism including nitrogen reductase (NR), glutamine synthetase (GS) and glutamate dehydrogenase (GDH) (Feng et al., 2010). Similarly, Cd application in wheat seedlings also damaged the leaf photosystem II (PS II) and structure of chloroplast which resulted in the reduction of chlorophyll contents and consequently inhibition of photosynthesis (Ouzounidou et al., 1997; Ci et al., 2009). In peanut (*Arachis hypogaea*) plants, Cd treatments inhibited the net photosynthetic rate which may be due to reduction of stomatal conductance and photosynthetic pigments and alteration in leaf structure (Shi and Cai, 2008). More recently, Gill et al., (2012) reported that Cd at higher dose reduced the photosynthesis and nitrogen metabolism in garden cress (*Lepidium sativum* L.). Similarly, in tomato (*Lycopersicon esculentum*) plants, low Cd (10µM) concentration did not affect the photosynthesis but higher Cd (100µM) in the nutrient solution decreased the photosynthetic rates and photosynthetic pigment concentrations (López-Millán et al., 2009).

Cd toxic effects on photosynthetic machinery depend not only upon the quantity of Cd but also depend upon the exposure period. For example, Chugh and Sawhney, (1999) reported that in one-month-old pea seedlings, different Cd treatments for six days had a more pronounced effect on the activity of PS II but on prolongation of the Cd exposure time for twelve days, the functioning of photosystem I (PS I) was also equally affected.

2.1.5.5 Cd induced oxidative stress in plants

Excess Cd generates free radicals and reactive oxygen species (ROS) those causes oxidative stress in plants (Khan et al., 2007; Mobin and Khan, 2007). ROS reacts with proteins and pigments and cause lipid peroxidation, inactivation of enzymes and membrane damage (Khan et al., 2007). Excess Cd in plants also resulted in the modification of antioxidant enzymes and sulfur assimilation activity (Shi et al., 2010; Gill et al., 2012). For example, Cd increased the activity of antioxidant enzymes such as production of ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and catalase (CAT) were increased in shoot of mustard (*Sinapis arvensis* L.) plants (Heidari and Sarani, 2011). Ascorbic acid concentration decreased in the shoots and roots of durum wheat cultivars by supplying 75 and 150 µM Cd in the nutrient solution (Ozturk et al., 2003). Excess Cd also decreased the activity of glutamine synthetase (GS) enzyme in several plant species (Balestrasse et al., 2006). Similarly, excess Cd caused an enhancement of malondialdehyde (MDA), an increase in activity of superoxide dismutase (SOD) and APX and a decrease in CAT activity (Shi et al., 2010b). Excess Cd also increased the production of H_2O_2 in pea plants and consequently enhanced activity of SOD and low activity of CAT, POD and APX (Pandey and Singh, 2012). However, physiological response of plant to Cd stress depends upon species and cultivars. For example, Shi et al. (2010a) showed that Cd exposure caused oxidative stress in both Cd-sensitive (Luhua 11) and Cd-tolerant (Luzi 101) peanut cultivars but the toxicity was more obvious in Cd-sensitive cultivar. Oxidative stress also depends upon the concentration of Cd i.e., in pea plants excess Cd induced a concentration dependent oxidative stress in leaves (Sandalio et al., 2001).

2.1.5.6 Miscellaneous toxic effects

Cd toxicity effected normal plant growth and development in a number of ways given above. However, there are still large numbers of toxic effects of Cd on plants. Excess Cd decreased the percentage of water content of shoot and root in pea plants (Lozano-Rodriguez et al., 1997). Influence of Cd on plant-water relationship is directly related to the reduction in the absorption surfaces by inhibiting the formation of root hairs (Pál et al., 2006). Cd application in wheat seedling also decreased the total soluble sugar concentration and increased the free amino acid concentration in both shoots and roots (Ci et al., 2009). Protein content in pea roots was reduced significantly in the presence of high cadmium concentrations (Bavi et al., 2011). In *Solanum nigrum*, high Cd concentration (48 mg Cd kg^{-1} soil) decreased the nitrate reductase activity (Wang et al., 2008). Excess Cd also limited the stomatal conductance in safflower (*Carthamus tinctorius* L.) plants (Shi et al., 2010b).

2.1.6 Cd tolerance mechanisms in plants

Plants developed a number of defense strategies to cope with Cd stress. The first defense mechanism is the reduced uptake by plants then immobilization in the cell wall, synthesis of phytochelatins (PCs) and sequestration into the vacuole (Pál et al., 2006). In plant cells, PCs are the best characterized metal-binding ligands and are frequently cited as metal protective proteins in plants (Maestri et al., 2010). In addition, activation of various antioxidants to cope with Cd-induced ROS production constitutes a secondary defense system (Shi et al., 2010a, 2010b; Heidari and Sarani, 2011; Sharma et al., 2012). However, plant responses to excess Cd vary depending on plant species, tissues, stages of development and Cd concentration (Sharma et al., 2012). Use of agronomic management practices also helps the plant to minimize the uptake and toxic effects of Cd in plant (Gao et al., 2010; Perilli et al., 2010; Rizwan et al., 2012). However, informations are still lacking in this respect and need further research.

2.1.7 Synthesis on Cd in soils and plants

Based upon the studies cited above we can conclude that:

Cd concentration in the agricultural soil is increasing continuously due to anthropogenic activities. Cd uptake and accumulation in plant is a function of the complex interaction of soil, plant and environmental factors which influence Cd phytoavailability. Cadmium behavior in soil depends upon soil and biological factors. Soil factors are: parent material, organic matter content, pH, CEC, redox conditions, competing ions and the amount of organic and inorganic ligands etc. Biological factors are: plants species, root morphology and microbial conditions. The main pathway of Cd uptake by plants is through roots. Once in roots it sequesters there or translocated to aerial parts by different pathways depending upon plant species. The studies reported to date have proved that durum wheat (*Triticum turgidum* L.) tends to accumulate higher Cd concentrations than other wheat varieties. Higher Cd accumulation in plants causes toxicity in plants. Cd decreased the seed germination, reduced root elongation and shoots and roots biomass. Higher Cd also resulted in leaf chlorosis, inhibition of photosynthesis and reduced the essential element concentration in different plant parts. Cd induced the oxidative stress in plants by producing the reactive oxygen species and modifying the production of antioxidant enzymes. However, intensity of these effects varies and depends upon the concentration of metal, duration of exposure and plant species and stage of plant. Plants have different mechanisms to cope with Cd stress. Agronomic practices also help the plants to cope with higher Cd stress.

However, information is still lacking concerning these practices and their role in minimizing metal toxicity in plants. Therefore, to produce crops with acceptable Cd concentrations requires an integrated approach to provide a safe and stable food supply, while ensuring the long-term sustainability of our soils.

2.2 Copper

Copper (Cu) is one of the oldest known metals and is the 25th most abundant element in the Earth’s crust. Cu is the world’s third most used metal. It is a transition metal with atomic number 29, atomic weight 63.5, density 8.96 g cm^{-3}, melting point 1083°C and boiling point 2595°C. In unpolluted soils, Cu concentration in the soil depends upon the amount of Cu in the parent material and is released by weathering of the parent material (Mantovi et al., 2003). In these soils its concentration is influenced by the parent material and reaches an average of 30 mg kg^{-1} (Baize, 1997; Adriano, 2001). Cu average concentration in crustal rocks is 55 mg kg^{-1} (Wuana and Okieimen, 2011). In agricultural soils, Cu concentration vary from 5 to 30 mg kg^{-1} of soil but the soils of wine growing areas contain from 200 to 500 mg kg^{-1} of Cu (Brun et al., 1998 and the references therein).

Copper was first identified as a plant nutrient in the 1930s (Sommer, 1931; Arnon and Stout, 1939). Cu is an essential element that is required for the healthy growth and development of plants. It is a constituent of protein and is required for the functioning of more than 30 enzymes, especially those participating in electron flow (Epstein and Bloom, 2005; Yruela, 2005). For optimum plant growth, Cu concentrations in plant shoots vary between 5-30 mg kg^{-1} dry weights (DW) (Kabata-Pendias and Pendias, 1992) with an average content of 10 mg

kg^{-1} DW (Yruela, 2005). However, Cu requirements of plants depend upon plant species (Mantovi et al., 2003). For example in Australia, Cu concentrations in shoots of chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris Medik*), faba bean (*Vicia faba* L.) and wheat (*Triticum aestivum* L.) were 2.6, 4.6, 2.8, and 1.5 mg kg^{-1} respectively (Brennan and Bolland, 2003). Plant response to Cu also depends on its growth stage (Vinit-Dunand et al., 2002). Cu concentration in many plant species was not correlated to the amount of Cu present in the soil (Nan and Cheng, 2001; Mantovi et al., 2003; Pip and Mesa, 2002). Cu deficiency occurs in plants when its concentration is below 5 mg kg^{-1} DW of shoots and at this level plant growth is reduced and deficiency symptoms can occur. In contrast, Cu concentration in the plant can reach a critical higher value from which toxicity symptoms appear in plants (Marschner, 1995).

Toxic levels of copper occur in many soils which are due to the parent material or as a result of anthropogenic (Figure 3) release of Cu into the environment (Yruela, 2005; Marschner, 2002). The continuous use of Cu fungicides, bactericides and pesticides to control plant diseases and pests has accumulated Cu in the surface layer of agricultural soils (e.g., in vineyards) (Brun et al., 1998; Scheck and Pscheidt, 1998; Zheng et al., 2004; Michaud et al., 2007; Mackie et al., 2012). In Europe, continuous spraying of Bordeaux mixture ($Ca(OH)_2$ + $CuSO_4$) used to control vine downy mildew has increased the heavy metal pollution of vineyards soils. For example, it has been estimated that a single application of the Bordeaux mixture introduced 3-5 kg Cu ha^{-1} in the region of Champagne (France) (Brun et al., 1998). Similarly, pig and poultry slurries rich in Cu concentration are contaminating livestock grazing pastures (Legros et al., 2010; Marschner, 1995). Other anthropogenic sources of Cu include waste water and sewage sludge irrigation. By these sources, Cu is accumulated in soil and as a result increased uptake by plants (Cao and Hu, 2000; Luo et al., 2003; Karami et al., 2009). These toxic levels of Cu entering into the food chain are toxic to plants, humans and animals (López Alonso et al., 2000; Michaud et al., 2007). When compared with other potentially toxic trace elements such as Cd and Zn, Cu is more toxic to plants and not so toxic to animals and humans (Wheeler and Power, 1995; Kinraide et al., 2004).

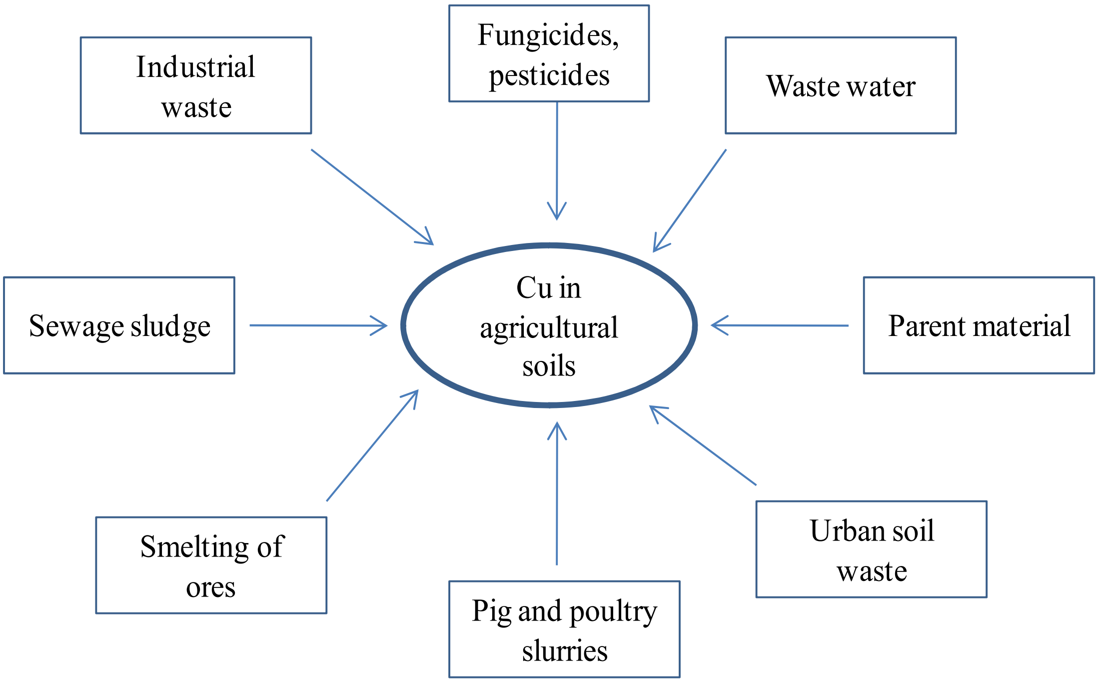

Figure 3. Sources of Cu in the agricultural soils.

2.2.1 Copper behaviour in soil

In soil Cu can be found in the liquid and solid phases. Copper is found under different forms in the solid phase which include water soluble, exchangeable and complexed in secondary minerals such as clays and Fe and Mn oxyhydroxides and organic matter and primary minerals (Kabata-Pendias and Pendias, 2001). Adsorption is the first process that controls metal concentration in the soils solution (Alloway, 1995). In uncontaminated soils, Cu concentration in the soil solution is generally very low with an average of 11µM and 0.8 µM in sandy and calcareous soils respectively (Mench, 1990). Available Cu in the soil depends upon soil type, soil physical and chemical properties (Brun et al., 1998; Chaignon et al., 2002; Ginocchio et al., 2002). Cu bioavailability is generally controlled by the total Cu concentration, CEC, soil organic matter (SOM) and pH of the soil (McBride, 1989; Sauvé et al., 2000a; Chaignon et al., 2002; Bravin et al., 2009; Wu et al., 2010). Bioavailability of Cu increases when the total Cu content in soil increases and the soil CEC decreases (Sauvé et al., 1997; Brun et al., 2001). Activity of free Cu^{2+} in the soil solution is increased when soil pH decreases (Sauvé et al., 1997). Its solubility is greatly dependent on soil pH and dissolved organic matter (DOM) content (Bravin et al., 2012) and is readily available at pH below 6 (Adriano, 2001; Brun et al., 2001). In addition, a number of studies comparing soils of varying pH showed that Cu bioavailability was greater in acidic than in more alkaline soils (Brun et al., 2001; Chaignon et al., 2002, 2003; Bravin et al., 2009). In soil solution, Cu is mainly complexed with organic matter and can be adsorbed onto the surfaces of clays, Fe oxyhydroxides and co-precipitated with carbonates and phosphates (McBride et al., 1997; Sauvé et al., 1997; Guan et al., 2011). Copper bioavailability also depends upon anthropogenic activities. For example, sewage sludge application in a calcareous soil increased the SOM, CEC and also increased the Zn and Cu bioavailability (Karami et al., 2009).

In addition, there is increasing evidence that plants can alter the Cu bioavailability by changing the mobility of Cu in the rhizosphere (Marschner, 1995). However, Cu bioavailability depends upon plant species. In the rhizosphere root released exudates of high or low molecular weight organic acids. Low molecular weight organic acids include sugars, organic acids, phenolic and amino acids (Marschner, 1995). These exudates may form complexes with metals such as Cu. However, the role of these organic anions exuded such as citrate, malate and oxalate in complexation process of metals is still a matter of discussion (Hinsinger, 1998; Yruela, 2009). In monocots, phytosiderophores released by plant roots also affect the Cu biavailability. For example, Chaignon et al. (2002) reported that under Fe and Zn deficiency, wheat plants (*Triticum aestivum* L.) accumulated higher Cu concentrations which may be due to the increased release of phytosiderophores. Plant roots may change the pH of rhizosphere and as a result affect the Cu bioavailability (Bravin et al., 2009). Cu bioavailability is also influenced by physical, chemical and biological processes that occur at the soil-root interface in the rhizosphere such as change in pH or the amount of dissolved organic matter (Hinsinger et al., 2009). Cu bioavailability is more influenced by rhizosphere pH than the bulk soil pH in an acidic Cu-contaminated soil (Chaignon et al., 2009).

On the other hand, Cu bioavailability is also affected by the concentration of other plant nutrients in the cultural medium. For instance, Fe deficiency in the nutrient solution resulted in higher Cu concentration in Chinese cabbage (Xiong et al., 2002), in maize (Nenova and Stoyanov, 1999), in wheat (Chaignon et al., 2002) and in pea plants (Welch et al., 1993; Cohen et al., 1998) while excess Fe in the nutrition solution decreased Cu concentration in Chinese cabbage (Xiong et al., 2002) and in apple plants (Vedina and Toma, 2000). Similarly, Fe and P deficiency in the nutrient solution stimulated Cu accumulation in plants while high P supply decreased Cu accumulation (Xiong et al., 2002). Recently it has been shown that P applications effectively decrease the bioavailability of Cu to maize and soybean from soil while N application in the soil significantly increased maize shoot Cu concentration (Xie et al., 2011).

2.2.2 Copper uptake and accumulation in plants

Plants take up Cu mainly from the soil solution through the root system. After absorption by roots Cu is transported to shoots via xylem. During plant uptake Cu is first adsorbed on the root surface then seems to dissociate from its complexed forms before absorption (Kabata-Pendias and Pendias, 1992). The root apoplast is one of the main compartments accumulating metals (Krzeslowska, 2011). Adsorption of Cu on the root surface takes place in cationic form with negative charges of the cell walls because cell walls consists a network of cellulose, pectins and glycoproteins and act as a specific ion exchangers (Allan and Jarrell, 1989). It can also be linked by non ionic form of reactions coordination groups containing phosphatase and peroxidases present in the cell walls (Marschner, 1995).

After adsorption on the root surface, Cu is absorbed by the roots but the mechanisms involved in the absorption of Cu in the roots are still poorly identified. However, the absorption of Cu in the root cells may be by passive (non-metabolic) and/or active (metabolic) transport through different transporters such as CTR (Copper Transporter) and COPT1 (Copper Transporter protein) or co-transporters (Marschner, 1995; Sancenon et al., 2004).

After absorption by the roots, Cu is transported towards xylem of roots. This xylem loading takes place mainly by two processes called apoplastic and symplastic pathways. The apoplastic transport of Cu takes place through the intercellular spaces. The transfer of low molecular weight solutes (i.e. trace metals complexed or not, amino acids, sugars) takes place by diffusion from soil solution to the intercellular spaces before being blocked by the Casparian strip in the endoderm (Marschner, 1995). This symplastic transport of Cu takes place through the plasma membrane of a cell with the help of different transporters. Cu is translocated to shoots through xylem vessels. In the xylem sap, Cu can form complexes with different organic substances such as carboxylate (aconitate, citrate, oxalate, malate, succinate and acetate etc.), polymers (proteins, pectins, DNA, RNA, polysaccharides and lignin) and amino acids (glutamic acid, histidine and cysteine etc.) (White et al., 1981).

Cu is sparingly mobile in plants; therefore, the highest concentration of Cu is present in roots compared to shoots of plants (Liu et al., 2001; Nan and Cheng, 2001; Chaignon et al., 2002;

Benimali et al., 2010; Guan et al., 2011). In roots, most Cu is present as divalent ion and major portion of Cu may be bound to cell wall and histidine like ligands (Shi et al., 2008). In roots, higher Cu concentration is located in the root epidermis (Kopittke et al., 2011).

2.2.3 Responses to copper deficiency

Copper is an essential micronutrient in plants that requires for the healthy growth and normal development of plants. It is a constituent of protein and requires for the functioning of more than 30 enzymes (Epstein and Bloom, 2005; Yruela, 2005). However, Cu deficiency causes abnormal plant growth and development. Cu deficiency in plants normally occurs when plant Cu concentration is lower than 5 mg kg^{-1} dry weight of plant (Marschner, 1995). Cu deficiency is reported in organic soils and on sandy soils containing low organic matter. In plants, Cu deficiency altered root and leaf architecture, decrease in chlorophyll pigments and photosynthesis (Yruela, 2005, 2009). Cu deficiency also inhibits many processes occurring within plants. For example, in sugar beet (*Beta vulgaris*) both PS II and PS I electron transports were inhibited due to Cu deficiency and PS II electron transport activity was not restored by adding artificial electron donors (Droppa et al., 1984). Moreover, under Cu deficient conditions, the expression of Cu/Zn superoxide dismutase (SOD) is down regulated and its function is compensated by Fe SOD in chloroplasts of higher plants (Yamasaki et al., 2008). Reduced Cu supply in the nutrient solution decreased the root and shoot biomass of pea plants (*Pisum sativum* L.) and restrictive supply with Mo and Cu to the growth medium strongly affected the activities of the enzymes (nitrate reductase & glutamine synthetase) involved at initial steps of nitrate assimilation and also decreased the plastid pigment content in the leaves (Hristozkova et al., 2006).

2.2.4 Responses to copper toxicity

Higher Cu concentration is toxic to plants causing different toxicity symptoms on plants except in some plants tolerant to higher Cu concentration (i.e. *Arabidopsis halleri* L. and *Elshotzia splendens*) (Shi et al., 2008; Li et al., 2009). Major toxic effects of Cu on plants are also summarized in figure 1.

2.2.4.1 Effects on seed germination

High Cu concentration in the nutrient medium negatively affects seed germination. For example, higher copper exposure to wheat (*Triticum aestivum* L.) seeds reduced seed germination (Singh et al., 2007). Similarly, Saravanan et al. (2001) reported that excess Cu drastically inhibited seed germination of soybean crop. In addition, seed germination of mung bean plant decreased with increasing Cu (50, 200 and 500 µM) concentrations in the nutrient solution (Verma, et al., 2011). However, Ouzounidou, (1995) showed that lower Cu^{2+} (8-16 µM) concentrations seemed to be necessary for seed germination while higher Cu^{2+} (80 and 160 µM) concentrations negatively affected the seed germination of *Minuartia hirsuta, Silene compacta, Alyssum montanum and Thlaspi ochroleucum*.

2.2.4.2 Effects on uptake of mineral nutrients

Increased concentrations of available Cu in soils have been shown to cause reduction in the uptake and translocation of other mineral nutrients (Marschner, 1995; Kopittk and Menzies, 2006). Excess Cu changes the distribution of calcium, potassium and magnesium in roots and shoots of cucumber plant (Alaoui-Sossé et al., 2004). Zn foliar spray increased the leaf Cu content in maize leaves indicating a synergism between Zn and Cu (Aref, 2011). Similarly, foliar application of Cu in combination with Mg on wheat plant grown in sandy soil increased the macro and micronutrients contents of plant (El-Metwally et al., 2010). In cowpea (*Vigna unguiculata*), concentrations of K, Ca, Mg, and Fe decreased in plant tissues as Cu concentration in solution increased (Kopittke and Menzies, 2006). Recently it has been shown that low level of Cu (50 mg kg^{-1} of soil) increased the biomass, macro and micronutrients of green gram (*Vigna radiata* (L.) Wilczek) grown for 45 days while excess Cu has opposite effect on these parameters (Manivasagaperumal et al., 2011). High concentrations of Cu (32-80 µM) in the cultural medium significantly decreased Ca, K and Fe concentrations in fifteen-day-old maize shoots and roots (Ouzounidou et al., 1995). In durum wheat excess Cu decreased Fe uptake by plants (Michaud et al., 2008).

2.2.4.3 Effects on plant growth and biomass

Toxic effects of Cu on plants growth and biomass have been widely reported. Under excess Cu root growth of plants is affected much strongly than shoot growth (Kulikova et al., 2011). Reduction of root growth under Cu stress has been reported in many plant species such as durum wheat (*Triticum turgidum durum* L.) (Michaud et al., 2007; Bravin, et al., 2009), cowpea (*Vigna unguiculata*) (Kopittke and Menzies, 2006), soybean (*Glycine max* L.) (Kulikova, et al., 2011) and Rhodes grass (*Chloris gayana Knuth*.) (Sheldon and Menzies, 2005). Toxicity of excess Cu caused some morphological changes in roots such as thickening of the root, a decrease in the root elongation and an increase in branching, the formation of breaks in the surface cell layers of the root tips and cracking of the epidermis and outer cortex and reduced root hair proliferation (Sheldon and Menzies, 2005; Kopittke and Menzies, 2006; Kopittke et al., 2009; Kulikova, et al., 2011). Shoot morphology is also negatively affected by excess Cu (Vinit-Dunand et al., 2002).

Plants grown in the presence of excess Cu exhibited different toxicity symptoms such as chlorotic symptoms (Cao and Hu, 2000; Yruela, 2005; Xu et al., 2006). For example, increased concentrations of available Cu in soil led to chlorosis symptoms in durum wheat plant (Michaud et al., 2007; Bravin, et al., 2009). Keller and Hammer, (2004) also observed chlorosis symptoms on *Thlaspi caerulescens* plants grown in calcareous soil with large Cu concentrations. Similarly, chlorosis symptoms were observed on durum wheat seedlings above 1 (Michaud et al., 2008) or 0.55 µM Cu^{2+} (Bravin et al., 2010) in the nutrient solution. Moreover, 10µM Cu concentration in the nutrient solution exhibited leaf chlorosis in fourteen-day-old maize plants (Mocquot et al., 1996). Increased Cu^{2+} concentrations at $\geq$5 µM also resulted in leaf chlorosis in Chinese cabbage (*Brassica pekinensis*) plant (Shahbaz et al., 2010).

Excess Cu also negatively affected plant biomass and grian yield (Cook et al., 1997; Yruela, 2009). This reduction in growth and biomass has been observed in many plants such as Sabi grass (Kopittke et al., 2009), maize (Mocquot et al., 1996), Chinese cabbage (Shahbaz et al., 2010), durum wheat (Bravin, et al., 2009), cowpea (Kopittke and Menzies, 2006) and soybean (Kulikova et al., 2011).

2.2.4.4 Effects on photosynthetic pigments

Excess Cu decreased the photosynthesis of plants. This reduction in photosynthesis is related to decrease in chlorophyll contents and structural damages to the photosynthetic apparatus (Cisato et al., 1997; Kabata-Pendias and Pendias, 2001). The Cu inhibitory effects on both photo-systems have been confirmed in a number of studies (Mishra and Dubey, 2005) and it was found that PSII is the most sensitive site to Cu toxicity (Yruela, 2009). Excess Cu decreased chlorophyll contents of leaves of many plant species such as wheat (Cisato et al., 1997; Cook et al., 1997; Quartacci et al., 2001; Singh et al., 2007), cucumber (Vinit-Dunand et al., 2002), sunflower (*Helianthus annuus* L.) (Zengin and Kirbag, 2007) and barley (Caspi et al., 1999). Similarly, there is increasing evidence that reduction in chlorophyll biosynthesis is related to structural damages of the photosynthetic apparatus at the thylakoid level under Cu stress (Cisato et al., 1997) and also to interference of excess Cu with chlorophyll organization (Caspi et al., 1999). Excess Cu also decreased the active photosystem II centers of illuminated leaves (Patsikka et al., 2002) and also resulted in lower photosynthetic electron transport activities (Vassilev et al., 2003; Yruela, 2009).

2.2.4.5 Cu induced oxidative stress in plants

Excess Cu can cause oxidative stress in plants by producing oxygen free radicals and as a result increased the antioxidant responses. Cu-induced oxidative stress influenced the production of enzymes in plants. For instance, increasing Cu^{2+} concentration increased the superoxide dismutase (SOD) activity in leaves and roots of garlic (*Allium sativum* L.) and maize (Mocquot et al., 1996; Meng et al., 2007) and decreased the peroxidase (POD) activity in leaves of garlic plant (Meng et al., 2007). Accordingly, it was observed that under Cu stress the enzymes involved in ascorbate-glutathione cycle increased due to Cu-induced oxidative stress in *Phaseolus vulgaris* (Gupta et al., 1999). Plant response to oxidative stress also depends upon plant species and cultivars (Yruela, 2009).

2.2.5 Tolerance mechanisms to copper toxicity

In order to avoid metal toxicity all plants possess some tolerance mechanisms to maintain correct concentration of essential metals like Cu in different active parts of plant (Yruela, 2005, 2009). First defense strategy is to avoid the accumulation of toxic concentrations at sensitive sites within the cell to prevent the damaging effects rather than developing proteins that can resist the heavy metal effects (Yruela, 2009). Plants may evolve different cellular mechanisms to cope with excess Cu such as reduction in metal uptake, immobilizing excess Cu in roots, vacuole sequestration, chelating Cu with phytochelatins of metallothionines, organic acids and proteins (Clemens 2006; Yruela, 2009). Plants also excrete different organic acids, proteins and carbohydrates. These compounds may complex with Cu and may facilitate or inhibit Cu uptake by plants (Yruela, 2009). P-type Cu transporting ATPases are thought to be involved in preventing toxic levels of Cu in essential cell fonctions (Yruela, 2005). However, importance of these mechanisms may vary with plant species, metal supplied and the exposure time. Once inside the plants, the excess metal will be sequestered into metabolically inactive parts like vacuole, apoplast and epidermal cell walls. High level of Cu can stimulate the accumulation of acids such as proline or histidine which can act as chelator (Sharma and Dietz, 2006).

2.2.6 Synthesis on Cu in soils and plants

Cu concentration in the agricultural soil is increasing continuously due to anthropogenic activities. Cu uptake and accumulation in plant depends upon soil and biological factors. Plants require small amount of Cu for their normal growth and development. However, high Cu concentration is toxic to plants and resulted in the inhibition of many physiological processes in plants. High Cu concentrations have been reported in soils, especially those exposed to long term use of Cu containing fungicides in vineyards, pig farming and industrial pollution. In Southern France, durum wheat is mostly grown in former vineyard soils contaminated with Cu. Recently different Cu toxicity symptoms are observed in the durum wheat plants grown in that area. This higher Cu in plant tissue may affect various physiological, morphological and biochemical functions in plants.

Therefore, the remediation of such Cu contaminated soils to prevent the entry of toxic metal into food chain still needs greater attention. Different techniques exist for the remediation of heavy metals in soils such physical and chemical remediation. However, these methods are often too expensive and inefficient in many cases. Therefore, to produce crops with acceptable Cu concentrations requires an integrated approach to provide a safe and stable food supply, while ensuring the long-term sustainability of our soils.

2.3 Silicon

Silicon (Si) is the second most abundant element on the surface of Earth's crust and in the soils. Si comprises about 28% of the earth's crust (Exley, 1998; Ma and Yamaji, 2006; Sommer et al., 2006). Although the contents of Si in plants are comparable to the levels of many macronutrients, but Si is not considered an essential element for the plant growth (Epstein, 1994, 1999). However, undeniable beneficial effects of this element have been observed in plants with respect to plant growth, better resistance to both biotic and abiotic stresses (Ma and Takahashi, 2002; Ma and Yamaji, 2006). In agricultural soils, Si generally comes due to application of silicate fertilizers, irrigation water and decomposition of crop residues (Figure 4). Generally, $210\text{-}224\times10^6$ tons of plant available Si are removed annually (Matichenkov and Bocharnikova, 2001). This removal of Si takes place due to plant exportation as shown in figure 4.

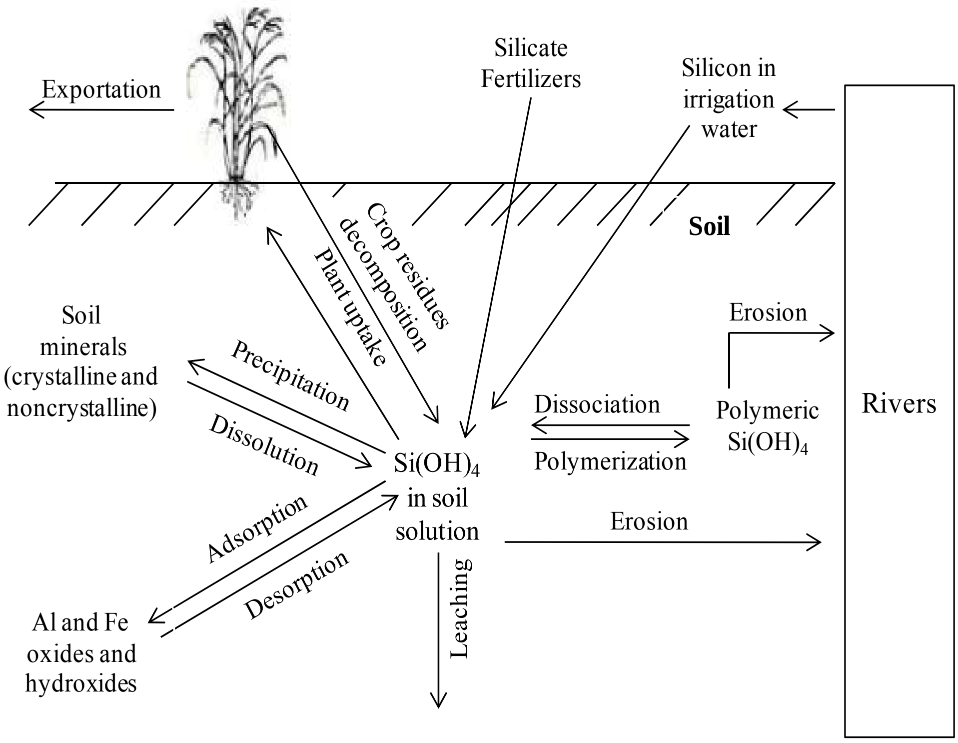

Figure 4. Biogeochemical Si cycle and processes influencing the Si concentration and solubility in the soil (modified from Savant et al., 1997 and Meunier et al., 2001). Where Si(OH)4 indicates a plant available silicon.

2.3.1 Forms of silicon in soil

Silicon compounds in the soil are usually present as SiO_2 and in various alumino-silicate forms. Silicon dioxide comprises about 50-70% of the soil mass (Ma and Yamaji, 2006). External sources of Si include calcium and magnesium silicates, silicate slag, dolomite and rock phosphate (Savant et al., 1999). Silicon compounds exist both in the liquid and in solid phase in the soil (Figure 5). In the solid phase, Si compounds are divided into amorphous and crystalline forms. In natural soil solution, Si is mainly present in the form of uncharged orthosilicic acid, H_4SiO_4 (Epstein, 1994; Sommer et al., 2006). Its concentration ranged from 0.1 to 0.6 mM and it is thought to be the only form which is taken up by plants (Epstein, 1994, 1999; Ding et al., 2005). The maximum solubility of $Si(OH)_4$ is 1.7 mM at 25°C at pH < 9 (Knight and Kinrade, 2001). The chemical similarity between silicate anion and the phosphate anion results in a competitive reaction between the various phosphates and monosilicic acid in the soil. Similarly, Polysilicic acids are an integral part of the soil solution. The mechanism of polysilicic acid formation is not well understood. Unlike monosilicic acid, polysilicic acid is chemically inert and essentially acts as an adsorbent, forming colloidal particles (Hodson and Evans, 1995). Polysilicic acids have been considered important in the soil structure formation (Matichenkov et al., 1995). Since polysilicic acids are saturated with water, they may indeed have an effect on soil water holding capacity.

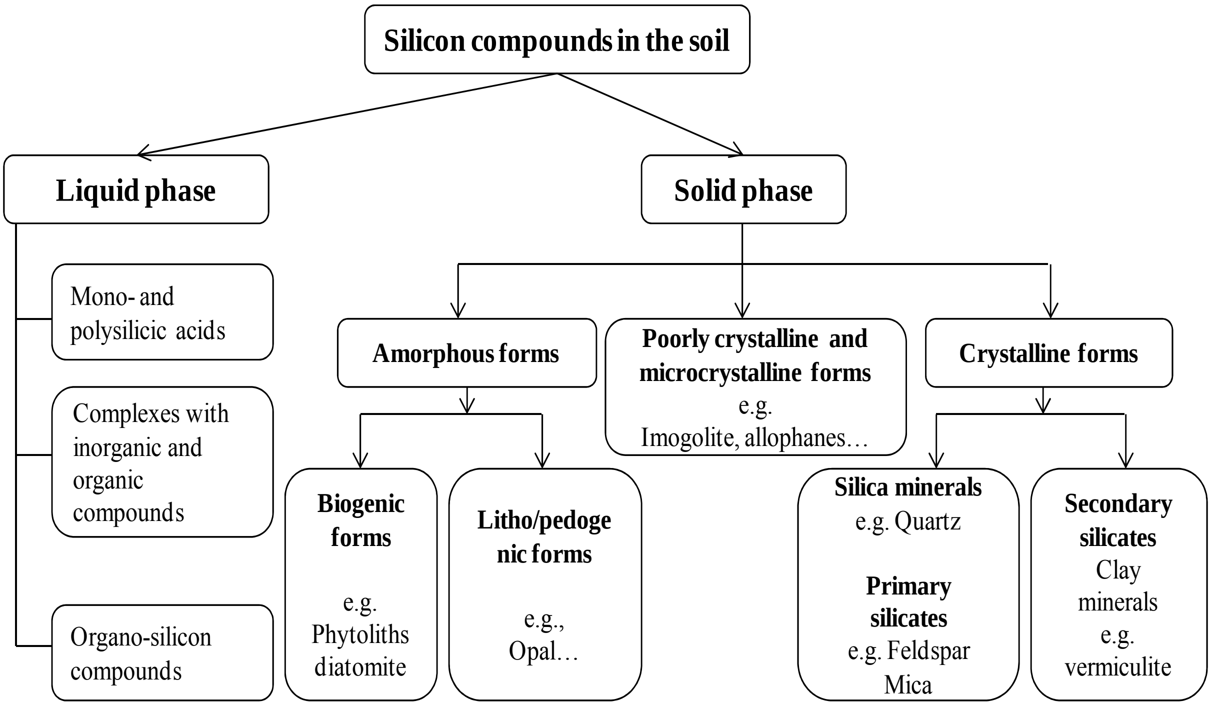

Figure 5. Classification of Si compounds in soil (modified from Matichenkov and Bocharnikova, 2001 and Cornelis et al., 2011).

2.3.2 Bioavailable silicon in soil

The question of Si bioavailability in soil is still not well understood. Most sources of silica are not bio-available (Savant et al., 1997). Solubility of Si is affected by a number of dynamic processes occurring in the soil and soil solution (Figure 4). Usually agronomists define bioavailable silica by the easily-leached silica extracted with an acetate buffered at pH = 4 (Ma and Takahashi, 2002). However, this procedure and others defined to assess the nutrient need of crop species do not dissolve amorphous biogenic silica (Savant et al., 1997) while it has been shown that phytoliths are a pool of Si which should not be neglected, and the amorphous silicon is the important pool of bioavailable silicon (Jones and Handreck, 1967). Amorphous silica is comprised of both ASi from plants including phytoliths and other inorganic forms of ASi (Sauer et al., 2006). Both under a tropical and a temperate forest, phytoliths have been shown to be the first pool of available Si for plants (Alexandre et al., 1997; Bartoli, 1981). The amount of biogenic silica (phytoliths) in soils ranges from 0.03 to 0.06 wt% (Desplanques et al., 2006). Grassland soils contain phytoliths up to only 1-3% of total Si pool (Blecker et al., 2006). The phytoliths are mainly composed of about 92 wt% silica and 6 wt% water with small amounts of carbon and traces of Al and Fe (Meunier et al., 1999). Isotopic studies on a bamboo forest also showed that Si in the soil solution comes mainly from phytoliths dissolution (Ding et al., 2008). Indeed, Fraysse et al. (2006, 2009) have shown that phytoliths dissolve faster than other silicates at pH>4 and solubility of phytoliths is about 17 times higher than that of quartz. Therefore, in natural ecosystems, soil phytoliths can be a major source of bioavailable Si for plants (Farmer et al., 2005).

Man ignored exogenous application of Si with the belief that the soil itself can sustain its supply but most recently Guntzer et al., (2012) showed that continuous cropping and removal of wheat straw reduced the bioavailable Si from the soil. Therefore, there is need of external plant available Si amendments to maintain higher level of bioavailable Si in soils. The practice of Si amendment on rice and sugarcane is already well developed in different parts of the world (Liang et al., 1994; Korndörfer et Lepsch, 2001). These amendments include calcium and magnesium silicates, silicate slag, dolomite and rock phosphate etc (Savant et al., 1999). However, due to low solubility of Si, there is a practical limit to increase silicic acid bioavailability in the field. In addition, some industrial Si amendments are toxic to crop plants and needs to be evaluated with considerable care (Côté-Beaulieu et al., 2009). Therefore, there is need to use non toxic sources of silicon with higher capacity to release bioavailable silicon. However, little is known about the ability of different minerals to release plant available silicon so there is still a need for further research to understand the efficacy of different minerals to release the phytoavailable silicon. In this thesis, the efficiency of different minerals to release plant available Si and its effect on Cu and Cd toxicity in durum wheat is assessed.

2.3.3. Amorphous silica determination methods

In order to determine the available Si to crop plant it is therefore necessary to measure the amount of phytoliths and other forms of amorphous silica in soils. There are two procedures to quantify amorphous silica particles: 1) physical extraction using heavy liquid density (Kelly, 1990) and 2) wet chemical extractions including acid and alkaline extractions (Kodama and Ross, 1991; Saccone et al., 2007). However, the use of chemical extractants has not been fully exploited yet. The physical extraction of phytoliths is problematic because phytoliths are probably not the only source of plant available silicon (Alexander et al., 1997; Blecker et al., 2006).

So far, many methodologies have been developed by different authors to evaluate plant available Si from the soil (Fox et al., 1967; Biermans and Baert, 1977; Khalid et al., 1978;

Korndorfer et al., 1999; Snyder et al., 2001). Recently, Sauer et al. (2006) reviewed different extraction techniques for the measurement of plant available Si from the soil. However, the validity of these techniques for measuring ASi in different soils needs to be tested because ASi concentration varies with soil type and horizon. Saccone et al. (2006) showed that samples pre-cleaned with acid and peroxide then extracted with Na_2CO_3 showed better extraction efficiency compared to not pre-cleaned samples. Similarly, Saccone et al. (2007) suggested that alkaline extraction methods especially sodium carbonate can be used on a variety of soils for the extraction of ASi because Si extracted by acid methods was two order of magnitude less than Si extracted by alkaline methods and pre-cleaning was not necessary for the extraction of soil ASi. On the other hand, Herbauts et al. (1994) indicated that the alkaline methods may attack non amorphous minerals and that correction is necessary when using these methods. However, assessing the amounts of potentially available Si in soils still remained problematic due to variability of ASi in each soil and horizon. Due to the growing demand for the use of Si, there is need to investigate the single soil extraction methods having high potential for determining the plant available Si in the soils of different origins. In this thesis, the efficiency of different single soil extraction methods is assessed for quantification of amorphous silica in soils of different origins.

2.3.4 Si contents in plants

Silicon concentration in crop plants varies from 0.1 to 10%wt weight of plants (Epstein, 1994, 1999; Hodson et al., 2005; Ma and Yamaji, 2008). Plant species, in general, are classified as high-, intermediate-, and non Si-accumulators (Takahashi et al., 1990). Plants containing more than 1% Si in the dry leaf matter are considered as Si accumulators (Epstein, 1994). Generally, graminaceous plants take up more Si as compared to other plant species and some dicotyledons such as legumes exclude Si from uptake (Ma et al., 2001a). It is widely accepted that wheat is a Si accumulating plant (Mayland et al., 1991; Rafi and Epstein, 1999; Casey et al., 2003; Rains et al., 2006). However, Si concentration in plant tissues varies widely and depends upon the characteristics of Si uptake and transport, variability between species and genotypes within a species (Jones and Handreck, 1967; Epstein, 1994; Deren, 2001; Ma and Yamaji, 2008). Similarly, a species or cultivar grown under different conditions will absorb different amount of silicon and different parts of plants generally have varying Si concentrations (Henriet et al., 2006; Ma and Takahashi, 2002).

2.3.5. Silicon uptake by roots

Plant roots take up Si in the form of un-dissociated H_4SiO_4 (Casey et al., 2003; Ding et al., 2008). Si uptake mechanisms differ between plant species (Ma and Yamaji, 2006). Ma and co-workers showed that absorption of silicon is achieved by lateral roots of rice and root hairs do not play a role in Si uptake (Ma et al., 2001b). The mechanisms of Si absorption by roots are still controversial. There are, however, three possible mechanisms of Si uptake by higher plants namely passive, active and rejective. Plants that take up Si by active process tend to have higher Si concentrations in the xylem than that of growth medium, those that take up Si by passive process have similar Si concentration both in xylem sap and in growth medium and those that exclude Si have a lower Si in xylem than that in the growth medium (Takahashi et al., 1990; Ma et al., 2004; Mitani and Ma, 2005). Passive uptake of Si takes place along the transpiration stream and it is shown that most dicotyledonous plants absorb Si passively (Takahashi et al., 1990; Ma et al., 2001a).

Active uptake of Si takes place by different transporters and appears to require the expenditure of metabolic energy (Jones and Handreck, 1965). Active Si uptake is dominant in many plant species such as rice, sugar cane and wheat (Jones and Handreck, 1967; Casey et al., 2003; Rains et al., 2006). Active transport mechanisms by Si-transporters have been studied in many plant species (Mitani and Ma, 2005). However, the molecular mechanisms of Si are still poorly understood. Recently, two transporters (Lsi1 and Lsi2) responsible for active Si uptake have been identified in rice (Ma et al., 2006; Ma et al., 2007). Similarly, Si influx transporters have also been identified in barley (HvLsi1) and maize (ZmLsi1) (Chiba et al., 2009; Mitani et al., 2009). Unfortunately, molecular mechanisms responsible for active Si uptake in wheat and other gramineous plant species are still unknown. In future, identification of more Si uptake transporters in different plant species will help to better understand the Si uptake mechanisms.

2.3.6 Silicon transport and deposition in plants

Once absorbed by roots, Si is translocated to shoots via xylem along transpiration stream; this assumes that the movement of Si takes place with the movement of water (Jones and Handreck, 1965). However, the transporters responsible for Si xylem loading have not been identified yet. In many plants, More than 90% of Si taken up by the roots is translocated to the shoots (Ma and Takahashi, 2002). During its passage through the xylem, Si has to remain in solution as unpolymerized form. However, the mechanisms which prevent polymerization are not well understood but they may involve association with organic compounds (Kaufman et al., 1981). However, Casey et al. (2003) found no evidence of organo-silicate complexes in wheat plants. In the xylem sap, Si is present at higher concentration and mostly in the form of monosilicic acid and some as disilicic acid with an approximate concentration ratio of 7 : 1 respectively (Casey et al., 2003; Mitani et al., 2005). Therefore, it is likely that transporters are also required for Si xylem loading, unloading and distribution. However, these transporters still need to be identified. Recently, Yamaji et al. (2008) identified Si transporter (Lsi6) responsible for xylem unloading, which subsequently affects the distribution of Si in the leaf. They also identified that this transporter is localized at the xylem parenchyma cells of leaf blades and sheaths.

After translocation to shoots, Si is ultimately deposited as amorphous silica, $SiO_2.nH_20$ mainly at transpiration sites (Ma and Takahashi, 2002). Once deposited in this form, Si is immobile and not redistributed (Hodson and Sangster, 1990). Deposition of Si takes place in different parts of plant such as epidermis of shoots but can also occur in cell walls of root endodermis (Marschner et al., 1990; Ma and Takahashi, 2002; Lux et al., 2003). Maximum deposition of Si takes place in the leaf apical region and then systematically decreases towards the base of the leaf (Jones and Handreck, 1967). In graminaceous plant species such as maize and sorghum, silica is deposited as silica bodies in the epidermis as well as in the stomata and leaf sheath (Currie and Perry, 2007). Mecfel et al. (2007) showed that in wheat plants Si was predominately deposited in the epidermis cells of the leaves and their cell walls. In rice plant, Si deposited as a thick layer in the space immediately beneath the thin cuticle layer, forming a cuticle-Si double layer in leaf blades (Ma and Yamaji, 2006). However, silica bodies formed and their localization varied between individual plant families. These silica bodies are formed by different processes in which polymerization of monomers to form stable nuclei with critical size takes place. Overall the process of polymerization of monomeric silicic acid to form larger silica particles (phytoliths) is given in figure 6.

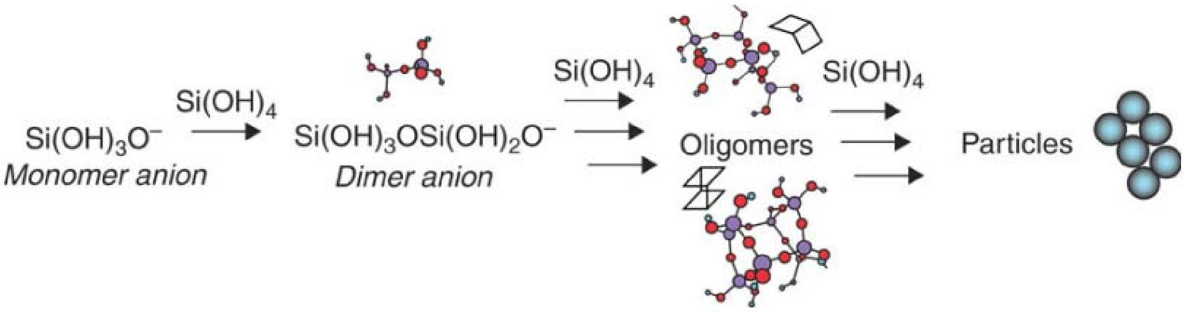

Figure 6. Polymerization of monomeric silicic acid to form larger silica particles precedes though various condensation reactions with dimers, oligomers and aggregates as intermediates. (Extracted from Currie and Perry, 2007).

In this thesis, it is hypothesized that silica uptake, translocation and deposition may be changed under metal stress but also silica may change metal uptake, translocation and deposition in plants. This may be helpful to mitigate the heavy metal stress mechanisms in durum wheat plants.

2.3.7 Silicon functions in plants

Although silicon has not been considered as an essential element for plants, it has been proved to be beneficial for the growth and development of many plants, particularly graminaceous plants such as rice and sugarcane (Epstein, 1994). Si protects the plants from a number of abiotic and biotic stresses given in figure 7 (Epstein, 1999, 2009; Bélanger et al., 2003; Ma and Yamaji, 2006; Côté-Beaulieu et al., 2009).

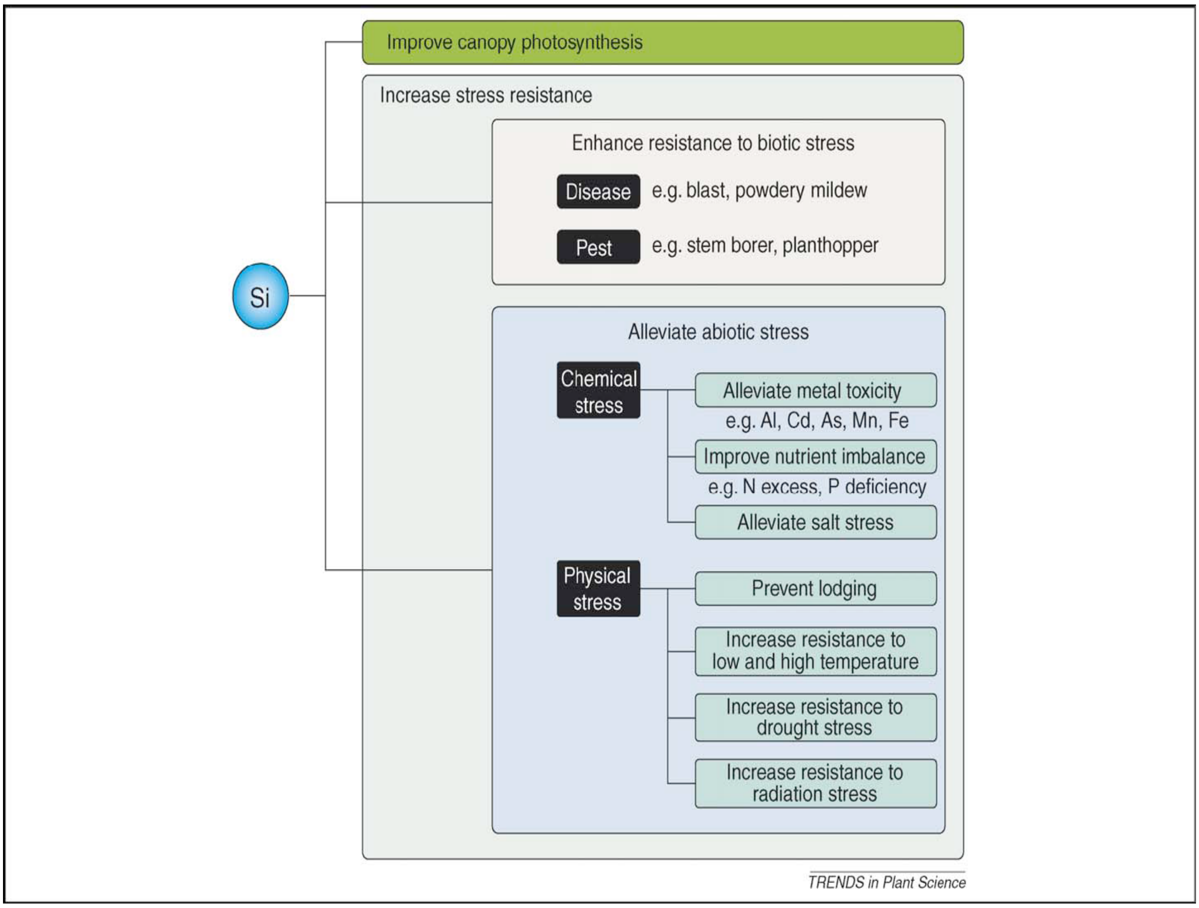

Figure 7. Beneficial effects of silicon on plant growth under biotic and abiotic stresses (Extracted from Ma and Yamaji, 2006).

Experiments showed that plants supplied with Si resist lodging by increasing mechanical strength of plants enabling them to achieve and maintain an erect habit. Epidermal tissue deposition of Si also protects plants against excessive loss of water by transpiration (Emadian and Newton, 1989). Reviews on the role of Si in plants therefore stress the association of Si with cell walls and discuss the increased rigidity of cell walls of plants grown with available Si (Raven, 1983; Jones and Handreck, 1967).

Also, Si is rapidly absorbed by wheat (*Triticum aestivum* L.) plants (Casey et al., 2003; Rains et al., 2006) but its role in wheat plant has not been widely studied. However, beneficial effects of Si are studied in many plant species other than wheat i.e. rice, cucumber, maize and bamboo (Rogalla and Römheld, 2002; Ma and Takahashi, 2002; Shi et al., 2005a; Zhang et al., 2008; Vaculik et al., 2009; Feng et al., 2010).

Silicon affects the plants in many ways as given in figure 7 (Ma and Yamaji 2006; Liang et al., 2007; Guntzer et al., 2011). However, in the following section we discussed the role of Si on the alleviation of metal toxicity in plants. We also highlighted the points which still need consideration for better understanding the mechanisms of Si-mediated alleviation of metal toxicities in durum wheat plants.

2.3.8 Silicon and metal toxicity

Several studies showed the ameliorative effect of Si on the toxicity of certain metals such as Zn, Mn, Al, Cu and Cd. All the studies showed that Si supply reduced the metal toxicity in many plants species. However, mechanisms of Si-mediated alleviation of metal toxicity remained poorly understood and needs further investigation to clarify the role of Si in metal tolerance mechanisms. The proposed hypotheses in the literature are presented below and it is shown that combination of more than one hypothesis is observed to explain the role of Si in metal tolerance.

2.3.8.1 Modification of environmental conditions

It has been shown that Si application in the soil affects the soil properties which in turn control heavy metals availability to plants (Liang et al., 2007). However, not many data is available regarding the effect of Si on soil factors which control metal bioavailability. Among soil factors, pH and organic matter are two of the most important factors that control Cd availability and it has been shown that Si decreased metal bioavailability by increasing soil pH (Chen et al., 2000; Barančiková et al., 2004; Liang et al., 2005; Treder and Cieslinski, 2005; Gu et al., 2011). Solubility of Cd is closely related to the acidity of the soil solution. Liang et al. (2005) showed that application of Si at a rate of 400 mg Si kg^{-1} reduced Cd availability because of a silicate-induced pH rise in the soil. More recently, Li et al. (2012) reported that addition of 800 mg sodium metasilicate kg^{-1} in Pb contaminated soil significantly increased soil pH and decreased exchangeable Pb in soil but that 100 mg kg^{-1} did not influence soil pH.

Silicon can also change the speciation of metals in soil solution by the formation of silicate complexes (Ma et al., 1997; Liang et al., 2007). Chen et al. (2000) reported that the higher Si concentration due to added furnace slag reduced Cd uptake by rice and wheat plants. In addition, more Cd was found in the form of oxides or adsorbed Fe-Mn oxides bound fraction in the Si-amended soil. These results suggest an external interaction between Si and Cd. Si application in the form of calcium silicate also altered the Cd and Zn distribution in the soil (de Cunha et al., 2008). Application of silicate fertilizer could effectively immobilize Cd in the soil and decrease its uptake in sweet basil (*Ocimum basilicum*) plant (Putwattana et al., 2010). Recently, Gu et al. (2011) reported that Si rich amendments immobilized Cu, Cd and Zn in multi-metal contaminated acidic soils by increasing in soil pH. They also showed that mobile metals were mainly deposited as their silicates, phosphates and hydroxides in amended treatments. However, to our knowledge, no other study is available on Si and Cu interactions in the soils.

2.3.8.2 Effect of silicon on the uptake of plant nutrients

Silicon plays an important role in the absorption of nutrients by the plants. However, little is known about the mechanisms of mineral nutrient absorption in the presence of Si. It plays an important role both in soil and in plants itself. Silicon increased the efficiency and utilization of essential plant nutrients. Si application in hydroponic solution increased the content and accumulation of phosphorus in maize seedlings and significantly alleviated the effects caused by low-P stress (Yang et al., 2008). Si significantly affected the adsorption and desorption of P in soils (Kewei et al., 2002, 2004) and also increased the P uptake from hydroponics (Corrales et al., 1997). However, Ma and Takahashi, (1990, 1991) reported that in rice plant silicate application negatively affected the phosphorus uptake both in soil and hydroponics. Recently it has also been shown that Si application in highly weathered soil increased P uptake and utilization in rice plant by enhancing the P/Mn and P/Fe ratios (Tavakkoli et al., 2011). Sodium silicate enhanced K concentrations in soybean plant in K-deficient seedlings (Miao et al., 2010). However, effect of Si on mineral nutrients under metal stress is rarely studied. Recently, Frantz et al. (2011) showed that Si influenced mineral nutrient concentrations, either as a main effect or an interactive effect, in zinnia (*Zinnia elegans*) plants under Cu stress. Si may increase the uptake of other essential nutrients under metal stress. Oliva et al. (2011) reported Si may enhance the uptake and translocation of Zn and Ca in *Erica andevalensis* plants under Cu stress which might contribute to reduce Cu content in shoots. There is, however, still need to evaluate the uptake of mineral nutrients in the presence of Si under metal stress. Moreover, the effect of Si on micro- (Zn and Mn) and macronutrient (Ca, Mg, K and P) under Cd and Cu stress has not been studied yet. Effect of Si on the uptake and accumulation of micro- and macronutrients under Cu and Cd stress may be helpful to find the possible mechanisms of Si-mediated alleviation of metal toxicity in durum wheat plant.

2.3.8.3 Increase in growth and biomass

Role of Si in the growth and biomass of plants has been widely accepted. Si supply may increase the biomass of plants under metal stress and may decrease the metal toxicity by dilution effect (same uptake but larger biomass). However, this mechanism is not well understood and is still controversial. Si supply increased the dry weight of both shoots and roots of many plant species such as maize (Liang et al., 2005; Da Cunha and do Nascimento, 2009; Vaculik et al., 2009), wheat (Rizwan et al., 2012), strawberry (Treder and Cieslinski, 2005), and rice (Gu et al., 2011) plants grown on Cd contaminated soils. Si supply also increased the biomass of roots and leaves of rice grown hydroponically under Cd (Nwugo, and Huerta, 2008; Zhang et al., 2008) and Zn stress (Song et al., 2011; Gu et al., 2012). Also in cucumber plants, Si supply also inhibited the Mn toxicity symptoms and improved the plant growth (Shi et al., 2005a; Feng et al., 2010). Similarly, Si supply increased length of leaves, leaf area and root length of maize plant grown in hydroponics under Cd stress (Vaculik et al., 2009). Si supply also increased the total root length, total root surface area of rice plants under Zn stress (Song et al., 2011). In addition, Corrales et al. (1997) observed that in maize plants, Si supply increased the shoot and root dry weights. Si also increased the biomass of maize plants under Mn stress (Doncheva, et al., 2009). Role of Si on growth and biomass under Cu stress has been rarely studied. Recently it has been shown that Si supply also increased plant biomass of *Arabidopsis thaliana* and *Erica andevalensis* under Cu stress grown in hydroponics (Li, et al. 2008; Khandekar and Leisner, 2011; Oliva et al., 2011). However, the effect of Si on growth and biomass of durum wheat under Cd and Cu stress is poorly under stood as compared to maize and rice.

2.3.8.4. Decrease in metal absorption and transport

Recently, Si-mediated removal of heavy metal toxicity in plants is widely accepted. However, to date, the mechanisms of Si-mediated alleviation of metal toxicity in plants are still poorly understood (Neumann and zur Nieden, 2001; Shi et al., 2010, Song et al., 2011). One of the major effects of Si on the reduction of metal toxicity is reducing the metal uptake by plants. This was observed in maize (Liang et al., 2005), rice (Shi et al., 2005; Li et al., 2011) and in *Brassica chinensis* L. (Song et al., 2009). This decrease in metal uptake in the presence of Si can be explained in many ways such as Si can enhance the uptake of calcium ions (Hammond et al., 1995) which compete with Cd for same uptake sites. Si may stimulate production of root exudates which can chelate metals and reduce their uptake by roots (Kidd et al., 2001). In addition this reduction in metal uptake can also be explained by the fact that Si reduced the apoplastic transport of metals by decreasing free metal concentration in the apoplast (Iwasaki et al., 2002; Rogalla and Romheld, 2002). Moreover, the physical barrier formed by the deposition of Si in the vicinity of the endoderm may reduce the cell wall porosity of inner root tissues, thus reducing the metal concentration in the xylem (Shi et al., 2005; da Cunha and Nascimento, 2009). Similarly, Si decreased apoplastic transport of Cd in *Kandelia obovata* through the enhanced adsorption of Cd on the cell walls (Ye at al., 2012).

However, it is unlikely that the mechanism reducing the concentration of metals in aerial parts is common to all species and is only due to the reduction of metal accumulation by plants. Moreover, in some studies, Si increased the metal concentrations in the plant while reducing their toxicity which may be due to co-precipitation of Si and metals or due to delayed development of endodermal suberin lamellae in the root endodermis in the presence of Si (Neumann and zur Nieden, 2001; da Cunha and Nascimento, 2009; Vaculik et al., 2009). However, Si effect on metal uptake and accumulation in plants depends on plant species and cultivars (Li et al., 2011).

In this thesis it is hypothesized that under Cd and Cu stress Si may decrease Cd and Cu uptake and accumulation by increasing the uptake and accumulation of other micro- (Zn and Mn) and macronutrients and by depositing higher Cd and Cu contents in root apoplast.

2.3.8.5 Modification of metal distribution in plants

Several studies have observed the localization of metals and silicon in shoots and roots of plants to understand Si detoxification mechanisms in plants. For the first time in 1957, Williams and Vlamis (1957) showed that Si application did not affect Mn concentration in barley leaves but Mn was evenly distributed in leaves instead of discrete necrotic spots, so in this way Si reduced the effect of Mn toxicity. Si may decrease the translocation of metals by depositing higher concentration of metals in roots. For example, Cd and Zn stress may be alleviated by the deposition of silica in the endodermis and pericycle of maize roots (Da Cunha and do Nascimento, 2009). Zhang et al. (2008) observed that Si decreased Cd accumulation in rice shoots by compartmentalization of Cd in the root cell walls. Similarly, Shi et al. (2005b) showed that in rice plant, Si application decreased the translocation of Cd from roots to shoots by 33%. They showed that Cd was mainly deposited in the vicinity of the endodermis and epidermis and Si was more deposited in the vicinity of the endodermis than in the epidermis. Deposition of Si in endodermis may play a role in the decrease of Cd translocation. Si also reduced metal toxicity in rice plant by stronger binding to less bioactive tissues (Gu et al., 2012). In addition, Neumann et al. (1997) reported that in *Minuartia verna*, Si accumulating dicotyledon, Zn was co-precipitated as Zn silicates in the cell walls of leaf epidermis.

More recently, Vaculik et al. (2009) showed that Si did not reduce the total Cd uptake in maize plant. However, Si may decrease metal toxicity through development of endodermal suberin lamellae in root endodermis of plants. Liang et al. (2005) reported that Cd concentration in xylem exudates of rice decreased in Si-amended Cd treatments compared with the non-Si amended Cd treatments which may be due to significant increase of xylem sap in the presence of Si under Cd stress. Similarly, Gu et al. (2012) reported that Zn concentration in the xylem exudates decreased due to silicate supply which may be due to Zn-Si precipitates in the cell walls of less active tissues. In addition, Si supply also increased xylem sap of banana plants grown in Pb contaminated soils and decreased Pb concentration in the xylem sap (Li et al., 2012). Doncheva et al. (2009) showed that Si increased the thickness of epidermis layers of maize plants under Mn stress. They suggested that Mn storage in non photosynthetic tissue could be a Mn tolerance mechanism in maize plant. In cucumber, Si increased the amount of Mn bound to the cell walls (90%) and reduces the symplasmic proportion (10%). It is therefore proposed by the authors that Si promotes binding of Mn in cell wall thereby decreasing its toxicity (Rogalla and Römheld, 2002a, b, Wang et al., 2000). Al toxicity in wheat and maize plants is ameliorated by the formation of hydroxylaluminumsilicates (HAS) in root apoplast of Si treated plants (Cocker et al., 1998; Wang et al., 2004).

Silicon may decrease the metal toxicity by sequestrating the metal in metabolically inactive parts of shoots. In leaves of cowpea, Iwasaki et al. (2002) observed a decrease in the amount of Mn in the apoplastic fluid combined with a highest rate of Mn adsorbed on the cell walls after the addition of Si 50 µM. Vaculik et al. (2009) showed that Si did not reduce the total Cd uptake by plant but Si may decrease metal toxicity through co-precipitation in shoots. Si may sequester Cd in the shoot cell walls of rice plant (Liu et al., 2009). More recently Vaculik et al. (2012) reported that Si mediated alleviation of Cd toxicity in maize may be attributed to enhanced apoplasmic binding of Cd in shoots. Neumann and zur Nieden (2001) showed that

Si may reduce Zn toxicity by co-precipitation with Zn in the form of Zn silicate in the vacuole and cell wall of *Cardaminopsis halleri* leaves.

On the other hand, while Si is reported to alleviate the toxicity of some heavy metals (Liang et al., 2007), its role in reducing the toxicity symptoms induced by excess Cu has been rarely studied. However, Li, et al. (2008) indicated that Si addition improved the resistance of *Arabidopsis* to Cu stress ranging from physiological changes to alterations of gene expression. Their study showed that Cu concentration in plants is not changed but the plant changed its strategy to Cu tolerance. Similarly, Khandekar and Leisner, (2011) proposed that in *Arabidopsis,* Si apparently allows plants to more efficiently respond to Cu toxicity by manipulating both Cu-binding molecules and expression of free-radical metabolizing enzymes. Oliva et al. (2011) showed that in Cu tolerant species *Erica andevalensis*, Si played an important role in diminishing Cu toxicity symptoms mostly by decreasing Cu translocation to the shoots and apparently increasing Cu fixation in root tissues. They also showed that leaf phytoliths might play some role by immobilizing Cu in leafs. However, there is still need to evaluate the mechanisms of Si-mediated alleviation of Cu toxicity in plants. Previous studies have focused mainly on the mechanisms of Si-mediated alleviation of metal toxicities in rice, maize and cucumber. However, little information is available on Si-mediated alleviation of metal toxicity in wheat plant despite the fact that wheat accumulates large amounts of Si.

2.3.8.6 Increase in photosynthetic pigment contents

Not many data is available on the effect of Si on photosynthetic pigments. Recently, it has been shown that Si supply increased chlorophyll pigment contents in cucumber plants. Si played an important role in protecting photosynthetic machinery from damaging (Feng et al., 2010). Similarly, Si supply increased concentrations of chlorophyll *a* and *b* and carotenoids in maize plants as compared to Mn treatment alone. This higher maintenance of chlorophylls concentrations may be related to slight alterations in the chloroplasts under Si supply as compared to without Si supply (Doncheva, et al., 2009). Moreover, Kaya et al. (2009) reported that Si supply increased chlorophyll contents in maize plants. Similarly, Si (0.6 mM) supply also increased photosynthesis in rice plants under Cd stress which may be due to improvement in light use efficiency in the presence of Si (Nwugo and Huerta, 2008). However, Effects of Si on photosynthetic pigments remained poorly understood.

2.3.8.7 Modification of gene expression

Plants have developed a complex antioxidative defense system including antioxidants. Stimulation of antioxidants under metal stress appears a mechanism that accounts for increased metal tolerance in plants. Effect of Si on antioxidant has been rarely studied. Si supply may enhance the activity of antioxidant (e.g. SOD, CAT, APX) under metal stress to help plants to cope with metal stress. This Si stimulation effect on antioxidants has been observed under Cd stress in *Brassica chinensis* (Song et al., 2009) and peanut (Shi et al., 2010), under Mn toxicity in *Cucumis sativus* (Shi et al., 2005b) and rice (Li et al., 2011; Tripathi et al., 2012) and under Cu toxicity in *Arabidopsis thaliana* (Khandekar and Leisner, 2011).

Recently, it has been shown that 1.5 mM Si application under Cu stress stimulated the genes responsible for the production of metallothioneins (MTs) that can chelate toxic metals. Similarly, Si may decrease the expression of PAL (phenylalanine ammonialyase) genes under Cu stress (Li et al., 2008). However their role in decreasing Cu toxicity is not well understood (Khandekar and Leisner, 2011).

2.4 Conclusion

Generally the above discussion showed that soils are continuously contaminated by Cd and Cu which is due to parent material and anthropogenic activities. These metals are also highly toxic to durum wheat plants and enter the food chain via plants and animals. Therefore, there is need to reduce metal toxicity in plants. Different actions can be undertaken to reduce the absorption of metals by plants but most methods are costly and not efficient. However, it is shown that silicon is not an essential element for plants but is beneficial for many plants. The beneficial effects of Si are distinct in many plants under biotic and abiotic stresses.

Although our knowledge of Si alleviation of metal toxicity in plants as well as in the soil-plant system has increased considerably in the recent years, there are still many gaps, to our knowledge, regarding the basic mechanisms of Si alleviation of metal toxicity in plants. Certainly more research is needed regarding the mechanism of Cd and Cu uptake by the root, translocation, and its deposition within plants in the presence of Si. Additionally, the above literature synthesized that most studies related to Si mediated metal stress are conducted in rice and maize plants but not in wheat. There is need to evaluate the role of Si in heavy metal tolerance in durum wheat plants. Similarly, most forms of Si are unavailable to plants and the capability of different minerals to release bioavailable Si and the methods to assess plant available Si in soil have not been well documented yet.

Chapter 3: Improving the methodology for understanding the soil/plant interaction of Si in cultivated lands

In order to determine if the Si uptake by plant is correlated with the pool of ASi from natural soils, we conducted a pot experiment on 3 different French soils. In the first part two acid extraction (0.5 M acetic acid and 0.2 M NH_4 oxalate buffered at pH 3.0) methods and two alkaline extraction (0.094M Na_2CO_3 and 0.1 M Tiron) methods were tested in order to characterize the soil material. In a second part we reported a long-term experiment of straw exportation where the variability of key parameters (ASi in soil and plants; DSi, pH) was measured.

3.1 Introduction

Availability of Si depends upon the silicic acid concentration in the soil solution, the physico-chemical conditions (i.e. pH) and the pool of easily leachable solid compounds (Savant et al., 1997; Ding et al., 2005; Henriet et al., 2008). Solid Si compounds consist of primary or secondary crystalline silicates and amorphous/ poorly crystallised silica (ASi) (Sauer et al., 2006). ASi includes lithogenic forms (volcanic glasses, non crystalline inorganic fractions of iron oxides/hydroxides and Si in alumino-silicates) and biogenic forms (phytoliths and microorganisms remains) (Cornelis et al., 2011).

Currently, different extraction techniques are used for the measurement of plant available Si from the soils (Sauer et al., 2006). The common methods used in soil science for the extraction of Si include acetate, oxalate, NaOH, Na_2CO_3 and Tiron (Sauer et al., 2006). The acid techniques are used for the determination of Si rapidly available to plants while alkaline techniques are more appropriate for the determination of ASi. Saccone et al. (2007) showed that acid techniques are able to extract only minor quantities of Si compared to alkaline techniques because phytoliths are not affected by acids. However, Cornelis et al. (2011) showed that in acid soils from temperate forests, Si extracted by oxalate partly extracted Si adsorbed onto amorphous Fe oxides and did not extract opal Si that is usually extracted by alkaline solution. Using pot experiment, Buck et al. (2011) found that extraction with Na_2CO_3 + NH_4NO_3 was more efficient than acid extractions for extracting plant available Si from solid fertilizer sources. Studies that use such combinations of extractants are encouraging for producing a better understanding of the Si bioavailability.

Here we would like to assess the significance of acid and alkaline methods used in determining the bioavailable Si in soil. More precisely, we would like to determine the applicability of the Na_2CO_3 extraction method which has been recently widely used for the determination of ASi (Clymans et al., 2011). In the first part, two acid extraction (0.5 M acetic acid and 0.2 M NH_4 oxalate buffered at pH 3.0) methods and two alkaline extraction (0.094 M Na_2CO_3 and 0.1 M Tiron) methods were tested on a diverse group of soil samples. Different soils were selected to provide a wide range of Si contents under diverse soil forming conditions. In the second part durum wheat (*Triticum turgidum* L.) was grown on a variety of cultivated soils containing various amounts of amorphous silica and with various physicochemical conditions. Durum wheat is a silicon accumulator species and is a commonly cultivated crop around the Mediterranean Sea. Amorphous silica content of the soil, soil solution and silicon content in plants were analyzed in order to better understand the impact of Si uptake and exportation of crops on soil Si pools.

3.2 Materials and methods

3.2.1 Materials

All soil samples were collected from different climatic regions. For the first four soils, the samples were taken from the surface (0-20cm depth) at several points and the O horizon was discarded when present. The properties of all soil samples are given in table 1. The soils are:

1. A Silandosol (further refered as Andosol) under a *Pinus sylvestris* forest located on the east slope of the Puy de la Vache volcano (Chaîne des Puys, Massif Central, France, 45.6698°N, 2.9666°E), at 1000 m a.s.l..
2. An aric Podzol from the Landes (Villenave d'Ornon, France) located at 10 m a.s.l., which was under maize cultivation at the time of sampling.
3. A Calcisol from the Arvalis experimental station at Gréoux-les-Bains, France (43.7513°N, 5.8579°E) at 340 m a.s.l.. This soil is cultivated with cereals and its pH is higher than the two other soils (~8).
4. A haplic Luvisol from Switzerland and more specifically Rafz, near Zurich airport (47.37°N, 8.32°E) at 419 m a.s.l.. This is an agricultural soil and was contaminated with sewage sludge in the 1960s with pH around 6.8 (Krebs et al., 1999).
5. Grounded quartz supplied by Sibelco as BE01.
6. Mascareignite: an andosol horizon from La Réunion Island containing mostly phytoliths (Meunier et al., 1999).
7. A Podzol from Champex (Switzerland) 46°01'55" N, 7°05'55" E at 1650 m a.s.l.. It is acid loamy sand. Three different horizons of this soil were selected based on their various iron and aluminum oxyhydroxide content: an E (eluvial) horizon (1-20 cm depth) and two illuvial horizons namely BPh (20-25 cm depth) and BPs (25-60 cm depth) (Keller and Domergue, 1996).

Table 1: Properties of the soil samples used in the experiment.

Soil type	Depth	pH_w	Organic C*	N*	Sand (%)	Silt (%)	Clay (%)	Exch. K**	Exch. Na**	Exch. Mg**	Mehra-Jackson extraction***		
	(cm)										Al*	Fe*	Si*
Andosol A horizon	0-20	5.5	88.3	6.45	34	53.6	12.4	0.165	0.238	2.22	28.5	48.6	6.36
aric Podzol L horizon	0-20	5.3	20.4	0.58	93.9	4.1	2.0	0.072	0.076	0.272	0.35	0.2	1.39
Calcisol Lhorizon	0-20	8.2	14.4	1.38	18.9	52.4	27.7	0.777	0.024	0.453	1.4	11.3	1.34
haplic luvisol L horizon	0-20	6.8	16	1.78			16.4				1.04	6.78	1.25
Quartz	-	-	-	-	-	-	-				-	-	-
Mascareignite	0-15												
Podzol E horizon	1-20	3.9	-	-	-	-	4				0.5	0.7	-
Podzol BPh horizon	20-25	4.6	30	1.9	-	-	4				7.4	15.8	-
Podzol BPs horizon	25-60	5.1	-	-	-	-	3				4.7	12.5	-

* in g kg^{-1}, ** in $cmol^{+}$ kg^{-1} and *** Citrate-bicarbonate-dithionite according to Mehra and Jackson (1960).

3.2.2 Methods

3.2.2.1 Long-term pot experiment

Three soils, Andosol, aric Podzol and Calcisol, were selected for this study. The soils were sieved at 5.0 mm and then placed in plastic pots of approximately 0.015 m^3, which represented between 3 and 10 kg of soil and left to settle for 3 months. Before seeding, each pot was each time fertilised with a 500-ml solution containing 130 mg L^{-1} N (as NH_4NO_3), 130 mg L^{-1} P (as $Ca(H_2PO_4)_2$), 180 mg L^{-1} K (as 50% KCl and 50% K_2SO_4) and 40 mg L^{-1} Mg (as $MgSO_4 \cdot 7H_2O$) as a basal dose. Five pots were prepared for each soil: two pots were left unplanted and considered as a control and 3 pots were sown with durum wheat (*Triticum durum*, cv. Claudio). The number of plants per plot varied for initial three runs: 6, 20 and 12 for first, second and third run respectively and for the last six runs nine plants per pot were maintained. Before sowing last run, soil in each pot was equally divided into two portions and one portion was mixed with diatomite (Clarcel 78® produced by CECA used as a source of amorphous silica (ASi)) at a rate of 15 ton ha^{-1} ASi. Each portion was sown in the same way as the previous runs. Each time 4 more seeds than desired were planted and approximately five days after germination, the smallest plants were removed from the pots to maintain the number of plants for each set.

Wheat was grown 9 times in a row: first from December 2007 to April 2008, second from April to August 2008, third from October 2008 to February 2009, fourth from March to July 2009, fifth from mid March to mid May 2010, sixth from end May to end July 2010, seventh from end February to end April 2011, eighth from start May to end July 2011 and ninth from mid May to Mid July 2012. Pots were left to settle for about six months after two cultivations each year. The first three cultivations were conducted in a climate chamber under controlled conditions with a natural full light spectrum (16/8 h 10°C/16°C day/night). The other runs were conducted in a greenhouse at Aix-en-Provence (France, 43.4944°N, 5.3444°E). Temperatures were set to stay between 16 and 25°C but it is likely that higher temperatures were reached during the experiment in the greenhouse. All pots were watered with distilled water at 80% of their field capacity. Soil solution samples were taken from the start of 5th run at different intervals up to last run with Rhizon soil moisture samplers (SMS, Rhizon®) which were installed in each pot just after seeding the pots in each run.

Durum wheat was harvested when all plants had at least one developed ear. The shoots were dried at 70°C, weighed and grounded, all organs being mixed. Roots were carefully separated from the soil, washed with distilled water and dried at 80°C till constant weight. After a careful separation from the roots, about 50 g of soil was taken after thorough mixing, and dried at 40°C. The remaining of the soil was returned to pot for use in the next run.

3.2.2.2 Plant analysis

The silicon content of wheat was extracted using tiron extraction (Guntzer et al., 2010). In brief, 50 mg of each plant sample (part above or underground) was extracted by adding 30 mL of 0.1M Tiron, [4,5-dihydroxy-1,3-benzene-disulfonic acid disodium salt, $(OH)_2C_6H_2(SO_3Na)_2$], solution buffered at pH 10.5 in polypropylene tubes, which were placed in a water bath at 85°C and horizontally shaken for 2 hrs with the caps loosely closed to prevent evaporation. The tubes were then cooled and 10 ml were sampled into pre-labeled 40 mL samples vials containing 10 mL of 30% H_2O_2 in order to destroy Tiron. This new solution was shaken again at 85°C in the water bath for about half an hour till the color disappeared and the solution was filtered at 0.2 µm and analyzed for Si by ICP-OES (Jobin-Yvon, Ultima-C).

3.2.2.3 Water analysis

pH of soil solution was measured from soil solution samples taken at different time intervals. Silicon was analysed by the molybdate blue colorimetry method (Hansen and Koroleff, 1999) with a spectrophotometer (Jasco V650) by absorption at an 810 nm wavelength.

3.2.2.4 Soil analysis

All soil samples were homogenized by hand and oven dried at 40°C for about 78 hours. After drying all samples were grounded to smaller size with mortar and pestle and not with a mill. This was done to avoid the formation of new reactive surfaces on the clay minerals and thereby overestimating ASi. After grinding all samples were passed through a 50 µm opening plastic sieve to ensure the sample particle size. Soil pH was measured (soil/water ratio of 1/2.5) before sowing and after harvesting of each run.

3.2.2.4.1 Acid methods

Two acid methods were selected in this study. The first one was 0.5 M acetic acid used by Korndorfer et al., (1999). In this method 1g of sample was shaken in 10 ml of extractant for 1 h then centrifuged and filtered through a Whatman No. 42 filter paper. The second method was 0.2 M NH_4-oxalate adjusted to pH 3.0 according to Tamm (1932) and modified by Schwertmann (1964): 1 g of sample was taken in 50 ml of extractant and immediately wrapped in aluminum foil to eliminate light and shaken for 1 h and then centrifuged and filtered in the same way as for the acetic acid extraction. All samples were analyzed for Al, Fe and Si by ICP-OES (Jobin-Yvon, Ultima-C).

3.2.2.4.2 Alkaline methods

Two different alkaline extractants were used to extract ASi from soils, that is Na_2CO_3 (ASi_{Na}) and Tiron (ASi_{Ti}). The Na_2CO_3 method was originally developed to measure biogenic silica in marine sediments (Demaster, 1981) and was applied to soils to estimate their amorphous silica content (Saccone et al., 2006, 2007). In this method 30 (±2) mg of sample was added to 40 ml of 0.094M Na_2CO_3 (pH = 11.2) and shaked for 5h at 85°C: samples were taken after 3, 4 and 5 hours and neutralized with 0.021 M HCl overnight. This method was used in our long term pot experiment because it is the most currently used method (Clymans et al., 2011)

Tiron method was used according to the method proposed by Biermans and Baert, (1977) and improved by Kodama and Ross, (1991). In this method, 25 ± 0.1 mg of the sample was added to 30 mL of 0.1 M Tiron solution buffered at pH 10.5 (prepared according to Kodama and Ross, 1991) and weighted (sample + solution + tube). Tubes were covered loosely with a cap and placed in a water bath at 80°C for 1 h with occasional shaking of the samples. After 1 h, the tubes were cooled in water bath and the outside of the tubes was dried before weighting. Loss in weight was compensated by adding distilled water. The samples were further mixed and then centrifuged for 10 minutes at 2700 rpm. An aliquot of 10 ml of supernatant was sampled and 10 mL of 30% H_2O_2 was added at 80°C to destroy the Tiron and analyzed for Al, Fe and Si by ICP-OES (Jobin-Yvon, Ultima-C) with standards and blanks having the same matrix composition.

3.2.2.4.3 ASi extraction by $ZnBr_2$

Additionally, amorphous silica particles were extracted from the soils using the physical gravimetric procedure (Alexandre et al., 997; Kelly, 990). The aim was to assess whether the phytolith contents of the soils had been altered by the cultivation. Six soil samples were chosen for phytolith extraction: a control pot with the Andosol and Calcisol after the first run, the same control pots after the fourth run and one with the planted Andosol and Calcisol pots after the fourth run. The physical gravimetric procedure is based on the density properties of soil mineral particles. The amorphous silica particles extracted with this procedure (ASi_{Zn}) were weighed and mounted on thin slices for observation with a Leica DM RXP microscope.

3.3 Results

3.3.1 Acid and alkaline methods

All soils extracted by acid methods contained different amounts of Si with maximum for Andosol and minimum for quartz (Figure 1). The extraction efficiency of these methods varied according to soils. NH_4-oxalate extracted larger amount of Si than acetic acid in the following order Mascareignite > Andosol > Pozdol BPs horizon > Calcisol > Podzol BPh horizon > aric Podzol > Haplic luvisol > quartz > Podzol horizon E. In Mascareignite and Andosol soil NH_4-oxalate extracted about 56 and 37 times more ASi than acetic acid. In Podzol horizons, extracted Si increased with increasing depth for both extraction methods with a minimum in E horizon and a maximum in BPs horizon. NH_4-oxalate extracted 1.2, 2.6 and 5.9 times more Si in E, BPh and BPs horizons respectively than acetic acid.

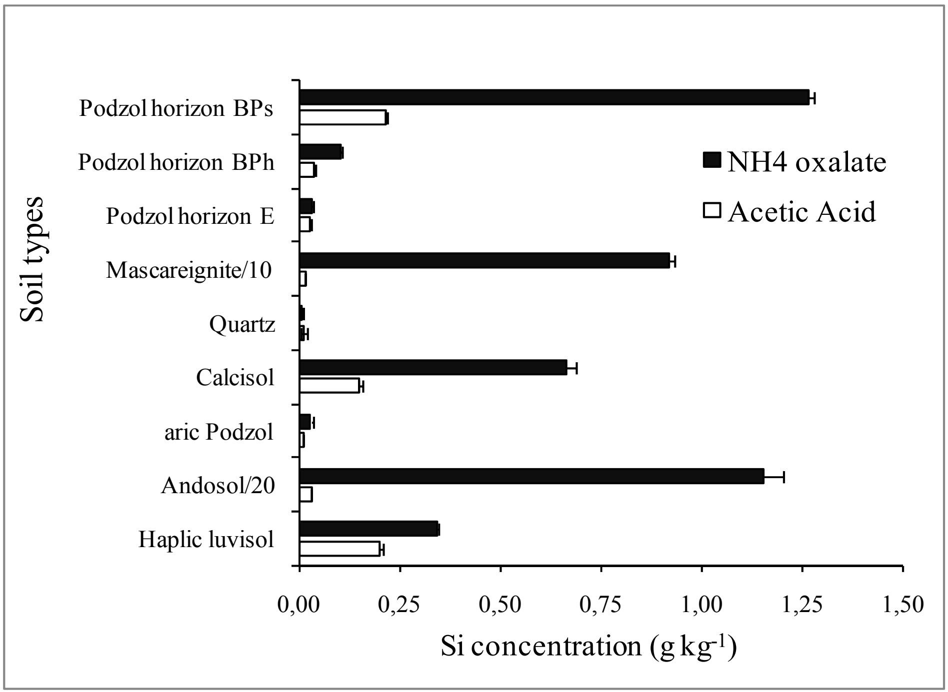

Figure 1: Si in g kg^{-1} extracted from different soils by NH_4 oxalate and acetic acid. Bars represent SD of three replicates. Mascareignite and Andosol values are divided by 10 and 20 respectively.

Al and Fe concentrations were larger in NH_4-oxalate than in acetic acid (Table 2). Lowest concentrations of both Al and Fe were measured in quartz samples extracted by both methods. In general, Al to Si and Fe to Si ratios were larger in NH_4-oxalate than acetic acid except in Andosol. The highest Al to Si ratio was found in Podzol BPh horizon for NH_4-oxalate and aric Podzol for acetic acid. The highest Fe to Si ratio was also obtained in Podzol BPh horizon for NH_4-oxalate and in the Andosol for acetic acid.

Table 2: Acid-extracted Al and Fe in g kg^{-1} (acetic acid and NH_4 oxalate). Values are means of three replicates. Al to Si and Fe to Si ratios are also given.

Soil Type	Acetic Acid (g kg^{-1})					NH_4 Oxalate (g kg^{-1})				
	Si	Al	Fe	Al/Si	Fe/Si	Si	Al	Fe	Al/Si	Fe/Si
Haplic luvisol	0.20	0.07	0.01	0.32	0.03	0.3	1.2	2.5	3.49	7.15
Andosol	0.62	1.47	9.34	2.38	15.19	23.1	52.5	31.0	2.28	1.34
aric Podzol	0.01	0.08	0.01	6.69	0.62	0.0	0.4	0.3	15.04	9.00
Calcisol	0.15	0.01	0.00	0.05	0.01	0.7	1.5	1.4	2.30	2.04
Quartz	0.01	0.02	0.00	1.18	0.31	0.01	0.02	0.01	2.00	1.47
Mascareignite	0.16	0.36	0.10	2.22	5.99	9.19	29.9	32.9	3.25	3.58
Podzol E	0.03	0.07	0.04	2.70	1.56	0.03	0.8	0.2	23.47	6.05
Podzol BPh	0.04	0.16	0.24	4.13	6.02	0.1	2.9	8.4	28.18	81.97
Podzol BPs	0.22	0.23	0.10	1.06	0.44	1.3	5.0	7.3	3.98	5.77

The alkaline methods extracted larger amounts of Si than the acid methods. Among the alkaline extractants, Tiron always extracted larger amounts of Si than Na_2CO_3 except in the Mascareignite (Figure 2). The extraction efficiency of these alkaline methods also varied according to soils. ASi_{Ti} was well correlated with all extraction methods studied while ASi_{Na} was not correlated with acid extraction methods (Table 3).

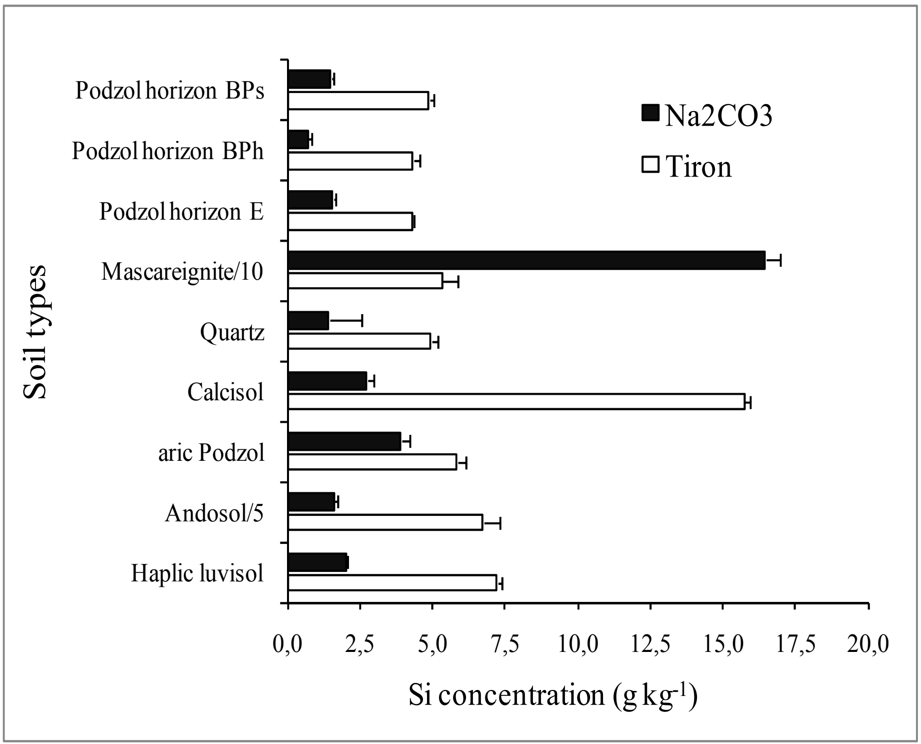

Figure 2. Silicon in g kg^{-1} extracted from different soils by alkaline methods (Na_2CO_3 and Tiron). Bars represent SD of three replicates. Mascareignite and Andosol values are divided by 10 and 5 respectively.

Table 3. Pearson correlation coefficients for Si extracted by different methods.

	pH	Acetic acid	NH_4 oxalate	Tiron 1H	Na_2CO_3	Tamm	Mehra-Jackson
pH							
Acetic acid	-0.081						
NH_4 oxalate	-0.239	0.897					
Tiron 1H	-0.271	0.507	0.715				
Na_2CO_3	-0.349	0.034	0.288	0.849			
Tamm	-0.437	0.952	1	0.945	0.956		1
Mehra-Jackson	0.043	0.873	0.964	0.953	0.955	1	

Al and Fe concentrations also varied between alkaline methods and also among soils (Table 4). Al and Fe concentrations extracted by Tiron were larger than those extracted by Na_2CO_3 for Andosol, Mascareignite, Podzol BPh and BPs horizons, while the opposite trend was observed for other samples. In the Podzol horizons, the smallest amounts of Al and Fe were extracted by both methods from upper soil (E horizon) and then increased with depth. The highest Al/Si ratios were observed in the Andosol extracted with Tiron and in the haplic Luvisol extracted with Na_2CO_3 (Table 4). The highest Fe/Si ratios were observed in the Podzol BPh horizon in Tiron extraction and in the haplic Luvisol extracted with Na_2CO_3.

Table 4: Si, Al and Fe in g kg^{-1} measured with alkaline extraction methods and Al to Si and Fe to Si ratios. Values are means of three replicates. AAl and AFe are amorphous Al and amorphous Fe respectively.

Soil Type	Na_2CO_3 (g kg^{-1})					Tiron (g kg^{-1})				
	ASi	AAl	AFe	Al/Si	Fe/Si	Si	Al	Fe	Al/Si	Fe/Si
Haplic luvisol	2.02	14.32	8.45	7.1	4.19	7.22	4.15	4.97	0.58	0.69
Andosol	7.97	14.14	3	1.77	0.38	33.69	55.41	40.64	1.64	1.21
Aric podzol	3.89	1.35	0.25	0.35	0.06	5.82	0.68	0.4	0.12	0.07
Calcisol	2.71	4.24	5.36	1.56	1.98	15.71	8.79	6.2	0.56	0.39
Quartz	1.43	0.22	0.11	0.16	0.08	4.94	0.05	0.06	0.01	0.01
Mascareignite	164.32	15.93	4.21	0.1	0.03	53.09	31.94	0.18	0.6	0.0
Pod. E (1-20cm)	1.55	3.75	0.58	2.42	0.38	4.33	1.49	0.48	0.34	0.11
Pod. Bph (20-25cm)	0.7	4.3	2.07	6.14	2.95	4.31	4.16	11.48	0.97	2.66
Pod. Pps (25-60cm)	1.5	6.96	4.97	4.65	3.32	4.86	6.72	9.96	1.38	2.05

3.3.2 Evolution of ASi and soil parameters in pot experiments

Soil amorphous silica

In general, there was no decrease in soil amorphous silica with wheat cultivation but in Andosol and Podzol there was a simultaneous decrease in the third and fourth runs (Figure 3). After addition of ASi in the 9th run, there was a significant increase in extracted ASi_{Na} in pots with plants as compared to the 9th run without ASi application with student test values of 0.04, 0.003 and 0.0015 for the Andosol, Podzol and Calcisol respectively. ASi_{Na} concentrations in the control pots were larger than in the planted pots except after the 1st run in all soils and in 2nd run of Podzol (Figure 3). In Podzol, ASi_{Na} concentrations in pots without plants were almost similar to those measured in pots with plants from the 5th run on. Addition of ASi also increased ASi_{Na} concentrations in the control pots of the 9th run as compared to the 9th run without ASi.

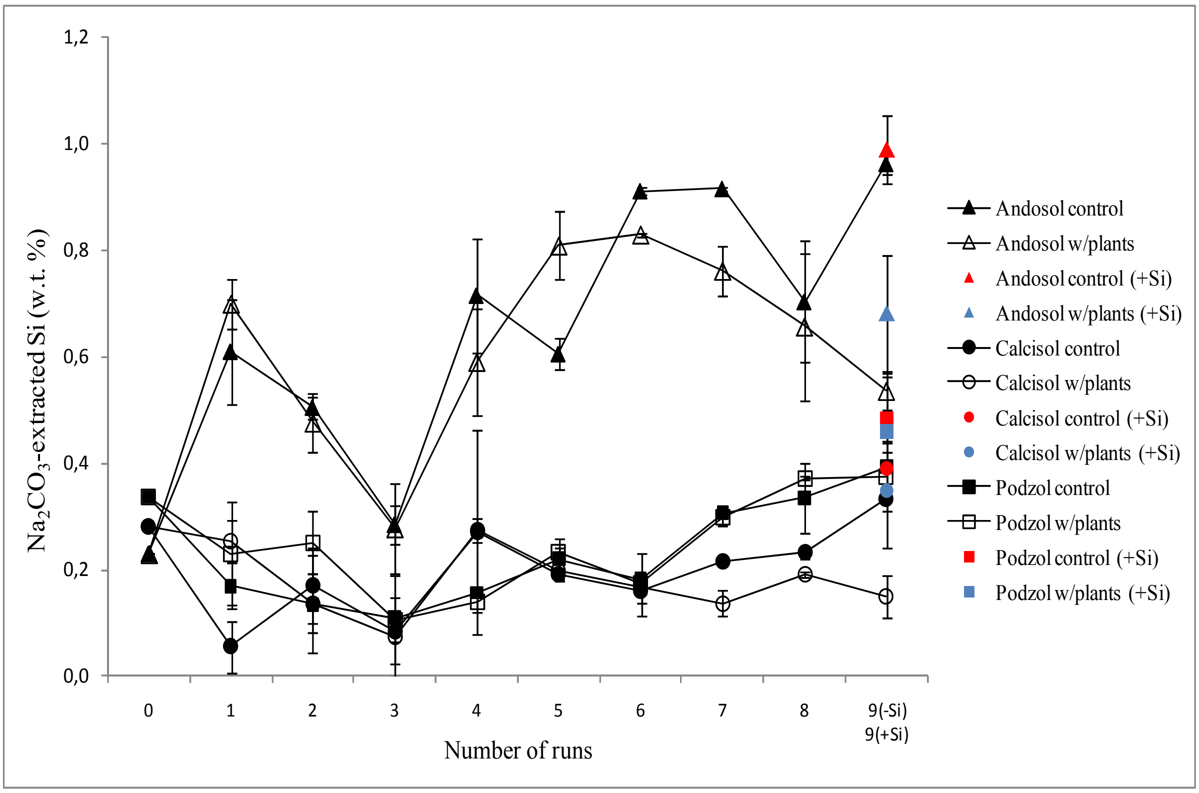

Figure 3. Na_2CO_3-extractable Si (w.t. %) in the pots with or without plants, according to soil types. Soil samples were taken at the end of the run, after plant harvest.

The quantification of amorphous silica by the physical gravimetric procedure was performed after the 4th run. This quantification was not possible for the Andosol due to loss of material during the organic matter oxidation. For the Calcisol, the silicon content coming from ASi_{Zn} method ranged from 0.09 to 0.32% (See annex 1 table 1) and was consistent with the sodium carbonate extraction results (Figure 3). In the Andosol, phytoliths with smooth surfaces were numerous, especially in the control pots, both at the beginning and the end of the experiment (Figure 4A). In the planted pot at the end of fourth run, phytoliths were small and exhibited many corroded surfaces (Figure 4B). No volcanic glass was observed. In the Calcisol samples (control after first and fourth runs and cultivated pots after the fourth run) the phytoliths were small, broken or corroded.

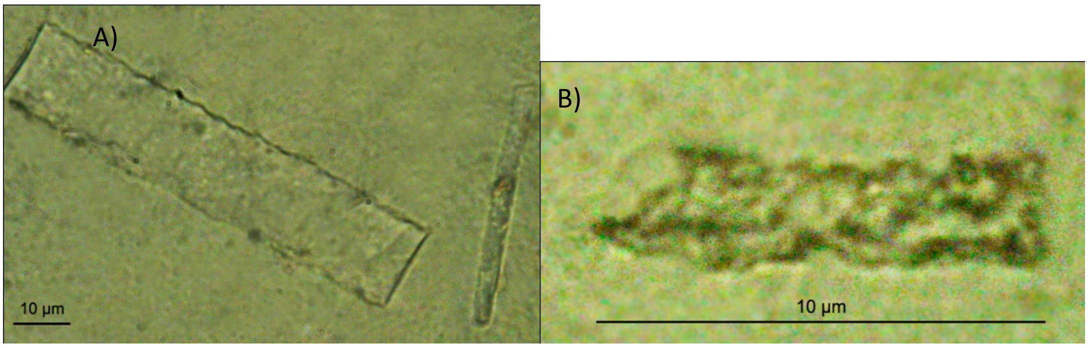

Figure 4. A) Phytoliths from the control pot of the Andosol at the end of the fourth run, B) Phytolith from the planted pot of the Andosol at the end of the fourth run.

Changes in soil pH

For the whole experiment, pH of the Andosol and Podzol was around 5 while that of the Calcisol was around 8 (Figure 5). pH of all sown pots was slightly larger than unsown pots throughout the experiment. Addition of ASi only significantly increased pH in the Podzol and Calcisol with plants as compared to the 9th run without ASi addition with Student test values of 0.009 and 0.02 for Podzol and Calcisol respectively.

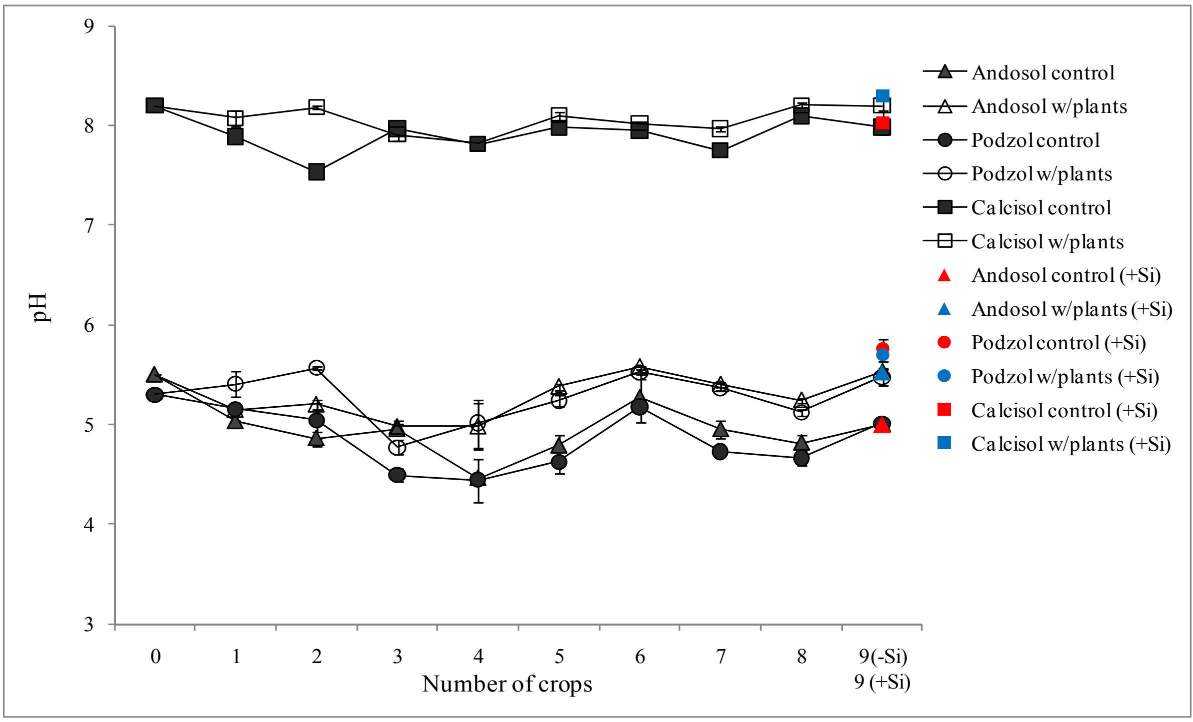

Figure 5. Soil pH in the planted and control pots during the whole experiment.

Changes in soil solution pH

Soil solution samples were taken from the start of the 5th run to the end of experiment at different time intervals during each run. In all soils, the soil solution pH of the sown pots was larger than unsown pots (Figure 6). Addition of ASi increased the pH of soil solution as compared to the same run without ASi addition except Podzol control and Calcisol with plant.

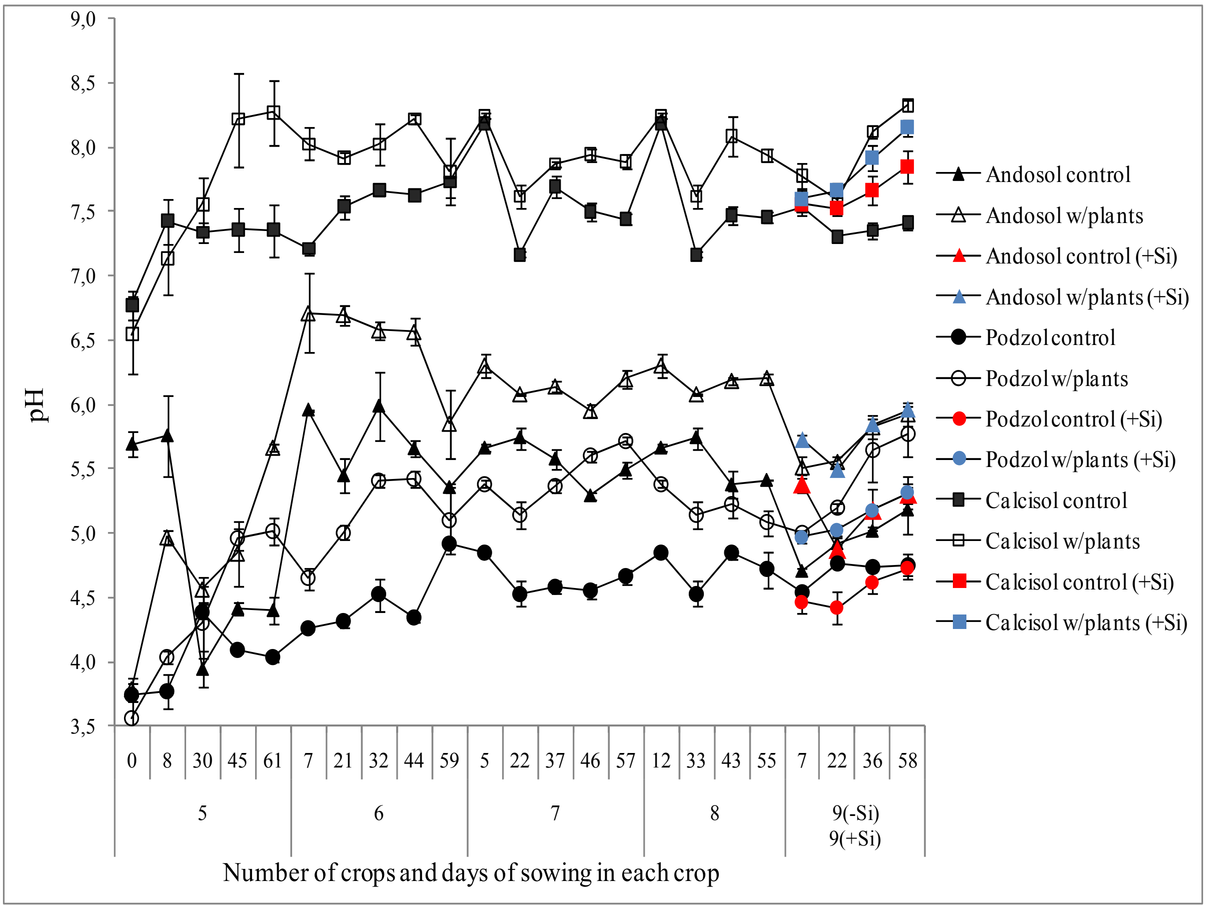

Figure 6. pH of soil solution in the planted and control pots starting from 5th cultivation till end of experiment.

Si in soil solution

In all soils, Si concentration in sown pots was smaller than in the respective unsown pots (Figure 7). Si concentration was lower in the Calcisol than in the two other soils and it did not vary throughout the runs. Larger Si differences were observed between sown and unsown pots of the Podzol followed by the Andosol and the Calcisol. Addition of ASi in the 9th run significantly increased Si concentration in the soil solution of the Podzol and the Calcisol both in sown and unsown pots while in the Andosol the increase was not significant.

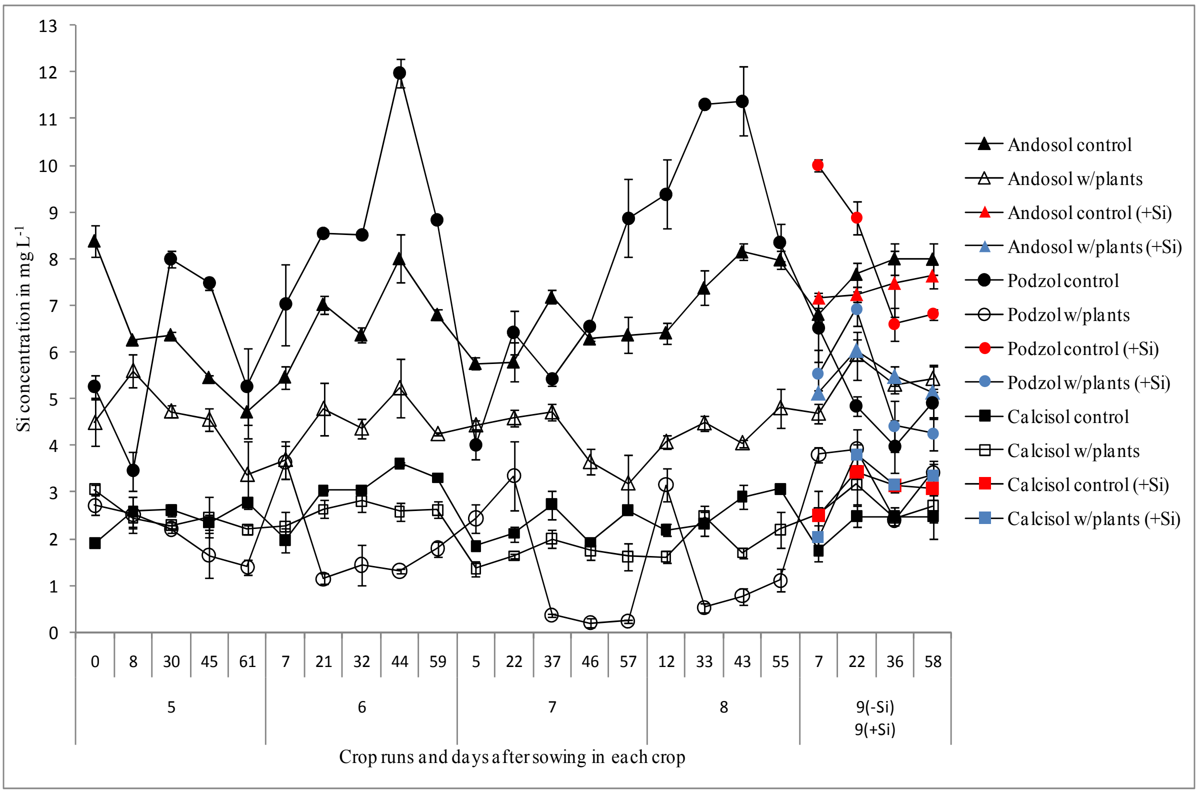

Figure 7. Si concentration in soil solution of the planted and control pots starting from the 5th run till the end of experiment.

Plant Si

Si concentration in plants depended on the soil in which plants were sown (Figure 8). The largest concentrations were found in the plants grown in the Andosol, then in the Calcisol and they were the lowest in the Podzol. From the 1st to the 4th run, Si concentration in plants decreased in all soils and the concentrations were quite low in the Podzol and Calcisol plants as compared to the Andosol plants. In the Podzol, plant concentrations in the last five runs were larger than in the first four runs. Addition of ASi significantly increased plant Si concentrations only in the Podzol (a Student test value of 0.002) as compared to plants of the 9th run grown without Si addition. In the Andosol, average Si shoot concentrations of all runs were significantly larger than those of the other two soils (Table 5). These larger Si concentrations in plants grown in the Andosol were related to a larger average Si concentration in soil solution and also a larger average ASi_{Na} concentration in the Andosol than in the other two soils (Table 5). This indicated that ASi_{Na} extracted from soil may be a source of Si for plants.

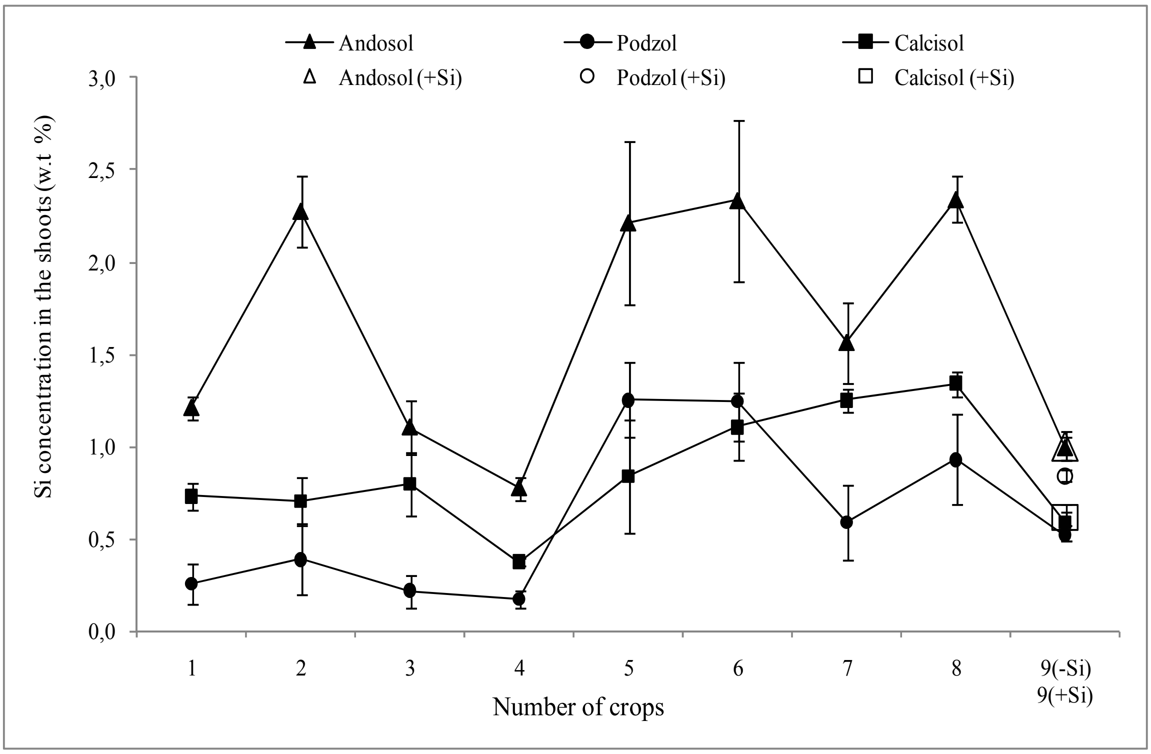

Figure 8. Si concentration in the shoots of each run.

Table 5: Average values of parameters studied in all runs in each soil. Different letters indicate significant differences among the treatments at a $P < 0.05\%$ for shoot and roots separately.

Soil type	Andosol		Podzol		Calcisol	
Soil	control	with plant	control	with plant	control	with plant
pH	4.90^{a} ± 0.22	5.27^{c} ± 0.22	4.82^{a} ± 0.28	5.28^{c} ± 0.26	7.88^{b} ± 0.17	8.05^{d} ± 0.14
ASi$_{Na}$ (%)	0.69^{a} ± 0.22	0.63^{a} ± 0.18	0.22^{b} ± 0.10	0.24^{b} ± 0.09	0.19^{b} ± 0.09	0.18^{b} ± 0.06
Soil solution						
pH	5.32^{a} ± 0.53	5.84^{b} ± 0.72	4.49^{c} ± 0.33	5.09^{d} ± 0.53	7.48^{e} ± 0.30	7.86^{f} ± 0.41
Si mg L^{-1}	6.87^{c} ± 0.65	4.56^{d} ± 0.46	7.26^{c} ± 2.16	2.01^{b} ± 0.84	2.52^{a} ± 0.30	2.29^{ab} ± 0.44
Plant						
Si (%age)	1.65^{a} ± 0.15		0.62^{b} ± 0.09		0.86^{b} ± 0.08	

Biomass and Si outputs

As the number of plants per pot varied during the experiment, we cannot conclude on a trend of the biomass through time. However, addition of ASi did not affect the biomass as compared to respective run without Si (Table 6). Total Si outputs were calculated over the nine runs in the three soils separately. Total Si outputs were larger in the Andosol than in the two other soils: from the Andosol wheat took up approximately 1.48 g of Si per pot while it took up 0.83 and 0.40 g Si from the Calcisol and the Podzol respectively. When compared to initial amorphous silica content of the soils measured at the beginning of the experiment, these values represent 16.1% of the amorphous silica stock removed by plants in the Andosol, 2.98 % in the Calcisol and 1.98% in the Podzol.

Table 6: Shoot weight (g pot^{-1}) of 9th run without and with Si addition.

Soil Type	(-Si)	(+Si)
Andosol	1.22 ± 0.14	1.30 ± 0.05
Podzol	3.25 ± 0.36	3.53 ± 0.45
Calcisol	1.83 ± 0.32	1.87 ± 0.17

3.4 Discussion

3.4.a Sources of plant available Si

Results of this study showed that NH_4-oxalate extracted about 37 and 55 times more Si from the Andosol and the Mascareignite respectively than acetic acid (Figure 1). It has been reported that NH_4-oxalate extracts Si bound to amorphous and poorly crystalline pedogenic oxides and oxy-hydroxides of Fe and Al (Sauer et al., 2006). This is well illustrated here with the highest values of Si extracted by NH_4-oxalate and larger Al and Fe concentrations found in Andosol. Acetic acid extractable Si represented a very small fraction of Si extracted by the alkaline methods used in this study suggesting that acetic acid is a weaker extractor than NH_4-oxalate and extracts only immediately soluble Si. Of the two alkaline methods, Tiron extracted larger amounts of ASi than Na_2CO_3 (Figure 2), as it was previously reported by Guntzer (2010). The alkaline Tiron solution was found more effective than Na_2CO_3 for extracting Al, Fe and Si, especially in the Andosol (Table 4). Tiron extracted larger amounts of Si from various Podzol horizons than Na_2CO_3, which was related to Fe accumulation in these horizons (Table 4). In Bph and Pps horizons, Al and Fe concentrations increased in NH_4-oxalate than acetic acid and in Tiron extraction than Na_2CO_3 extraction. Larger Tiron Si concentrations measured in these horizons can also be explained by Tiron extracting poorly crystalline and noncrystalline aluminosilicates as well as hydrous oxides of Fe, Al and Si including pedogenic opaline silica, as proposed by Kodama and Ross (1991). ASi_{Na} concentrations were correlated with ASi_{Ti}, Tamm and Mehra and Jackson Si concentrations while they were not correlated with the Si concentrations obtained with the acid methods (Table 3). Tamm extraction is usually used for the determination of Si bound to amorphous and poorly crystalline oxides and oxy-hydroxides of Fe and Al and the Mehra-Jackson extraction is used in soil science to dissolve pedogenic sesquioxides, thereby releasing occluded Si (Sauer et al., 2006). Na_2CO_3 extracted significantly larger amount of ASi from Mascareignite samples as compared to Tiron extraction (Figure 2). This indicated that Tiron is more efficient to dissolved poorly crystalline aluminosilicates than soil phytoliths while Na_2CO_3 can better extract Si from phytoliths than aluminosilicates. It has been reported that Na_2CO_3 method was found to be well adapted for ASi values below 70 g kg^{-1} approximately and should be used with caution in soils and continental sediments because the concentration and the dissolution properties of aged phytoliths may be distinct from the marine material for which the method was originally designed (Meunier et al., 2012).

3.4.b Evidences of ASi as an indicator of bioavailable Si for plants

In the second part of this study, we reported that the soil type and growing conditions influenced the silicon content, as the measured Si concentrations varied in the same cultivar of durum wheat not only differing according to soil type but also with time for a given soil (Figure 8). In the Andosol, both the ASi_{Na} and the silicon content of wheat shoot reached higher levels than in the two other soils. Average shoot Si concentrations were larger when ASi_{Na} and soil solution Si concentrations were also larger (Table 5). This indicates that ASi_{Na} extracted from soils can be a source of plant available Si. In the Calcisol, the pH was higher (between 7.7 and 8.2), allowing for a fast dissolution of amorphous silica. Even if amorphous silica concentration in the Calcisol was lower than in the Podzol, it was more available for plants and increased in shoots from the 5th to the 8th run (Figure 8). Addition of ASi in the last run showed that ASi was soluble in soils as indicated by larger Si concentrations in soil solutions as compared to the same soil without Si addition (Figure 7). As a result, the amount of Si was taken up by plants was larger in the ASi treated pots (Figure 8). The Si concentration in shoots did not impact the yields throughout the experiment: although ASi addition in the soil increased shoot Si concentrations, the yields were not larger than the yields without Si addition (Table 6). It has often been reported that Si can have no beneficial effect if there is no stress (Eneji et al., 2005; Hattori et al., 2005; Henriet et al., 2006, 2008).

3.5 Conclusion

Combination of acid and alkaline extractions showed that ASi_{Na} and ASi-oxalate extract different pools of Si. ASi_{Ti} is able to extract Al and Fe from Andosols which is in good agreement with the hypothesis of Sauer et al. (2006). Moreover, in the pot experiment ASi_{Na} was also well correlated with the Si in plants shoots indicating that ASi_{Na} can also be used as a good proxy for quantification of phytoavailable Si.

Chapter 4: Capacity of different minerals to release bioavailable silicon and the mechanisms of silicon-mediated alleviation of cadmium and copper toxicity in durum wheat (*Triticum turgidum* L. cv. Claudio W.)

One of the main objectives of this thesis is the evaluation of the capacity of different minerals to release bioavailable silicon and to find the mechanisms of Si-mediated alleviation of Cd and Cu toxicities in durum wheat. We highlighted in the second chapter (review of literature) that Si-mediated alleviation of metal toxicities has been studied in many plants such as rice, maize and cucumber but not in wheat and durum wheat and the mechanisms behind this are poorly understood despite the fact that wheat is Si accumulator plant. Therefore, we need to explore the mechanisms of Si-mediated alleviation of Cd and Cu toxicities in wheat as the wheat receives a large amount of Cd and Cu mainly due to anthropogenic activities such as phosphate fertilizers, sewage sludge, fungicides etc.

We have also seen in the previous chapter that continuous cropping and removal of wheat straw reduced bioavailable silica in the soil so there is need to use external sources of silicon to maintain high bioavailable silica pools in the soil. Recently different Si sources have been used by the researchers such as silicate fertilizers, silicate slag, fly ash and rock phosphate which contain small amounts of bioavailable Si (Gu et al., 2011; Putwattana et al., 2010; Savant et al., 1999). An important consideration is that these silicate sources which are derived from industrial by-product often contain high level of heavy metals (Berthelsen et al., 2001) which are highly toxic to plants so we need environment friendly sources of silicon with high solubility.

This chapter is divided into three parts with the following objectives:

- In the first and second part, the effects of Si on growth and physiology of wheat under Cd and Cu stress were evaluated in the two hydroponic studies separately at different time periods.
- In the third part the capacity of different minerals to release bioavailable silicon and its effect on reducing Cd and Cu toxicities in durum wheat was tested.

In the first and second parts, two hydroponic studies were performed during twenty days at different time periods. Durum wheat was grown for ten days with and without Si (1mM) and then different Cd (0, 0.5, 5.0 and 50 μM) and Cu (0, 0.7, 7.0 and 30 μM) concentrations were added for more ten days. After harvesting plants were analysed to diagnose Cd and Cu toxicity with respect to plant biomass, photosynthetic pigments, mineral nutrients, anions and the effect of Si was seen to alleviate Cd and Cu toxicity on these parameters and by localizing Cd, Cu and Si in roots to find possible mechanisms of Si-mediated alleviation of metal toxicity. After hydroponics, a pot experiment was done and for this diatomite, clay and quartz minerals were selected and were mixed according to different proportions in weight of quartz vs clay and quartz vs diatomite. After mixing these were divided into three different groups for metal treatment which include control, treated with 2 mg kg^{-1} Cd and treated with 20 mg kg^{-1} Cu. Wheat plants were grown for 60 days. After harvesting plant shoots and roots were analysed for Cd, Cu and Si and soil was analysed for pH and DTPA-TEA extractable Cd and Cu to find the possible plant available silicon sources and their effects on alleviation of metal toxicity.

I. Effects of silicon on growth and physiology of wheat seedlings (*Triticum turgidum* L. cv. Claudio) under Cd stress: a hydroponic study.

Abstract

A hydroponic study was performed to investigate the role of Si in enhancing Cd tolerance in durum wheat (*Triticum turgidum* L.) under different levels of Cd toxicity. Plants were grown for ten days without and with 1 mM Si followed by increasing levels of Cd for eleven days with 0, 0.5, 5.0 and 50 µM and without and with 1.0 mM Si. After harvesting, plant growth, chlorophylls and carotenoids contents, Cd and Si concentrations in shoot and roots, mineral nutrients in shoots and roots as well as concentration of inorganic (Cl, NO_3, SO_4 and PO_4) and organic (malate, citrate and aconitate) anions in the cell sap from shoots and roots were analyzed. The results showed that application of Cd concentrations in nutrient solution caused reduction in growth parameters, photosynthetic pigments and mineral nutrients both in shoots and roots. The magnitude of reduction increased with increasing Cd levels in the hydroponic solution. Concentrations of organic anions in shoots and roots increased and those of inorganic anions decreased except chloride with increasing Cd concentrations in the hydroponic solution. These adverse effects of Cd were mitigated by Si application in the nutrient solution. Si supply increased shoots and roots lengths, photosynthetic pigments and decreased Cd concentration in shoots while increased apoplasmic adsorbed Cd in roots. Si supply increased the Zn uptake by roots and decreased the concentrations of organic anions in shoots and roots and increased the concentration of inorganic anions in plant shoots and roots. Cd was mainly detected in the cortex while Si was mainly detected in the root endodermis. Co-precipitation of Cd together with Si was not found to be a major mechanism, neither at the shoot nor at the root level. X-ray Photoelectron Spectroscopy (XPS) analyses performed on freeze dried root surfaces demonstrated an enrichment of the most surface (< 100 Å) root layer by both Si and Cd in solution containing 50 µM Cd and 1 mM Si compared to the same treatment without Si addition. This suggested the existence of a root protection mechanism consisting in armoring the root surface by Si, Cd-bearing compounds; the distribution of these Cd-retention sites was not homogeneous over the root surface.

Key words: Durum wheat, Cd, Si, photosynthetic pigments, mineral nutrients, anions, µXRF, SEM-EDX, XPS

The results presented in this section have been prepared for submission in “Journal of Experimental Botany”

I.1 Introduction

Cadmium (Cd) is a highly toxic trace element at very low concentrations for plants and animals that enters the environment mainly through industrial processes, sewage sludge and phosphate fertilizers (di Toppi and Gabbrielli, 1999). High Cd causes several biochemical and physiological disorders in plants (Das et al., 1997). One of the main path ways for heavy metals to enter the plants is through the roots because root is a primary organ that participates in the heavy metal uptake due to the direct contact with the soil solution, containing these metals (Lasat 2002; Lux et al., 2011). Thus, Cd toxicity inhibits root growth and affects root morphology (Ci et al., 2009). It has been suggested that organic acids may participate in the uptake and translocation of Cd in many plants species. For example, Cieslinski et al. (1998) reported that organic acids increased the uptake of Cd by solubilization of particulate-bound Cd into the soil solution. Similarly, Zorrig et al. (2010) suggested that in lettuce, citrate might play a role in the translocation of Cd through the xylem vessels from roots to shoots. Organic acids also increased the Cd mobilization, plant availability and accumulation in maize plants (Nigam et al., 2001; Han et al., 2006). After absorption by roots Cd is translocated to shoots and is reported to inhibit the photosynthesis via reduction chlorophyll biosynthesis and function of photosystems (Ouzounidou et al., 1997; Ci et al., 2009, 2010). Cd toxicity also causes nutrient imbalance by decreasing the uptake of mineral nutrients by plants (Jalil et al., 1994).

It has been widely reported that durum wheat accumulates Cd in grains to a greater extent than bread wheat (Meyer et al., 1982; Hart et al., 1998, 2002; Greger and Löfstedt, 2004) and that this may exceed the concentration limits being considered by international regulatory agencies (Chaney et al., 1996; Norvell et al., 2000). This high Cd uptake and translocation rate from roots to shoots is the main source of Cd contamination in food (Hart et al., 2002; Greger and Löfstedt, 2004; Liu et al., 2007). Consequently, there is a need to develop agronomic management practices to minimize/reduce soil solution to plant transfer and translocation of Cd within the plant in addition to reduction of Cd toxicity symptoms that may impact yield (Hasan et al., 2009; Gallego et al., 2012).

Silicon, on the other hand, has not been considered as an essential element for higher plants, but has been proved to be beneficial for the growth and development of many plants (Epstein, 1994; Ma et al., 2006). Silicon is described as an effective substance that can reduce the negative effects of some toxic metals, such as Mn toxicity in many plants like in cucumber by increasing the binding of Mn to the cell wall or by homogeneous distribution of Mn in the leaves of cucumber (Rogalla and Römheld, 2002) and maize (Doncheva et al., 2009). Si also reduced Zn toxicity in rice seedlings by enhancing antioxidant defense capacity and by reducing Zn translocation to shoots (Song et al., 2011). Recently it has also been described that silicon application decreased the toxic effects of Cd in some plants species like maize (da Cunha et al., 2008; Vaculik et al., 2009), cucumber (Feng et al., 2010), strawberry (Treder and Cieslinki, 2005) and rice (Shi et al., 2005; Nwugo and Huerta, 2008; Liu et al., 2009). However, experiments on the effect of Si to ameliorate Cd toxicity in wheat plant are scarce. Recently, Rizwan et al. (2012) reported that Si application in soil in the form of amorphous silica under controlled condition can reduce Cd toxicity in durum wheat by immobilizing Cd in roots and/or by increasing plant biomass. However, the mechanisms behind this reduction of Cd toxicity by Si are still poorly understood. It is still unclear whether the possible mechanisms of Si-mediated Cd tolerance in durum wheat that may lie in soil or in the plant itself. More precisely we lack information on the localization of Cd and Si in plant organs and in cell compartments whose respective distribution may help to better understand the Si-induced tolerance mechanism. In addition, we have little information on the role of Si on photosynthetic performance like amount and type of chlorophylls and the contents of organic and inorganic anions in wheat plant under Cd stress which are important factors in understanding physiological alterations induced by Cd and possible recovery with Si treatment.

This study was thus designed to investigate the toxic effects of Cd in durum wheat (*Triticum turgidum* L. Cv. Claudio) and the impacts of Si to ameliorate these toxic effects with regard to uptake, root-to-shoot translocation and chlorophylls contents and anions and cations homeostasis through possible apoplasmic binding of Cd and micronutrients like Mn and Zn, and specific localization of Cd and Si in shoots and roots.

I.2 Materials and methods

I.2.1 Plant culture and treatments

Durum wheat (*Triticum turgidum* L. cv. Claudio W.) was grown in hydroponic condition. Before germination, the seeds were washed with distilled water then sterilized with sodium hypochlorite containing 2.6% active chloride for 3 minutes and then thoroughly washed with distilled water 5-6 times to remove excess chloride from seeds. Thereafter they were imbibed in water for 4 hrs at room temperature. During imbibition the seeds were aerated by a pump to avoid fermentation and then transferred for germination in petri-dishes for about 2 and a half days at 23°C/20°C day and night temperature respectively

After germination, seedlings were transferred to 12L plastic containers filled with a modified Hoagland nutritive solution containing: 0.5 mM KH_2PO_4, 0.5 mM K_2HPO_4, (these two also act as a buffer to maintain pH), 0.5 mM $MgSO_4$. $7H_2O$, 1 mM KNO_3, 1 mM $Ca(NO_3)_2$. $4H_2O$, 0.125 mM KCl, 50 µM H_3BO_3, 12 µM $MnSO_4$. H_2O, 0.7 µM $CuSO_4$. $5H_2O$, 1 µM $ZnSO_4$. $7H_2O$ and 0.25 µM MoO_4Na_2. $2H_2O$. For Fe, 100µM Fe_{III}-EDTA-Na was used for ten days and then replaced by 10 µM Fe-HBED when Cd treatments were started to avoid the complexation of Cd with EDTA. Plants were grown under a short-day cycle (8/16 h 23°C/20°C day/night), 70% humidity and 187 µmolphotonm^{-2}S^{-1} light intensity. There were 8 containers each containing 100 wheat seedlings. Half of the containers were supplied with 1mM Si in the form of monosilicic acid from potassium-metasilicate $Si(KOH)_2$, which has a K:Si mole ratio of 2 (Metso 400 - YARA) (Voogt et al., 2001). Additional K produced by $Si(KOH)_2$ was subtracted from KNO_3 and the resultant nitrate loss was supplemented with dilute nitric acid. The pH of the solution was adjusted to 6.5 ± 0.2 with 1 mM MES (2-morpholinoethanesulphonic acid) buffer when required. Each pot was aerated with continuous air bubbling produced by aeration pump. Nutrient solutions were changed every three days over the course of entire experiment to keep the nutrients concentrations in the solution as stable as possible. After 10 days adaptation with silicon, the plants were exposed to 0, 0.5, 5.0 and 50 µM $Cd(NO_3)_2$. $4H_2O$ (that is 0, 0.056, 0.56 and 5.6 mg L^{-1} Cd) to the nutrient solution, which were tested without and with Si application. Maximum 50 µM Cd concentration was selected as it is the optimum concentration for studying Cd stress in wheat seedlings according to previous reports (Ci et al., 2009, 2010). In total, the plants were cultivated in the growth chamber for 21 days: 10 days with Si application when required and 11 days with Cd and Si applications.

During each renewal, nutritive solution was sampled at the beginning (before contact with wheat plants) and just before the renewal, filtered on 0.2 µm membrane and was analysed for

Dissolved Organic Carbon (DOC), total Cd and Si to check for any possible contamination or element depletion.

I.2.2 Plant harvesting and biomass

After 11 days of Cd treatments, the plants of each treatment were harvested and separated into shoots and roots. Analyses were performed on individual plants with shoots and roots analyzed separately. Shoots were washed with distilled water and then divided into two parts: one subsample was frozen in liquid N_2 prior to measuring its organic and inorganic contents and localization of Cd and Si while another subsample was used to measure fresh weight then oven-dried at 80°C until constant weight was reached, weighted and ground to smaller size for further analysis. Roots were also divided into 2 parts: one subsample was washed with dilute acid to measure metals bound to root cell walls (root apoplasmic metals) as described by Chaignon et al. (2002): briefly, 0.4 g of roots (on a fresh weight basis) was shaken end-over-end with 20 ml of 1 mM HCl for 3 min, then 180 µl of 1 M HCl were added to yield a final concentration of 10 mM HCl. After shaking for another 3 min, the roots were recovered and gently dried between two layers of filter paper then oven dried at 80°C. Another subsample was washed only with distilled water and some fresh roots were stored in liquid nitrogen and others were used to measure again fresh weight then oven dried at 80°C till constant weight and weighted then grounded to smaller size for further analysis.

I.2.3 Anion assays

Anionic profiling was performed for both shoots and roots of wheat plants. For this, fresh plant samples (approximately, 1/8 FW/V ratio) were incubated in ultrapure water heated at 70°C for about 40 min then the samples were cooled at room temperature, centrifuged 3 times at 14×10^3 rpm at 4°C for 10 min each and the supernatant solution was recovered. The concentration of major inorganic and organic anions in the supernatant was determined by high performance ionic chromatography (LC20, Dionex) using an IonPaq AS11 column and a NaOH linear gradient. NaOH concentration was raised linearly from 3.5 to 5 mM over 3 min then from 5 to 33.5 mM over 8 min. Measurement of each anion was performed by comparing the retention times and peak areas, using the Chromeleon software (Dionex), with the standards.

I.2.4 Determination of photosynthetic pigments

Chlorophylls (a & b) and carotenoids contents in the fresh leaves (about 1.0 g) were extracted in the dark with 10 ml of 80% acetone by continuous shaking until color had completely disappeared from the leaves. Light absorbance at 663, 645 and 470 nm was determined by spectrophotometer (Hitachi U-2800). The concentrations of chlorophylls and carotenoids were calculated by using the adjusted extinction coefficients (Lichtenthaler, 1987).

I.2.5 Determination of Cd, micro and macro elements and silicon in plant samples

For the determination of Cd, micro (Mn and Zn) and macro (K, P, Ca and Mg) elements, finely ground plant (above or underground parts) samples (0.25g) were digested in 4 mL of concentrated HNO_3 70% (Fluka, Buchs, Switzerland) in a block of mineralization with a rise in temperature up to 95°C for 6 hrs then filtered at 0.2 µm (Whatman cellulose nitrate membrane) (modified from Keller et al., 2003).

Si concentration in plant shoots and roots was measured using Tiron extraction method (Guntzer et al., 2010). In brief, 50 mg of each plant sample (part above or underground) was extracted by adding 30 mL of 0.1M Tiron, [4,5-dihydroxy-1,3-benzene-disulfonic acid disodium salt, $(OH)_2C_6H_2(SO_3Na)_2$], solution buffered at pH 10.5 in polypropylene tubes, which were placed in a water bath at 85°C and horizontally shaken for 2 hrs with the caps loosely closed to prevent evaporation. The tubes were then cooled and 10 ml were sampled into pre-labeled 40 mL samples vials containing 10 mL of 30% H_2O_2 in order to destroy Tiron. This new solution was shaken again at 85°C in the water bath for about half an hour till the color had disappeared and the solution was filtered at 0.2 µm.

The micro (Mn and Zn), macro (K, P, Ca and Mg) elements, Cd and Si from the plant digests and extracts were measured by inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES Jobin-Yvon, Ultima-C).

I.2.6 Extraction of phytoliths

Phytoliths were extracted from shoots by dry ashing to remove organic matter (Parr et al., 2001a, b). This technique includes the following steps: 1) rinsing 10-20 g of plant material in Milli-Q ultrapure water; drying it in an oven at 50°C and grinding during 3 min in an agate mill; 2) heating the plant material in the crucible at 500°C for 8 hours; 3) Removing from crucibles and transferring the content to polypropylene tubes (0.2 g per tube); 4) adding 10 mL of 10% HCl to each tube; heating in water bath at 70°C for 20 min; 5) Centrifuging at

3500 rpm for 5 min followed by decantation; 6) rinsing with Milli-Q ultrapure water and centrifuging at 3500 rpm for 5 min followed by decantation; 7) adding 10 mL of 15% H_2O_2; heating in water bath at 70°C for 20 min; 8) centrifuging at 3500 rpm for 5 min and decanting; 9) rinsing with Milli-Q ultrapure water via repeated centrifugation at 3500 rpm during 5 min; 10) drying at 50°C during 48 hrs in an oven. The residual solid phases were then thoroughly rinsed in de-ionized water and freeze dried at -55°C. The resulting mineral phases were characterized by scanning electron microscopy (SEM) using a Jeol JSM840a, and by X-ray diffraction using an INEL CPS 120 $Co_{K\alpha}$, with a scan speed of 0.02^o s^{-1}.

I.2.7 Localization of Si and Cd by µXRF

One shoot sample containing 1mM Si in the nutrient solution and two roots, Cd 50 µM and Cd 50 µM + 1 mM Si, samples were selected for chemical micro-analyses by µXRF. Frozen samples were freeze dried at -55°C for about 72 hrs and then embedded in an epoxy resin. The embedded samples were then cut transversely with a diamond wire saw at about 5 cm distances from the bottom of shoots to get the comparable root cross-sections. The thickness of the obtained cross-sections was about 200 µm. The measurements were carried out on a microscope (HORIBA XGT^{7000}) equipped with an x-ray guide tube producing a finely focused and high-intensity beam with a 10-µm spot size Rh X-ray tube, accelerating voltage of 30 kV and a current of 1mA). X-rays emitted from the irradiated sample were detected with energy-dispersive X-ray (EDX) spectrometer equipped with a liquid nitrogen cooled high-purity Si detector. Only elements with an atomic number Z>11 (Z_{Na}), can be detected. Analyses were performed in sample chamber vacuum mode. Elemental maps (256 px^2, pixel size of 8 mm), showing in particular Cd, Si, S and K distribution in cross sections, were recorded with a total counting time of 30*1000 s under total vacuum mode (to enhance Si detection). Images were obtained from Kα emission line intensities of Cd, Si, K, Ca, Zn and Mn.

I.2.8 Scanning Electron Microscopy (SEM-EDX)

Root sample treated with 50 µM Cd + 1 mM Si was divided into two portions and one was freeze-fractured in a cryo specimen chamber, sputted-coated with a mixture of gold and palladium in argon atmosphere. The other part of the sample was freeze dried at -55°C for about 72 hrs and then cut horizontally and also vertically into small pieces. Both samples were examined using a Philips XL30 SFEG scanning electron microscope (SEM) at nitrogen liquid temperature (North Billerica, MA) coupled to an Oxford Instruments (Oxfordshire, UK) energy dispersive X-ray spectrometer (EDX). The SEM was operated at 20 kV with a counting time of 300 s per point. With SEM-EDX, chemical microanalysis results were obtained with a special resolution of about 2 to 5 µm and a penetration depth of about the same range.

I.2.9 X-ray Photoelectron Spectroscopy (XPS)

XPS is a surface sensitive technique which is probing approximately the first 10 nm of a sample, due to the attenuation length of travelling photoelectrons in the solid. Experiments were performed in a Kratos AXIS ULTRA^{DLD} vacuum chamber, using monochromated $Al_{K\alpha}$ X-rays (energy = 1486.6 eV, power = 150 W) to obtain chemical surface composition of freeze-dried samples. Wide spectra and high-resolution scans were recorded with pass energy of 160 eV and 20 eV respectively. Data were analyzed using the commercial software casaXPS, with Shirley background correction and binding energy calibration using C 1s at 285 eV. Different chemical environment for one given atom will give rise to shift in binding emergy of emitted electrons (for example, it is possible to distinguish a C atom bound to another C atom with one bound to an O atom). No X-ray beam damage was noticed during the analysis.

I.2.10 Statistical analysis

All the data were statistically analyzed using one-way ANOVA at a significance level of $P \leq 0.05$ with SPSS 16.0 software for windows. Single step multiple comparisons of means was performed via Tukey’s post hoc test. Different letters on the histograms indicate that the means were statistically different at $P \leq 0.05$ level. Data presented are from eight treatments and with 3 or 5 replicates as described above.

I.3 Results

I.3.1 Wheat growth

Increasing Cd concentrations in the nutrient solution reduced shoot length and induced slight chlorosis symptoms on leaves of plants treated with 5µM Cd and pronounced chlorosis on plants grown in 50µM Cd (Figures 1B and 2). There were no visible symptoms on the leaves in the presence of Si (Figure 1B). Cadmium treatments reduced the length of primary seminal roots as compared to control (Figures 1A and 2) and the effect was significant for the highest Cd treatment. At the same time, Cd stress increased the number of lateral seminal roots (Figure 1A). Addition of Si significantly increased root length especially in 5.0 and 50 µM Cd compared to Cd-only treated plants (Figures 1A and 2). Addition of Si also reduced the branching of seminal roots when compared with Cd-only treated roots (Figure 1A). Differences were observed in the fresh and dry weights of shoots and roots among the treatments (Figure 3). Higher (5.0 and 50 µM) Cd treatments significantly reduced the shoots and roots fresh and dry weights as compared to control irrespective of Si addition.

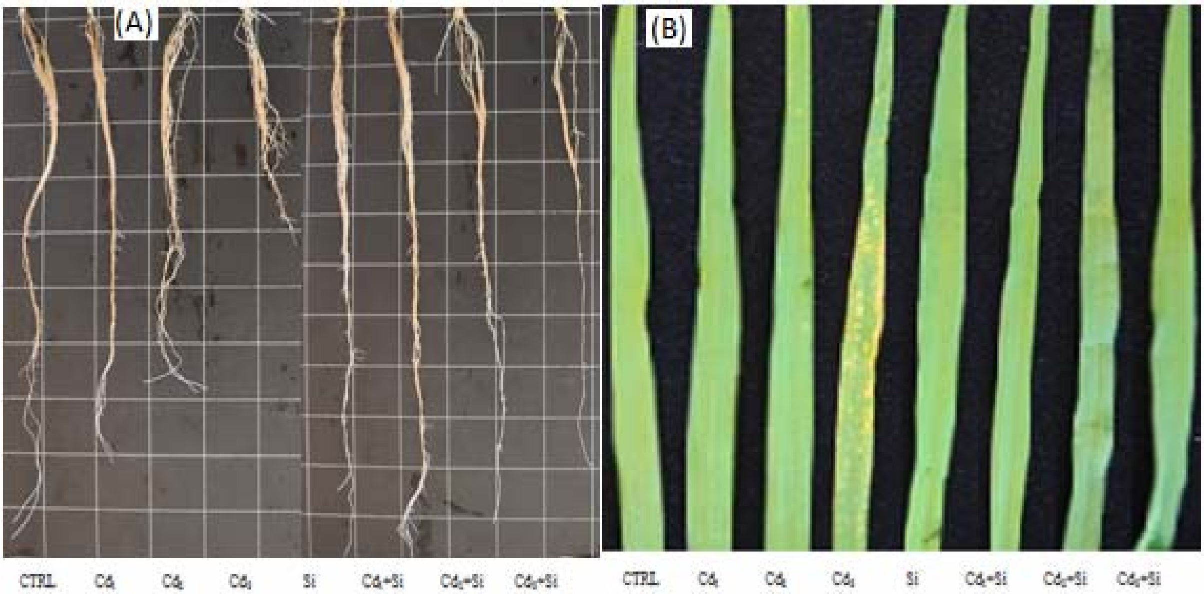

Figure 1. Effect of exogenous Cd and silicon on the wheat roots and leaves. (A) shows the effect of Cd and silicon treatment on roots (length between two lines is 3 cm) and (B) shows chlorosis induced by increasing concentrations of Cd on Cd-only treated wheat plants as compared to plants treated with Cd+Si exhibiting no visible symptoms on 2nd fully developed leaves CTRL=Control; Cd1= 0.5 µM Cd; Cd2= 5.0 µM Cd; Cd3= 50 µM Cd; Si = 1mM Si; Cd1+Si= 0.5 µM Cd + 1mM Si. Cd2+Si= 5.0 µM Cd + 1mM Si and Cd3+Si= 50 µM Cd + 1mM Si.

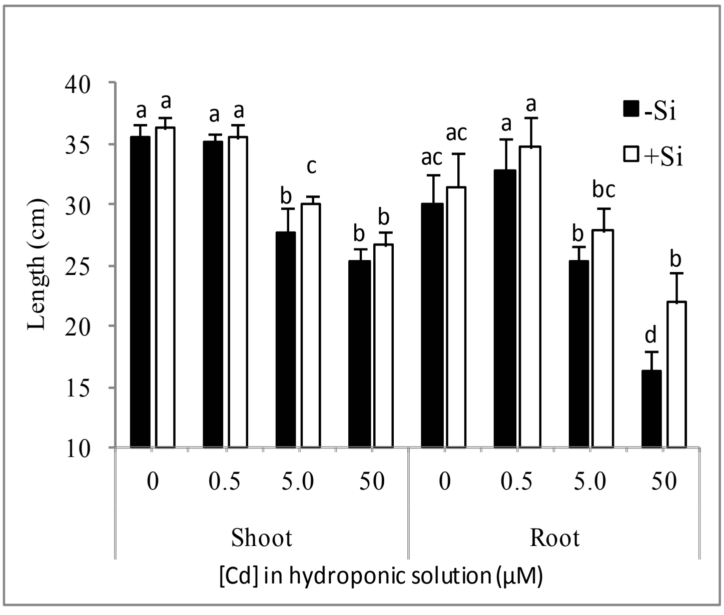

Figure 2. Length of shoots and primary seminal roots of young wheat plants grown hydroponically exposed to various Cu concentrations without and with 1mM Si addition. Bars represent SD of five replicates. Different letters indicate significant differences among the treatments at a P < 0.05% for shoots and roots separately.

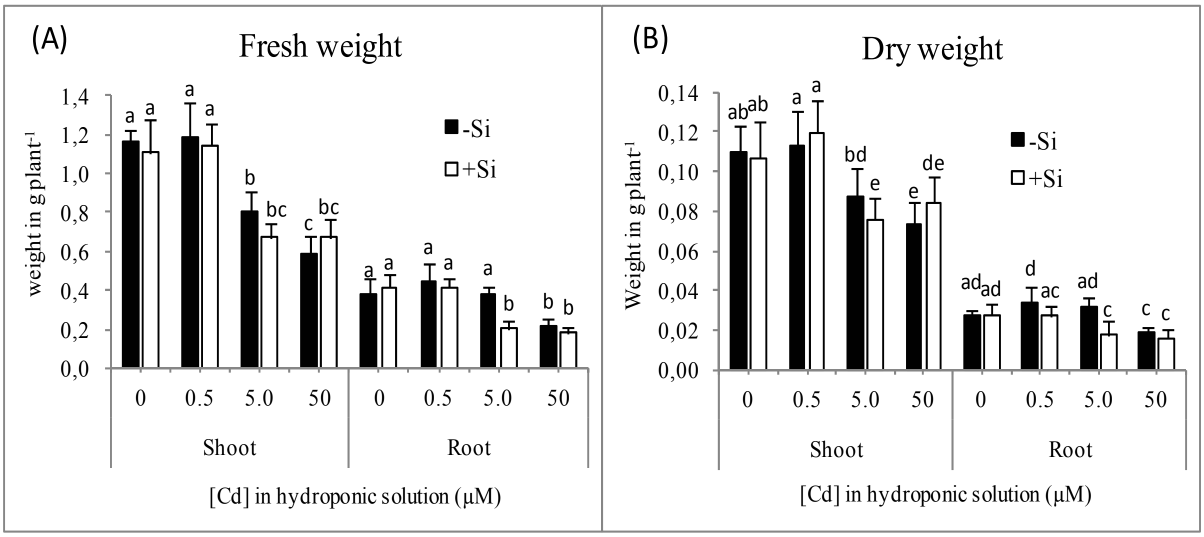

Figure 3. Fresh and dry weights of wheat plant shoots and roots grown in hydroponic conditions with increasing Cd concentrations (0. 0.5. 5.0 and 50 µM) treated or not with 1mM Si. Bars represent SD of seven replicates. Different letters indicate significant differences among the treatments at a P < 0.05% for shoot and roots separately.

I.3.2 Chlorophylls and carotenoids concentrations

Chlorophyll a (Chl-a), chlorophyll b (Chl-b) and carotenoid as well as chlorophyll a+b concentrations significantly decreased in leaves of wheat plant with increasing Cd concentrations in the nutrient solution (Figure 4). Application of 1 mM Si increased these pigments concentrations compared to respective Cd-only treated shoots.

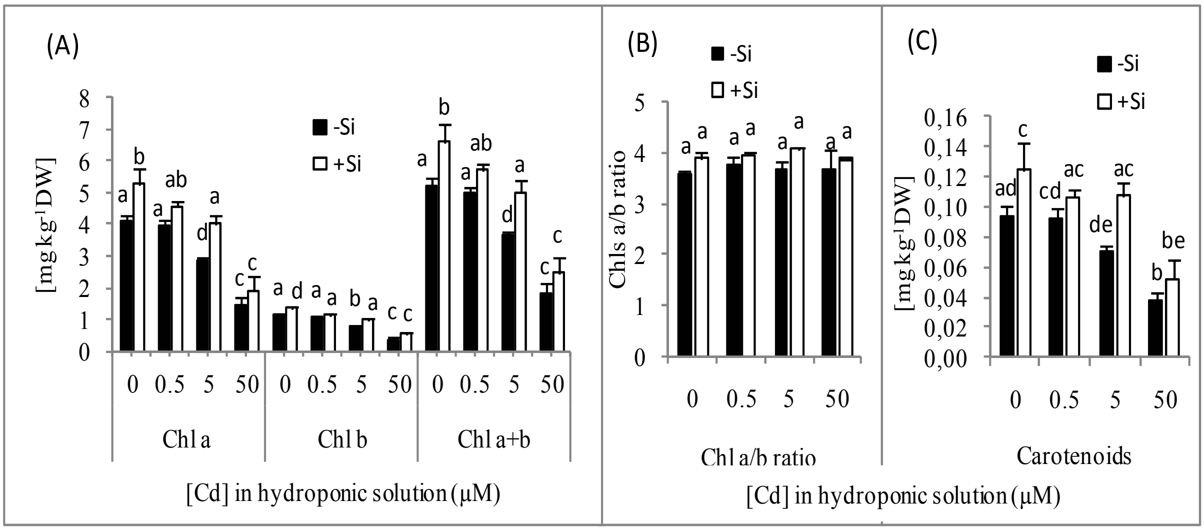

Figure 4. (A) Chlorophyll a and b concentration in plant shoots. (B) Chlorophyll a/b ratio in plant shoots and (C) Carotenoids concentration in shoots. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments at a $P < 0.05\%$.

I.3.3 Silicon concentration in shoots and roots

The distribution of Si between plant organs was not uniform when Cd was present in the nutrient solution (Figure 5). Si concentration in shoots significantly decreased with increasing Cd levels in the nutrient solution while no significant effect was observed in roots. Si concentrations were also measured in the wheat plants for which Si was not applied in the nutrient solution and the results showed that there was a small amount of Si in shoots of plants in control and 0.5 µM Cd treatments while in other treatments, Si concentration was below quantification limit of the instrument.

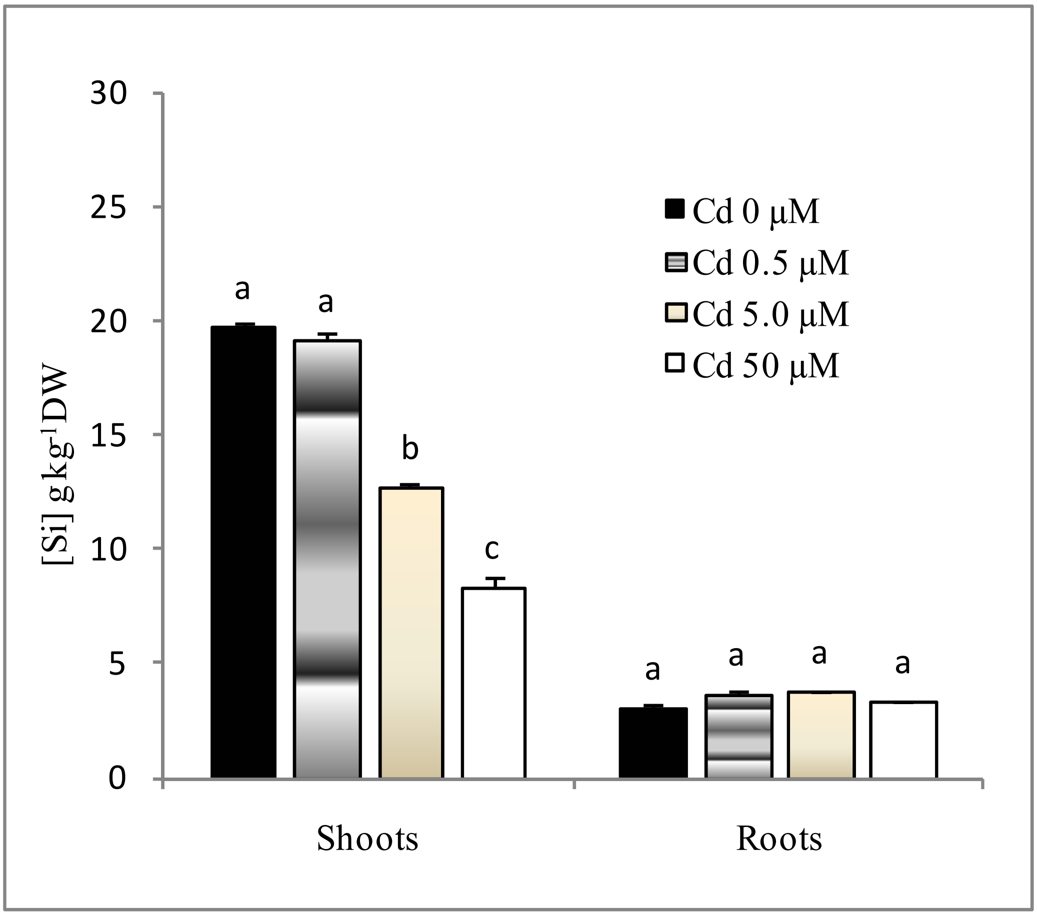

Figure 5. Silicon concentration in wheat plant shoots and roots. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments at a $P < 0.05\%$ for shoots and roots separately.

I.3.4 Cd and micronutrient (Mn, Zn) concentrations in shoots and roots

Cadmium concentrations significantly increased in shoots with increasing Cd levels in the nutrient solution (Figure 6). Si application decreased Cd concentrations in shoots as compared to the same Cd treatments without Si application, the decrease being significant in the highest Cd treatment. Mn concentrations significantly decreased in shoots with increasing Cd levels while Zn concentrations almost remained constant or slightly decreased with increased Cd levels (Figure 6). Si addition did not significantly changed Mn and Zn concentrations.

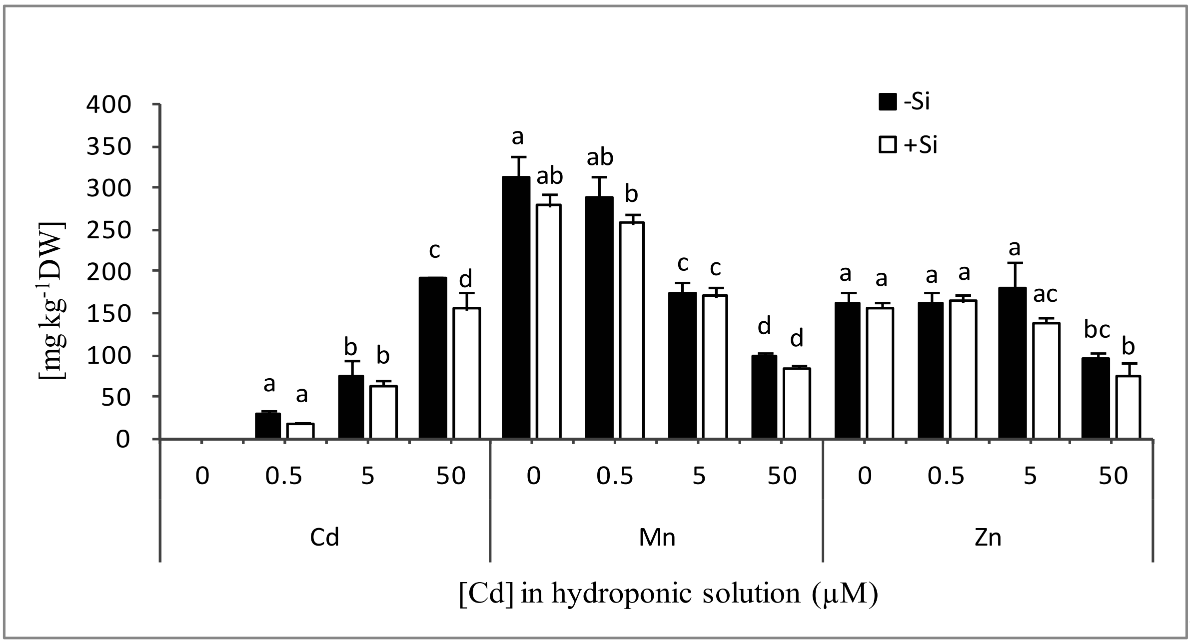

Figure 6. Cd. Mn and Zn concentrations in mg kg^{-1} DW in wheat shoots. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments at a P < 0.05%.

Cd concentrations significantly increased in acid washed (symplasmic Cd) roots with increased Cd levels in solution treated or not with Si (Figure 7). Si application did not affect Cd uptake by the roots as compared to the respective treatments without Si supply. Mn concentration decreased while Zn concentration almost remained constant or slightly increased with increasing Cd levels in solution. Mn concentration was lower in the +Si treatments except for the 0.5 µM Cd treatment while Si addition significantly increased Zn concentrations in higher Cd (5 and 50 µM) treatments.

Cd adsorption on the root surface increased with increasing Cd concentrations (Figure 8) and Si application further increased Cd adsorption on the roots surface especially in the 50 µM Cd treatment. Mn adsorption did not vary much across Cd treatments, but Si application increased adsorption of Mn onto the roots surface except in the highest Cd treatment. Zn adsorption increased in all Cd treatments while Si application significantly decreased Zn adsorption in the highest (5 and 50 µM) Cd treatments keeping Zn concentrations constant across the Cd+Si treatments.

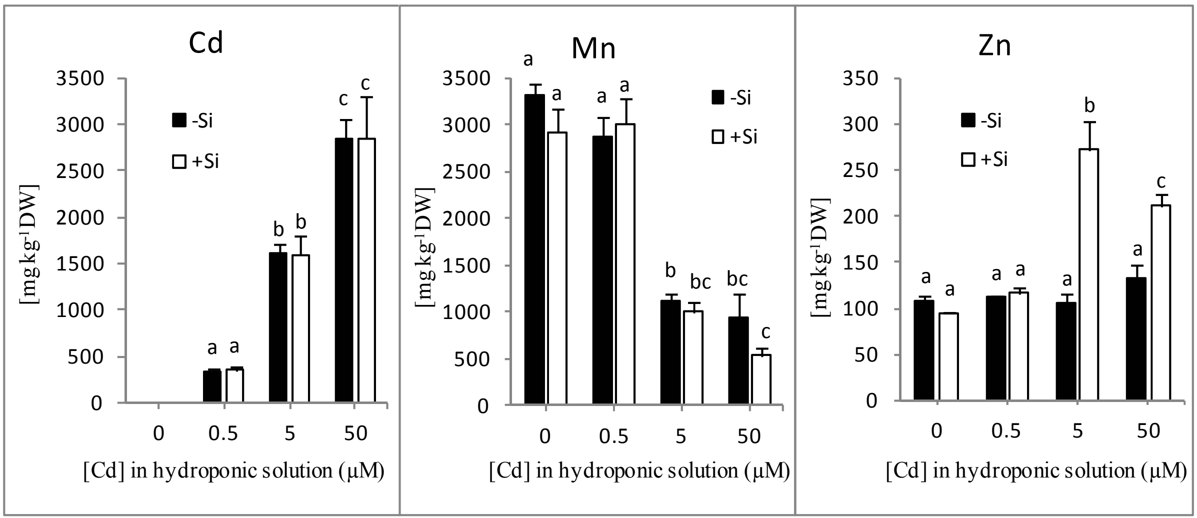

Figure 7. Cd. Mn and Zn concentrations in mg kg^{-1} DW measured in acid washed roots. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments at a P < 0.05%.

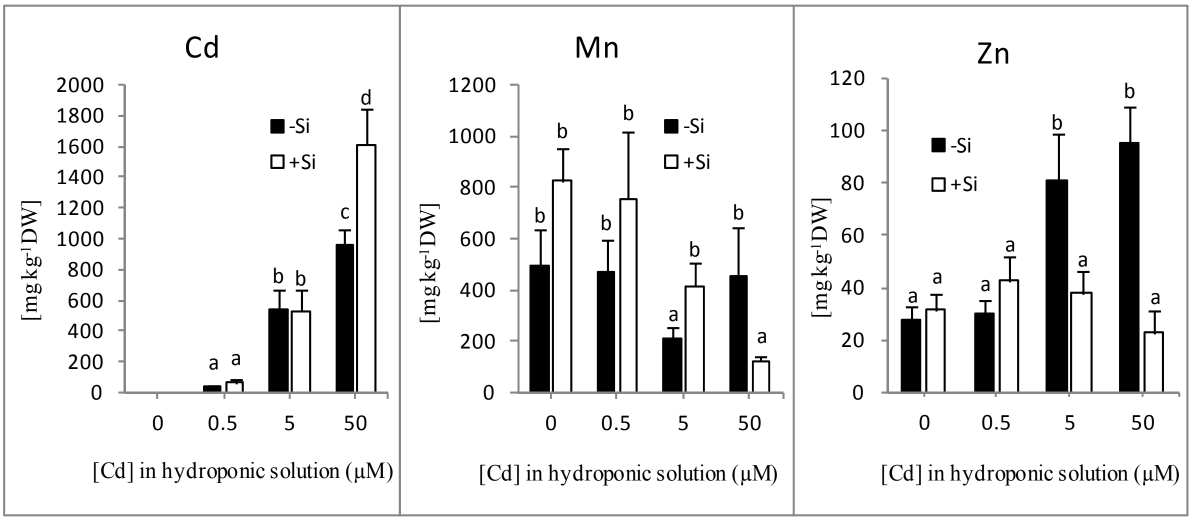

Figure 8. Apoplasmic adsorbed Cd, Mn and Zn concentrations in mg kg^{-1} DW in roots measured as the difference between total elemental concentration in roots and the concentrations measured in acid-washed roots. Bars represent SD of three replicates Different letters indicate significant differences among the treatments at a P < 0.05%.

The total plant Cd contents were larger in both shoot and root of the wheat plant without Si addition as compared to plants with Si supplementation. Shoot-to-root ratio decreased in the presence of Si as compared to without Si supply in all Cd treatments except 5.0 µM Cd treatment (Table 1).

Table 1: Total average content of cadmium (µg plant^{-1}DW) in shoot, root and shoot+root as well as shoot-to-root ratio of wheat plants grown in hydroponics for 10 days with and without Si and then more 11 days with increasing Cd treatments treated or not with 1mM Si. Values are mean ±SD (N=3). (*) denotes significance relative to the respective control as determined by Student's t-test at a $p < 0.05$ and (**) at $p < 0.01$.

	Cd (µM)	Shoot	Root	Shoot + Root	Shoot/Root
(-Si)	0.5	3.55 ± 0.4	12.66 ± 0.3	16.21	0.28
	5.0	6.51 ± 1.6	68.79 ± 3.1	75.3	0.09
	50	14.0 ± 0.2	69.79 ± 4.7	83.79	0.2
(+Si)	0.5	2.2* ± 0.1	11.29* ± 0.4	13.5	0.2
	5.0	4.89 ± 0.4	37.32** ± 3.0	42.22	0.13
	50	13.15 ± 1.7	70.86 ± 6.6	84.01	0.19

I.3.5 Other mineral and organic compounds

Increasing Cd concentrations in the nutrient solution decreased K, P, Ca and Mg concentrations in both in shoots and roots except Ca in roots at the highest Cd concentration in solution. Addition of Si Si did not significantly change both concentrations and trends (See annex 2 figure 1). Increasing Cd concentrations in the solution decreased significantly the concentration of inorganic (Cl, NO$_3$, SO$_4$) anions in shoots and roots except for Cl in roots which significantly increased with increasing Cd levels (Figure 9). Phosphate remained constant across the treatments. Si addition did not modify significantly the anions concentrations except for the PO$_4$ concentrations in roots that were larger in all treatments.

Concentrations of malate, citrate and aconitate were larger in shoots than in roots and increased both in shoots and roots in response to increasing Cd levels in solution with a significant increase in both shoots and roots under highest Cd stress as compared to control (Figure 10). Concentrations of citrate and aconitate slightly decreased while malate increased in +Si treated shoots as compared to Cd-only treated. At highest Cd treatment, Si addition significantly decreased the concentration of these anions in roots as compared to same treatment without Si. However, in lower Cd treatment Si addition slightly increased the concentration of these anions in roots as compared to respective treatments without Si.

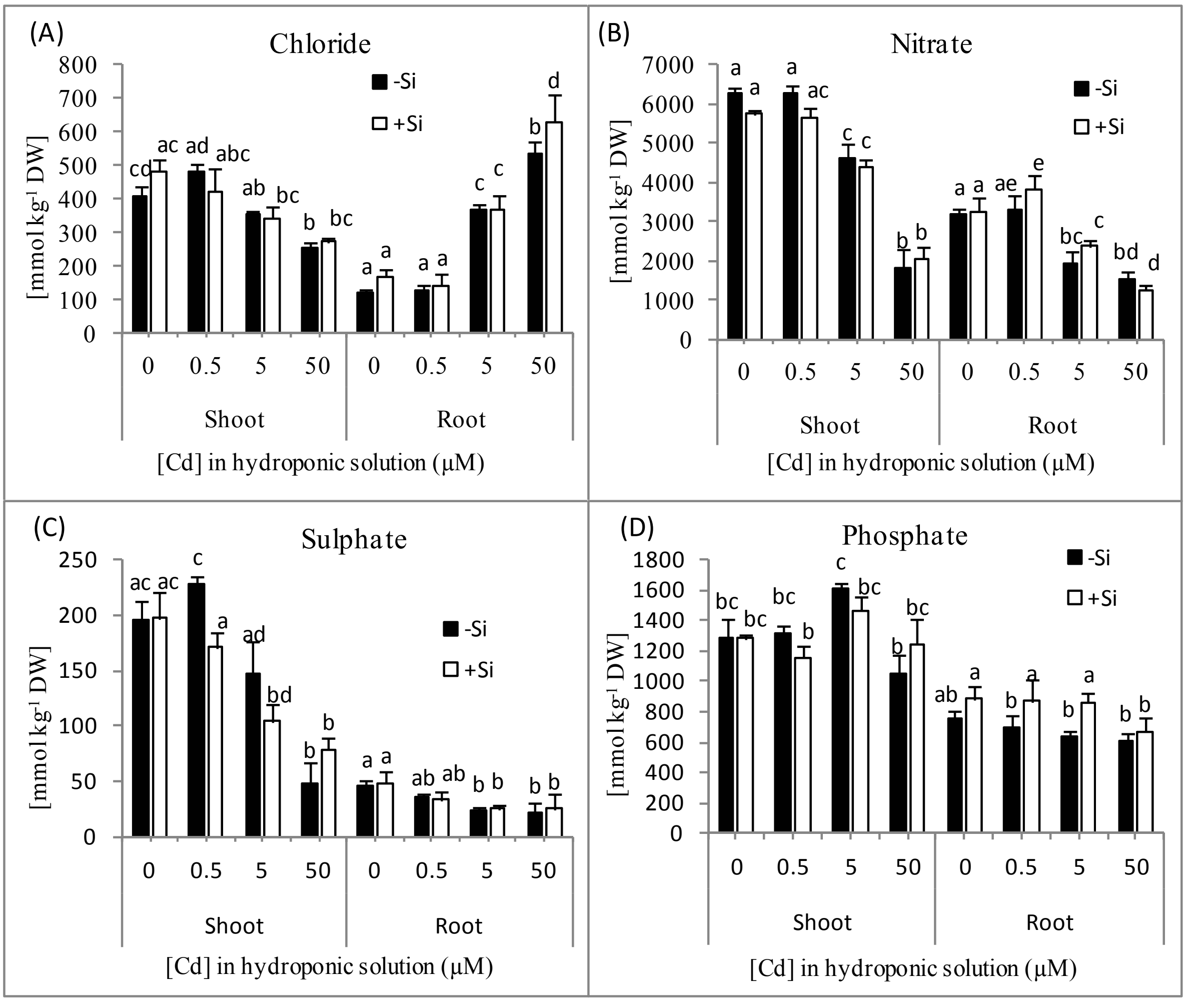

Figure 9. Inorganic anions (Cl, NO_3, SO_4 and PO_4) concentrations in plant shoots and roots. Bars represent SD of five replicates. Different letters indicate significant differences among the treatments at a $P < 0.05\%$ for shoots and roots separately.

I.3.6 Phytoliths composition

The phytoliths extraction procedure was applied to shoots of all plants with and without Si supply. We observed small amount of phytoliths in control and 0.5 µM Cd treatments without Si addition. Leaves supplied with Si in nutrient solution contained Si as amorphous silica (Figure 11). The dry ashing method of phytoliths extraction used in this study did not produce any preparation artefacts: the electronic microscopic observations of freshly extracted and phytoliths did not reveal any heat-generated smoothing and melting features on the surface and the X-ray diffraction analysis on the phytoliths material did not identify the presence of any crystalline phases (data not shown). SEM and optical observations of phytoliths showed bodies of various shapes. EDX microanalysis of phytoliths revealed that such bodies contain mostly Si and O. Traces of magnesium, Ca, K and Cl were also detected but no Cd.

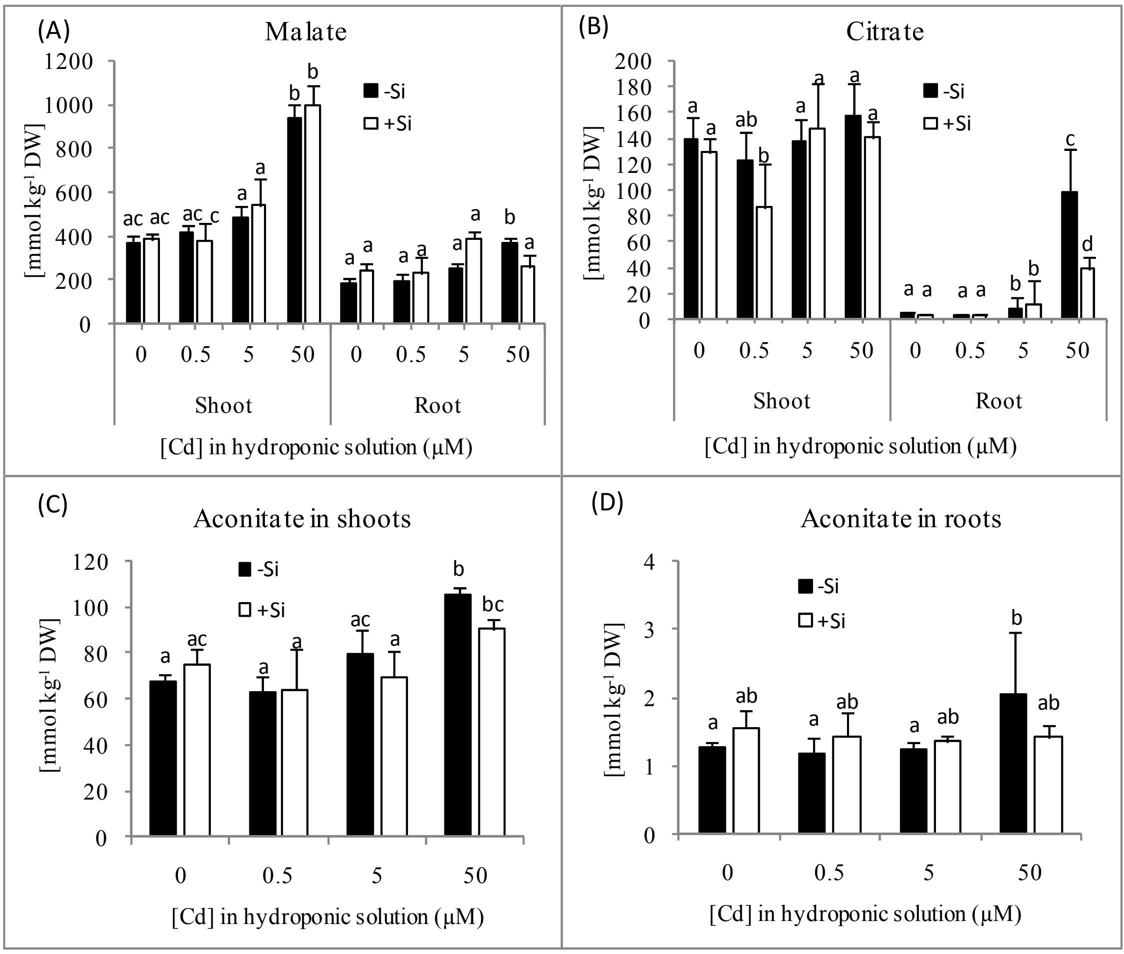

Figure 10. Organic anions (malate, citrate and aconitate) concentrations in plant shoots and roots. Bars represent SD of five replicates. Different letters indicate significant differences among the treatments at a P < 0.05% for shoots and roots separately.

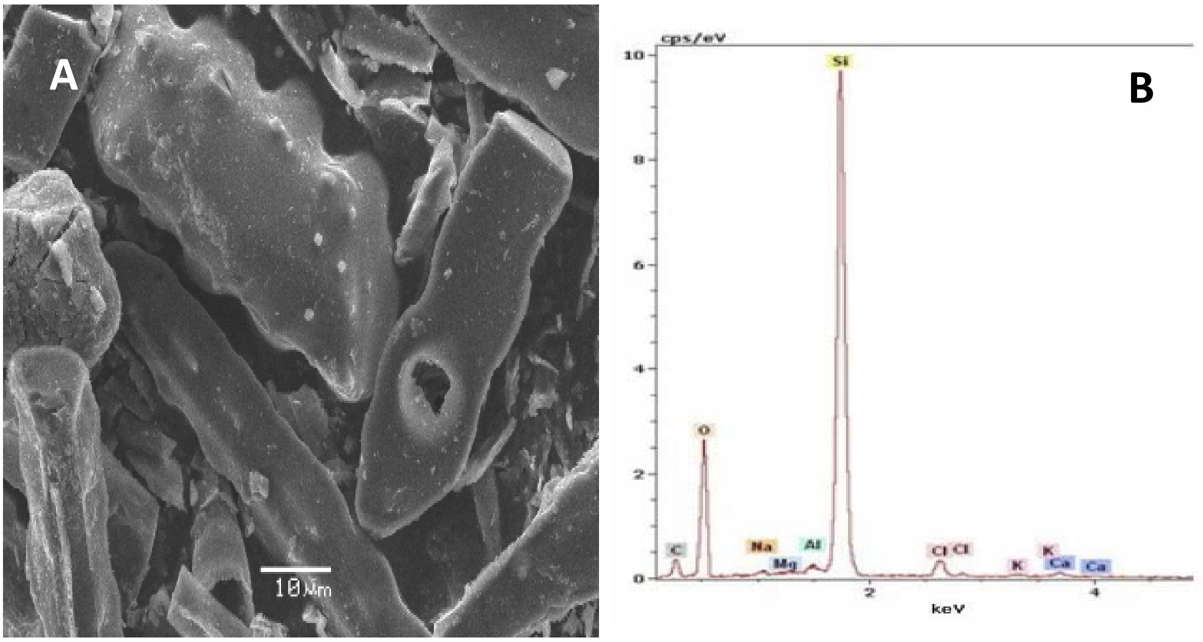

Figure 11. Scanning electron microscopy (SEM) microphotograph (A) and EDX spectrum (B) of phytoliths from ashed leaves of wheat plant grown in Si + 50.0 µM Cd.

I.3.7 Cd and Si localization

Detection and localisation of Cd and Si in roots by µXRF did not yield the expected results because Cd and Si concentrations in roots were below detection limits and because of interferences between Cd, K and Rd (data not shown). However, analysis shoot cross section treated with 1mM Si by µXRF showed that Si was mainly detected inside the cells of lower and upper epidermis of leaf in the form of dense spots (Figure 12).

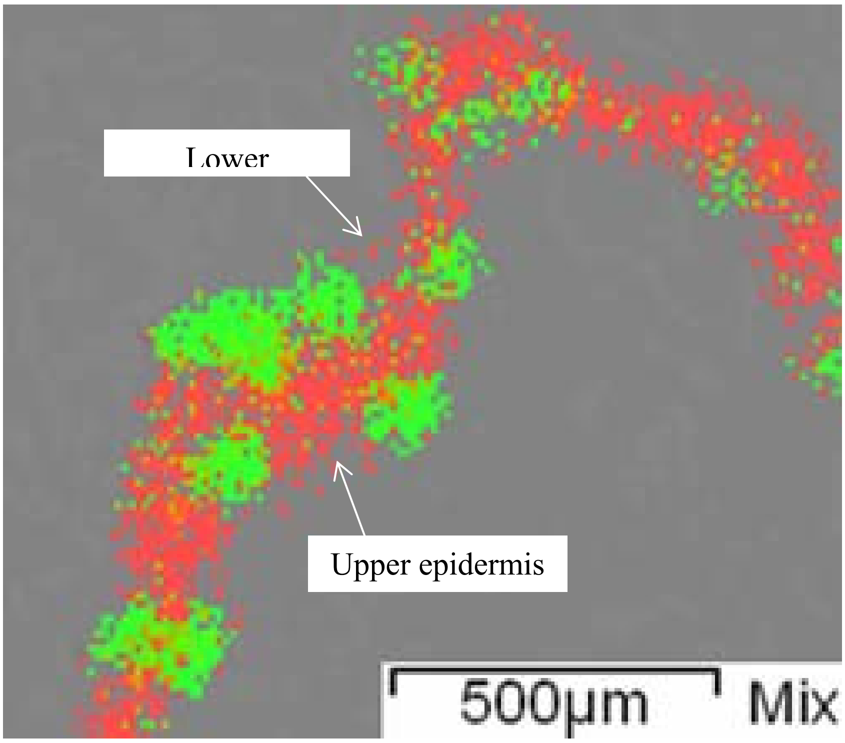

Figure 12. µXRF of shoot cross section from 1 mM Si+0µM Cd treatment. Two color image combing Si (green) and S (red).

SEM observations were then performed on 50.0 µM Cd+1mM Si treated roots. Different root cross-sections were examined by cutting horizontally and vertically the roots at different positions. Some roots were freeze dried at -55°C while some were freeze fractured in a cryo specimen chamber and then analyzed by SEM. Elements analyzed by EDX were Si, Cd, C, O, Na, Mg, P, S, Cl, K, Ca, Mn, Fe and Zn and the semi quantitative concentrations of these elements were measured in %age. EDX analysis showed that relatively large amounts of Cd were present in the cortex region near epidermis and in the epidermis (Figures 13 and 14). Small amounts of Cd were detected in the xylem or in phloem while in endodermis cells only trace amounts were detected. Si was mainly detected in endodermis cells while small amounts were present in the cortex cells. Only traces of Si were detected in the epidermis cells. From these results, Si and Cd concentrations did not appear correlated, indicating that there was no Cd-Si co-localization in the roots (Figure 14).

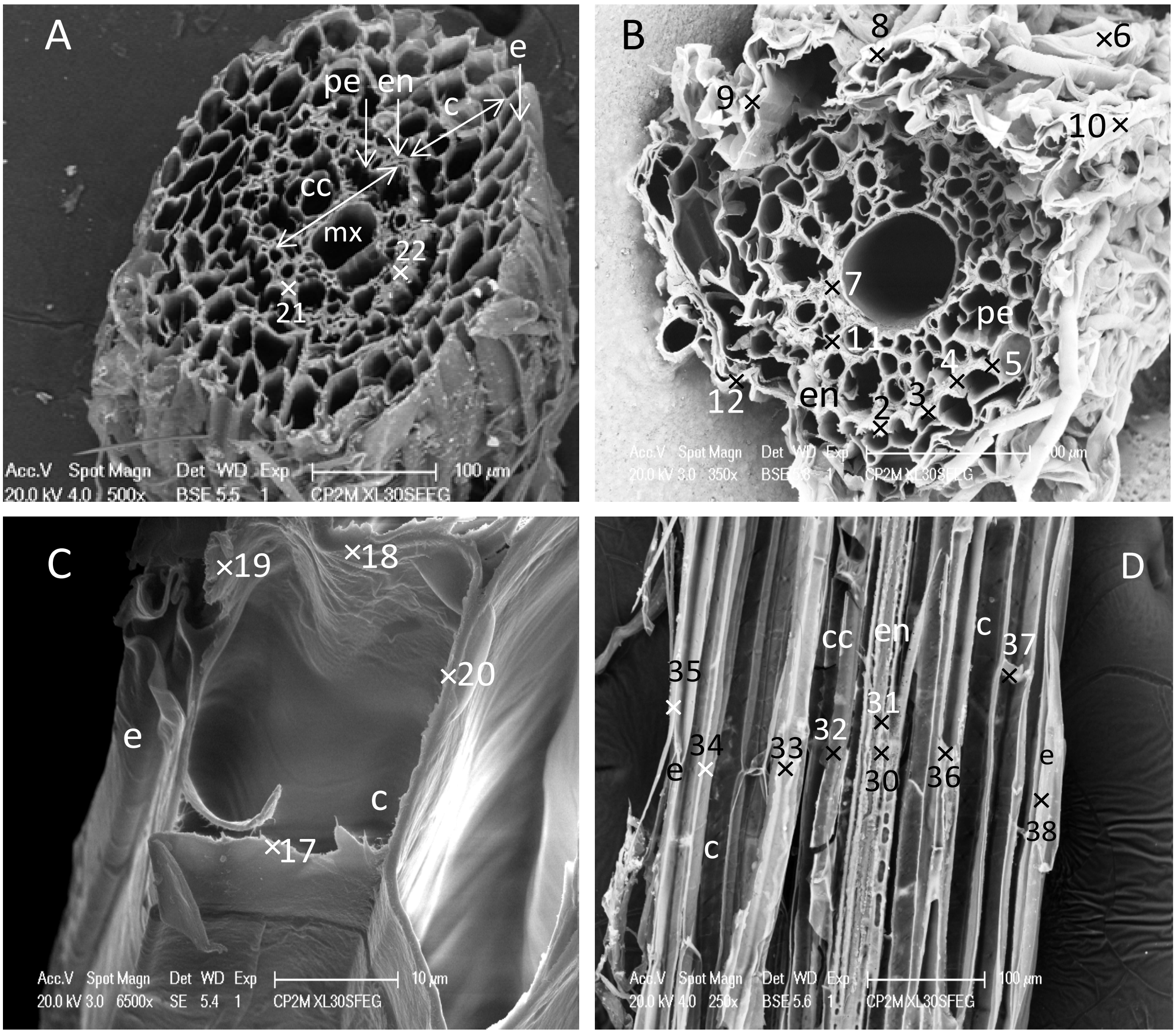

Figure 13. Scanning electron microscope (SEM) microphotographs of root cross-sections of wheat exposed to 50 µM Cd+1 mM Si. A, B, C, D- scanning electron microscopy micrographs. Different letters indicate different layers of root cross section i.e. e - epidermis, c - cortex, en - endodermis, pe - pericycle, cc - central cylinder and mx - metaxylem. Different numbers indicate sites at which EDX spectra were taken. A- 500×, B- 350×, C-6500×, D-250×.

I.3.8 Chemical composition of the most surface layer of roots by XPS analyses

Results of microscopic analysis described above were further corroborated by XPS analyses that allowed examining the most surface (< 100 Å) elementary composition of freeze-dried roots. It can be seen that the surface concentration of Si in Si-rich (1 mM) solutions was significantly higher than that without Si addition in the nutrient solutions (Table 2). The most important result was the enrichment of Cd on roots produced in Si-rich solution compared to Cd-only solutions (0.156 and 0.079 atomic percentage respectively). Similarly, Cd/C ratio on the surfaces of root grown in 50 µM Cd solution increased from 0.0014 in without Si solutions to 0.0025 in the presence of 1 mM Si. Noteworthy, the P concentration increased (and P/C ratio) in roots grown in 50 µM Cd + Si solutions by a factor of 2 as compared to Cd-only solutions. Another important result was highly heterogeneous Cd and Si distribution over the root surface: for sample in 50 µM Cd + 1 mM Si treatment, Cd concentration measured at 2 different spots in 2 different roots ranged from 0.08 to 0.31. The concentration of other measured components did not exhibit any significant variation among samples subjected to different treatments. High resolution spectra of C 1s reveal 3 types of carbon at the surface: C-C bond at 285 eV, C atom bounded to 1 O atom (C-O) at 286.5 eV and C atom bounded to 2 O atom (O-C=O) at 289 eV. No significant changes were noticed after all treatments in the ratio of these functional groups compared with the untreated roots (Table 2).

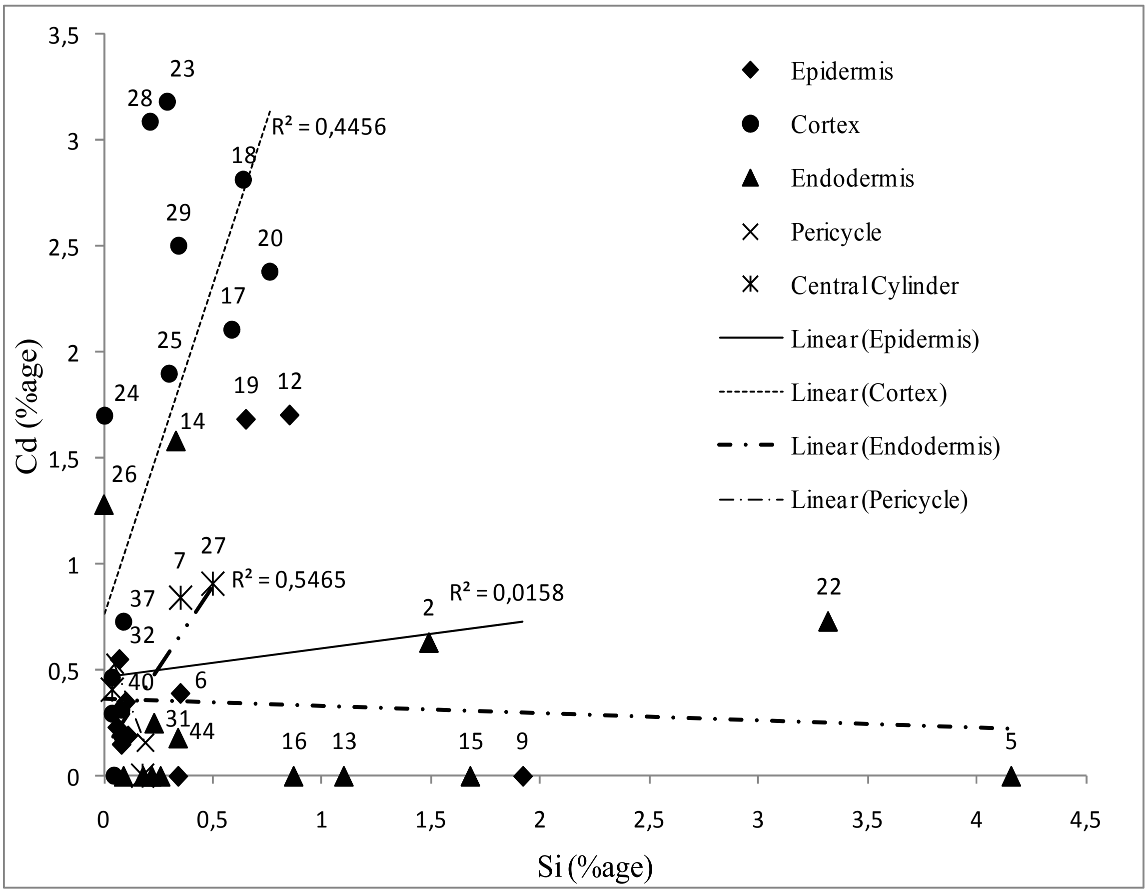

Figure 14. Correlation between Si and Cd concentrations (%age) at different points. Different numbers indicated on the markers corresponds to the numbers mentioned on the SEM micographs (figure 13).

Table 2. Chemical composition (%age) of the most surface layer of roots by XPS analyses. Values are means of four replicates.

Treatment	C	O	N	P	Si	Si/C	S	K	Cd	Cd/C
Cd 0µM+ Si 0mM	66.12±3.3	20.44±2.6	9.38±0.8	0.68±0.21	0.20±0.06	0.0030±0.001	0.15±0.21	3.05±1.46	0.00±0.0	0.00
Cd 0µM+ Si 1mM	65.64±2.9	26.05±1.2	5.56±2.4	0.52±0.31	0.69±0.01	0.010±0.001	0.21±0.02	1.35±0.44	0.0±0.0	0.0
Cd 50µM+ Si 0mM	60.79±14.6	31.81±13.4	5.93±1.8	0.32±0.18	0.18±0.14	0.0027±0.002	0.13±0.1	0.75±0.35	0.08±0.02	0.00143
Cd 50µM+ Si 1mM	66.96±10.1	23.69±9.2	6.62±1.0	0.64±0.15	0.72±0.39	0.010±0.004	0.11±0.13	1.10±0.59	0.16±0.10	0.00

	C-C	C-O	O-C=O	N (organic)	NO3 (nitrate)
Cd 0µM+ Si 1mM	50.47±7.69	33.94±2.8	15.60±4.9	100.0	traces
Cd 50µM+ Si 0mM	45.69±2.15	40.74±2.36	13.58±4.5	100.0	0.0
Cd 50µM+ Si 1mM	41.95±3.0	39.63±1.63	18.43±1.4	100.0	0.0

I.4 Discussion

I.4.1 Diagnosis of Cd toxicity

Increasing Cd concentrations in solution induced visible toxicity symptoms on both roots and shoots including decreased shoots and roots fresh and dry weight (Figure 3), shoot and roots lengths and chlorosis (Figures 1 and 2). These results are in agreement with previous studies in which toxicity symptoms were observed in wheat plants with 50 µM Cd concentration in the nutrient solution (Ci et al., 2009, 2010). They also included reduction of plant biomass in a broad set of plants such as maize (Wójcik and Tukiendorf, 2005), tomato (Haouari et al., 2012), rice (Shi et al., 2005) and wheat (Jalil et al., 1994), inhibition of shoots and roots elongation in wheat (Ci et al., 2009), as well as in maize (Wójcik and Tukiendorf, 2005; Zhang et al., 2008; Vaculık et al., 2009, 2012), rice (Liu et al., 2009) and cucumber (Feng et al., 2010). Chlorosis symptoms were observed in leaves of tomato (*Lycopersicon esculentum*) and pea plants with 10 µM and 50 µM Cd concentration in the nutrient solution respectively (López-Millán et al., 2009; Bavi et al., 2011). The inhibitory effect of high Cd concentration on root length has been previously explained by alteration of cell division at roots apex, inhibition of several enzyme activities (Zhang et al., 2008) and breakdown root cortex cells (Sridhar et al., 2007) leading to an increasing Cd flux into the plant (Cosio et al., 2005).

Cd toxic effects on roots were alleviated by the addition of Si and the effect was more pronounced when Cd was the highest in solution. These results are in agreement with the studies reporting that Si addition under Cd stress increased roots elongation (Vaculık et al., 2009, 2012). They speculated that Si mediated increase in cell-wall extensibility of young apical parts of maize roots may be considered as one of the causal factors for the better root elongation in Cd + Si treatment compared with the roots exposed to Cd-only treatment.

Addition of 1 mM Si in solution alleviated the visual toxicity by increasing roots fresh and dry mass (Figure 3) and increasing growth of primary seminal roots (Figure 1A). The increase in shoot biomass may be partially associated with suppression of chlorosis symptoms (Figure 1B) and lower Cd concentrations in shoots in the presence of Si (Figure 6). This implies that Si significantly reduced the Cd translocation from roots to shoots by increasing root to shoot ratios from 11.9, 28.9 and 19.9 to 22.4, 32.9 and 28.7 in 0.05, 5.0 and 50 µM Cd treatments respectively. Similar results were also reported previously in rice (Shi et al., 2005; Zhang et al., 2008), cucumber (Feng et al., 2009) and maize (Liang et al., 2005). By contrast, (Vaculik et al., 2009, 2012) measured larger Cd concentrations in maize shoots supplied with 35 and 5 mM Si respectively. Kulikova and Lux, (2010) reported that root to shoot ratio varied among maize hybrids treated with Cd + Si treatment as compared to Cd-only treatment. This showed that the mechanism of Si-mediated inhibition of Cd translocation varied among plant species.

Photosynthetic pigments can be used as indicators of growth under stress conditions as they are the key components of photosynthesis. We reported that Cd treatments significantly decreased Chl-a, Chl-b, carotenoids as well as total chlorophyll concentrations in wheat leaves (Figure 4) in accordance with the visual chlorosis symptoms. Toxic effects of Cd on chlorophylls biosynthesis have been already observed in wheat (Ouzounidou et al., 1997), tomato (López-Millán et al., 2009) and in pea plants (Sandalio et al., 2001). This reduction in chlorophylls biosynthesis may be due to the substitution of a central magnesium ion of chlorophyll structure with Cd due to their similar charges (Küpper et al., 1998) and/or may be due to damage or functional lost of the photosynthetic machinery (Feng et al., 2010). The carotenoids are accessory pigments that help in light harvesting and also protect chlorophylls and photosynthetic apparatus from oxidative damage and excess heat (Britton, 1995). Due to decreased carotenoid concentration under Cd stress, chlorophyll damage may be increased and consequently reduction in photosynthesis occurred. Reduction in photosynthetic pigments imply direct reductions in photosynthetic activity (Sandalio et al., 2001; Mobin and Khan, 2007) that resulted in lower shoot biomass production under Cd stress and chlorosis (Figure 3). The addition of Si significantly increased the concentration of these pigments as compared to Cd-only treatments (Figure 4) probably contributing to a growth recovery. Similar effects of Si on photosynthetic pigments have also been observed in cucumber (Feng et al., 2010). These authors also demonstrated that Si plays an important role in the protection of chloroplast ultrastructure and nitrogen metabolism from damaging in cucumber under 100 µM Cd stress. Nwugo and Huerta, (2008) reported that Si addition improved in chlorophyll-fluorescence parameters and light use efficiency in rice under 5 µM Cd stress. In the present study, Si addition lowered Cd concentration in wheat shoots reducing chlorophyll damages and helping to maintain biosynthesis of chlorophylls and the photosynthetic machinery.

I.4.2 The effect of Cd and Si on cations and anions uptake

Increasing Cd concentrations induced a decrease in Ca, Mg, K and P concentrations in both shoots and roots except for Ca concentrations in roots at the highest Cd treatment (Annex 2 figure 1). It has also been reported that 50 µM Cd concentrations in nutrient solution significantly decreased the concentrations of Mg, K and P both in shoots and roots of pea (Sandalio et al., 2001) and potato plants (Haouari et al., 2012). Jalil et al. (1994) reported that 0.5 µM or above Cd reduced K concentration both in shoots and roots of wheat plants. The decrease in major elements concentrations with addition of Cd may be attributed to antagonistic processes on the binding sites and transporters, in particular those with the same charge (Gussarson et al., 1996; Sandalio et al., 2001; Dong et al., 2006). In present study, Si did not significantly change the concentrations of macro elements except Ca concentration in roots. Song et al. (2009) suggested that Si can increase uptake of Ca in pak choi plants which competes with Cd for uptake sites in plant roots and inhibits Cd uptake and translocation to shoots. This might be the case in the 50 µM Cd treatment + Si for which the difference between +Si and –Si was significant.

Micronutrients distribution was also impacted both by the increasing Cd concentrations and the Si supply. Decrease in Zn concentrations in shoots and roots with increasing Cd concentrations in plant is traditionally explained by a competitive inhibition of Zn uptake when there is an excess Cd and vice versa (Grant et al., 1998; Harris and Taylor, 2001). Indeed, Zn and Cd share a common transport system at the root cell plasma membrane in both bread and durum wheat (Hart et al., 2002). Especially in the highest treatments, larger amounts of Zn were internalised and less were adsorbed onto the apoplasm (Figures 7 and 8) in plants supplied with Si. Larger Zn absorption in the presence of Si may increase Zn/Cd competition at the plasma membrane helping to reduce Cd concentration in the symplasm.

A significant increase of malate, citrate and aconitate concentration in shoots and roots of durum wheat exposed to different Cd treatments (Figure 10) are in agreement with the previous studies with Al and Cd (Saber et al., 1999) and with Al (Ma et al., 1998, 2001).

Ueno et al. (2005) indicated that Cd was mainly coordinated with malate in the leaves of *Thlaspi caerulescens*. In our study, higher concentration of organic anions in the presence of Cd may indicate that a plant physiological response against Cd toxicity. Larger production of inorganic and organic anions in the presence of Cd may chelate Cd with anion ligands in the cytoplasm and detoxify Cd and allocate it to metabolically inactive parts of the cells. In the presence of Si, the concentration of organic anions decreased both in shoots and roots in the 50 µM treatment (Figure 10) which may be due to lower total uptake of Cd in shoot in the presence of Si and the larger adsorbed Cd pool in roots as proven by XPS analyses that may induce a lower potentially assimilated "sensed" Cd concentrations (Figures 6 and 7) as discussed below.

The concentration of organic anions is also important for Cd translocation from roots to shoots. Previous findings indicate that Cd cannot move freely in cells or in xylem vessels. Gong et al. (2003) found that phytochelatins are considered to be involved in the long distance root to shoot transport of Cd in *Arabidopsis*. Larger production of organic anions in roots under Cd stress without Si application may complex Cd in roots and may translocate it from roots to shoots. Other compounds might also play a role, including Cd-organic acids complexes. Recently, in oil seed rape plants the level of amino acids and organic acids detected was enough to bind Cd transported via the xylem (Nakamura et al., 2008). Similarly, significant increase in citrate synthesis and Cd-citrate complexes have been reported to be present in the xylem sap of tomato plants treated with Cd (Senden et al., 1995; López-Millán et al., 2009). Zorrig et al. (2010) also reported that Cadmium-citrate complexes could play a particular role in Cd translocation from roots to shoots in lettuce. Our findings indicate that higher values of Cd in shoots without Si might be due to more Cd complexed and transferred by organic acids and chloride. Our results showed that Si application reduced Cd stress in plants by decreasing Cd in shoots and by increasing adsorbed Cd in roots (Figure 6 and 8) consequently less organic anions were produced in the presence of Si (Figure 10).

The results also highlighted the changes in the uptake and distribution of Si in wheat plants (Figure 5) indicating that Si uptake is also impacted by Cd concentration in the medium. With a shoot to root ratio shifted from 6.5 to 2.5 between the 0 and the 50 µM Cd in solution. Reduced Si uptake with increasing Cd concentration in the nutrient solution indicated that Cd may disturb the plant metabolism which resulted in reduced Si uptake by plant roots which is an active process via transporters as characterized by Mitani et al. (2009) in maize plants. Since Si potentially alleviates a whole range of stresses, this suggests that a Cd contamination may weaken the Si effect and, as a consequence, may reduce the resistance of wheat to other stresses. But unlike for shoots, there was no effect of Cd on Si concentrations neither in the bulk of the roots (Figure 5) or in the most surface layer (Table 2) resulting in a shift in the Si shoot/root ratio towards lower ratios, which may indicate an increasing Si immobilisation in roots with increasing Cd in roots. Whether this reduced Si transfer to the shoot is a consequence of Cd toxicity or is an active process aiming at reducing Cd toxicity at the root level remains to be clarified.

It can be noted that the increase of root surface Cd concentration in the presence of Si as inferred from XPS analysis may suggest some armoring protection mechanisms consisting in non-homogeneously distributed granular-like Cd, Si-bearing precipitates. Extremely small thickness of these newly formed compounds does not allow detecting them with classic microscopic/macroscopic techniques. Interesting that the increase of surface phosphorus concentration in the presence of Si (see table 2) may also lead to additional immobilization of Cd in the form of Cd-phosphates; however the synergetic mechanisms of Si and P immobilization remains unclear.

I.4.3 Cd-Si distribution in plant: coprecipitation vs compartmentation

An additional effect of Si that needed to be/could be tested was a Si-induced change in Cd allocation within the plant shoot tissue in less metabolically active compartments. When treated with Si, shoots were the main sites for Si accumulation in the form of phytoliths (Figure 11) which mainly deposited in the upper and lower epidermis in the form of dense spots (Figure 12) as expected for a Si-accumulating plant. Si deposition in the form of dense spots indicates that Si may be deposited near stomata and/or in specific bulliform cells, where water evaporates from the plants (Motomura et al., 2000; Sommer et al., 2006; Currie and Perry, 2007). Ma et al. (2001) proposed that Si deposition may decrease transpiration by depositing in surface layers of rice. This reduction in transpiration may reduce Cd translocation to shoots as observed in Figure 6. However no change in dry and fresh weights was associated to that possible reduced transpiration rate that would be expected to be visible in control plants with or without Si. Alternatives mechanisms associated to the decrease in Cd toxicity when plants are supplied with Si would be enhanced apoplasmic binding of Cd as proposed for maize (Vaculik et al. (2012) and rice (Wang et al., 2000; Liu et al., 2009), altered Cd subcellular distribution and reduced the Cd content in cell organelle fractions of leaves as identified for peanuts (Shi et al., 2010). Neumann and zur Nieden, (2001) also reported that Zn toxicity in *Cardaminopsis halleri* leaves may be reduced by co-precipitation of Zn and Si in the form of Zn silicate in the vacuole and cell wall. Although apoplasmic binding could not be ruled out, the EDX spectra obtained from phytoliths of the studied wheat plants contained no Cd or in concentrations below EDX detection limits, which indicates that there was no significant co-precipitation of Cd and Si in wheat leaves. Consequently, compartmentalization of Cd in shoots rather than co-precipitation may be a possible mechanism of Si reduced Cd toxicity in wheat plants, together with reduced total Cd concentration.

At the root level, increasing Cd levels in nutritive solution increased Cd concentration in roots but, unlike in shoots, Si application increased total Cd concentrations in roots, essentially due to an increase in adsorbed Cd as measured by the desorption experiment (Figures 6 and 8) and as also seen from the XPS data (table 2). However, total Cd uptake was lower both in shoot and roots in the presence of Si as compared to without Si addition except in roots of 50 µM Cd treatment (Table 1). This indicates that Si may act as a physical barrier and/or reduce Cd transfer from apoplasm to symplasm. However, the present results are not consistent with previous reports indicating that Si did not affect the distribution of apoplasmic and symplasmic Cd in roots of peanut (Shi et al., 2010), rice (Shi et al., 2005) and maize plants (Vaculik et al., 2012). Therefore, in the presence of Si, wheat plants might evolve a specific mechanism to reduce toxic effects of Cd by compartmentalization of Cd in metabolically inactive parts such as root apoplast.

SEM-EDX analysis indicated that Si was mostly in the endodermis cell walls while Cd was mainly found in the cortex region, in small amount in the central cylinder and in trace amounts in the vicinity of endodermis (Figures 13 and 14) of water-washed roots. This heavy deposition of Si in the vicinity of endodermis may reduce the cell wall porosity of the casparian strips and reduced metal apoplastic transport as suggested for Cd tolerance in rice (Shi et al., 2005) and maize (da Cunha and do Nascimento, 2009) and may provide an increased selectivity against metal entering the stele of roots (MacFarlane and Burchette, 2000). Root-to-shoot Cd translocation via xylem is the major process of shoot and grain Cd accumulation in rice (Uraguchi et al., 2009). In the present study, Si deposition in the endodermis may reduce Cd xylem loading and consequently reduced translocation to shoots as indicated by reduced shoot Cd concentration in the presence of Si (Figure 6). The fact that Cd was not accumulated at the endodermis level like Si disagrees with previous reports suggesting that Si deposition in root endodermis may restrict the apoplastic transport of Cd by co-precipitation of Si and Cd in the endodermis and alleviates Cd toxicity to the cytoplasm in rice (Wang et al., 2000; Shi et al., 2005) and maize (da Cunha and do Nascimento, 2009). Gu et al. (2012) localized Zn and Si around endodermis of roots and suggested that formation of Zn-Si precipitates is a possible reason for the lower Zn concentration in xylem sap of rice plants.

I.5 Conclusion

In conclusion, Si supply in solution decreased Cd translocation and toxicity symptoms in durum wheat seedlings by increasing root apoplasmic bound Cd and Zn uptake. Si-mediated lower uptake and translocation of Cd decreased physiological stress in plants that resulted in a larger production of photosynthetic pigments and lower production of organic acids especially citrate and aconitate in shoots and roots which could decrease the root to shoot translocation of organically bound Cd. While Cd increasing concentrations impacted a whole range of physiological parameters, Si counterbalanced these effects either directly through Si accumulation at the endodermis and decreased Cd translocation to shoot or indirectly as a consequence of reduced Cd concentrations in shoots. Co-precipitation of Cd together with Si was not found to be a major mechanism, neither at the shoot nor at the root level. The overall balance of Cd and Si showed that in the presence of Si total uptake of Cd decreased than without Si addition.

These results highlight the importance of Si in the nutrient status of plants and its role in alleviating Cd stress and the induced toxicity symptoms. Addition of Si to Cd contaminated soils may help to reduce Cd in wheat shoots and as a consequence in grain transfer to the food chain.

II. Effects of silicon on growth and physiology of wheat seedlings (*Triticum turgidum* L. cv. Claudio) under Cu stress: a hydroponic study.

Abstract

A hydroponic study was performed to investigate the influence of silicon (Si) in enhancing copper (Cu) tolerance in durum wheat (*Triticum turgidum* L.) plants under different toxic levels of Cu. Plants were grown for ten days without and with 1 mM Si followed by increasing levels of Cu for ten days with 0, 0.7, 7.0 and 30 µM and without and with 1.0 mM Si. Results showed that Cu significantly decreased Si concentration in shoots while it significantly increased Si concentration in roots. Si supply significantly alleviated the Cu toxicity in durum wheat at highest toxic level of Cu. Si partly overcame the reduction in shoot and root growth due to Cu which was correlated with reduction of Cu uptake and translocation, increase in photosynthetic pigments, macroelemnts and organic anions (malate, acetate and aconitate) in roots. Si increased adsorption of Cu on the roots surface while decreased adsorption of Mn and Zn. In addition, µXRF analyses showed that in the presence of Si, Cu was localized in the epidermis while Si in the endodermis. Cu was localized in the central cylinder when Si was not applied. This study suggested that the Si-mediated Cu tolerance in durum wheat is mainly due to the reduction of uptake and translocation of excess Cu and stronger binding of Cu in the epidermis of roots. Higher concentration of citrate and aconitate in roots might also play some role in complexing Cu in the roots.

Key words: Durum wheat, copper, silicon, photosynthetic pigments, mineral nutrients, localization, anions, µXRF

The results presented in this section have been prepared for submission in “New Phytologist”

II.1 Introduction

Excess copper (Cu) in soils results not only from industrial or mining activities, but also from Cu-based fungicides and pesticides as well as from the application of Cu rich pig and poultry slurries (Marschner, 1995; Xiong and Wang, 2005; Legros et al., 2010). In Europe, excessive use of so-called Bordeaux mixture ($Ca(OH)_2+CuSO_4$) to control vine downy mildew and use of pig and poultry slurries rich in Cu concentration has increased the Cu pollution in these soils (Brun et al., 1998; Brun et al., 2001; Legros et al., 2010; Mackie et al., 2012). Cu is an essential micronutrient which is required for normal growth and development of plants. It is a constituent of proteins and is also involved in electron transfer reactions (Epstein and Bloom, 2005; Yruela, 2005). However, when compared with other potentially toxic elements such as Cd and Zn, Cu is highly phytotoxic at slightly larger concentrations (Marschner, 1995; Wheeler and Power, 1995; Kinraide et al., 2004).

In plants, it has been widely reported that high accumulation of Cu may cause many biochemical, physiological and structural changes such as inhibition of photosynthesis, leaf chlorosis, root damage, disturbance in the uptake of other essential nutrients and inhibit the plant growth and biomass (Ouzounidou et al., 1995; Cook et al., 1997; Caspi et al., 1999 Yruela 2005; Kopittke and Menzies, 2006; Michaud et al., 2007, 2008; Azooz et al., 2012). Cu may eventually enter in the food chain through crop plants and can put a threat to human health (Kabata-Pendias and Pendias, 2001). Wheat (*Triticum aestivum* L.) is one of the most important cereals and is the most produced cereal crop in Europe and the second most produced in the world, with 228 and 686 MT produced in 2009, respectively (FAOSTAT, May 2011). Among wheat varieties durum wheat (*Triticum turgidum* L.) is cultivated mainly in the Southern France (Michaud et al., 2007 and the references therein). Recently, several authors have observed Cu phytotoxicity symptoms in durum wheat plants both in hydroponic and in the field studies (Michaud et al., 2007, 2008; Bravin et al., 2010). This higher accumulation of Cu in wheat plants is directly related to wheat safety. Remediation strategies are, therefore, needed for Cu-contaminated soils under durum wheat cultivation. Different actions can be undertaken to reduce the Cu absorption by plants such as metal excluding plants (Poschenrieder et al., 2001; Wei et al., 2005) and using different soil amendments (Roy et al., 2005; Li et al., 2008; Wuana and Okieimen, 2011). However, these actions may not be efficient in all cases. There is still a need for a range of more efficient and economical approaches for remediation of metal toxicity in plants.

Silicon, on the other hand, is not considered to be an essential element for plants (Epstein, 1994). However, there is increasing evidence that it has a number of beneficial effects on plant growth under biotic or abiotic stresses (Epstein, 1999; Ma, 2004; Guntzer et al., 2011). In the aspect of abiotic stresses, Si can reduce the toxic effects of some metals in many plant species such as Mn toxicity in cucumber (Rogalla and Römheld, 2002) and maize (Doncheva et al., 2009), Al toxicity in wheat (Cocker et al., 1998) and of Cd toxicity in some plants species such as maize (da Cunha et al., 2008; Vaculik et al., 2009, 2012), cucumber (Feng et al., 2010), strawberry (Treder and Cieslinki, 2005), rice (Shi et al., 2005; Nwugo and Huerta 2008; Liu et al., 2009) and wheat (Rizwan et al., 2012).

Compared with studies examining the effects of Si on Mn, Al and Cd, little information is available about the role of Si in Cu tolerance although it has been shown that Si can alleviate Cu toxicities in *Arabidopsis thaliana* by physiological changes and alterations of gene expression (Li et al., 2008; Khandekar and Leisner, 2011) and in *Erica andevalensis* by inhibiting Cu upward transport (Oliva et al., 2011). Cu concentration and its interactions with Si have also been recently assessed in bamboo species (Collin et al., 2012). However, mechanisms behind Si-mediated alleviation of Cu toxicity are not comprehensively investigated. In addition, it remains unclear whether Si detoxifies Cu toxicity in wheat and durum wheat seedlings, despite the fact that Si is actively absorbed by wheat plants and transported to different plant organs (Rafi and Epstein, 1999). In this study it was hypothesized that silicon application may also be effective in enhancing the Cu tolerance to durum wheat plants. Therefore, the objective of this study was to examine and find the possible mechanisms of Si on Cu detoxification in durum wheat.

Therefore, based upon the above information, we conducted a hydroponic study in controlled conditions to examine the effect of Si on Cu detoxification in durum wheat (*Triticum turgidum* L.) plant. In order to identify the possible Cu tolerance mechanisms in Durum wheat seedlings supplemented or not with Si, we investigated the localization and distribution of Cu in wheat shoots and roots and Si effects using several combined approaches: photosynthetic pigments, mineral and anion analyses.

II.2. Materials and methods

II.2.1 Plant culture and treatments

Durum wheat (*Triticum turgidum* L. cv. Claudio W.) was grown in hydroponic condition. Before germination, the seeds were washed with distilled water then sterilized with sodium hypochlorite containing 2.6% active chloride for 3 minutes and then thoroughly washed with distilled water 5-6 times to remove excess chloride from seeds. Thereafter they were imbibed in water for 4 hrs at room temperature. During imbibition the seeds were aerated by a pump to avoid fermentation and then transferred for germination in petri-dishes for about 2 and a half days at 23°C/20°C day and night temperature respectively.

After germination, seedlings were transferred to 12L plastic containers filled with a modified Hoagland nutritive solution containing: 0.5 mM KH_2PO_4, 0.5 mM K_2HPO_4, (these two also act as a buffer to maintain pH), 0.5 mM $MgSO_4$. $7H_2O$, 1 mM KNO_3, 1 mM $Ca(NO_3)_2$. $4H_2O$, 0.125 mM KCl, 50 µM H_3BO_3, 12 µM $MnSO_4$. H_2O, 0.7 µM $CuSO_4$. $5H_2O$, 1 µM $ZnSO_4$. $7H_2O$, 0.25 µM MoO_4Na_2. $2H_2O$ and 20 µM Fe-HBED. Plants were grown under a short-day cycle (8/16 h 23°C/20°C day/night), 70% humidity and 450 µmol photons $m^{-2}S^{-1}$ light intensity. There were 8 containers each containing 120 wheat seedlings. Half of the containers were supplied with 1mM Si in the form of silicic acid neutralized with KOH. Additional K produced by Si (K_2SiO_3) supplementation was subtracted from KNO_3 and the resultant nitrate loss was supplemented with dilute nitric acid. At the start of the experiment two basins were not supplemented with Cu and of these two basins one was supplied with 1mM Si. The other basins were initially supplied with 0.7 µM Cu in the form of $CuSO_4$. $5H_2O$. Solution pH was adjusted to 6.5 ± 0.2 with 1 mM tampon MES (2-morpholinoethanesulphonic acid) when required. Aeration was done in each pot with continuous air bubbling produced by aeration pump. Nutrient solutions were changed every three days over the course of the entire experiment to keep the nutrients concentrations in the solution as stable as possible. After 10 days adaptation with silicon, the plants were exposed to 0, 0.7, 7.0 and 30 µM $CuSO_4$. $5H_2O$ (that is 0, 0.044, 0.44 and 1.9 mg Cu L^{-1}) to the nutrient solution, which were tested without and with Si application. In total, the plants were cultivated in the growth chamber for 20 days: 10 days with Si application when required and 10 days with Cu and Si applications.

II.2.2 Plant harvesting and biomass

After 10 days of Cu treatments, the plants of each treatment were harvested and separated into shoots and roots. Analyses were performed on individual plants with shoots and roots analyzed separately. Shoots were washed with distilled water and measured for the fresh weight and then divided into two parts: one subsample was frozen in liquid N_2 prior to measuring its organic and inorganic contents and localization of Cu and Si while another subsample was oven dried at 80°C until constant weight was reached, weighted and grounded to smaller size for further analysis. Roots were also divided into 2 parts. One subsample was washed with dilute acid to measure metals bound to root cell walls (root apoplasmic metals) as described by Chaignon et al. (2002): briefly, 0.4g of roots (on a fresh weight basis) was shaken end-over-end with 20 ml of 1 mM HCl for 3 min, then 180 µl of 1 M HCl were added to yield a final concentration of 10 mM HCl. After shaking for another 3 min, the roots were recovered and gently dried between two layers of filter paper then oven dried at 80°C. Another subsample was washed only with distilled water and some fresh roots were stored in liquid nitrogen and others were used to measure fresh weight then oven dried at 80°C till constant weight and weighted then grounded to smaller size for further analysis.

II.2.3 Anion assays

Anionic profiling was performed for both shoots and roots of wheat plants. For this, fresh plant samples (approximately, 1/8 FW/V ratio) were incubated in ultrapure water heated at 70°C for about 40 min then the samples were cooled at room temperature, centrifuged 3 times at 14×10^3 rpm at 4°C for 10 min each and the supernatant solution was recovered. The concentration of major inorganic and organic anions in the supernatant was determined by high performance ionic chromatography (LC20, Dionex) using an IonPaq AS11 column and a NaOH linear gradient. NaOH concentration was raised linearly from 3.5 to 5mM over 3 min then from 5 to 33.5 mM over 8 min. Measurement of each anion was performed by comparing the retention times and peak areas, using the Chromeleon software (Dionex), with the standards.

II.2.4 Determination of photosynthetic pigments

Chlorophylls (a & b) and carotenoids contents in the fresh leaves (about 1.0 g) were extracted in the dark with 10 ml of 80% acetone by continuous shaking until color had completely disappeared from the leaves. Light absorbance at 663, 645 and 470 nm was determined by spectrophotometer. The concentrations of chlorophylls and carotenoids were calculated by using the adjusted extinction coefficients (Lichtenthaler, 1987).

II.2.5 Determination of micro and macro elements and silicon from plant samples

For the determination of micro (Cu, Mn and Zn) and macro (K, P, Ca and Mg) elements, finely ground plant (above or underground parts) samples (0.25g) were digested in 4 mL of concentrated HNO_3 70% (Fluka, Buchs, Switzerland) in a block of mineralization with a rise in temperature up to 95°C for 6 hrs then filtered at 0.2 µm (Whatman cellulose nitrate membrane) (modified from Keller et al., 2003).

Si concentration in plant shoots and roots was measured using Tiron extraction method (Guntzer et al., 2010). In brief, 50 mg of each plant sample (part above or underground) was extracted by adding 30 mL of 0.1M Tiron, [4,5-dihydroxy-1,3-benzene-disulfonic acid disodium salt, $(OH)_2C_6H_2(SO_3Na)_2$], solution buffered at pH 10.5 in polypropylene tubes, which were placed in a water bath at 85°C and horizontally shaken for 2 hrs with the caps loosely closed to prevent evaporation. The tubes were then cooled and 10 ml were sampled into pre-labeled 40 mL samples vials containing 10 mL of 30% H_2O_2 in order to destroy Tiron. This new solution was shaken again at 85°C in the water bath for about half an hour till the color had disappeared and the solution was filtered at 0.2 µm.

The micro (Cu, Mn and Zn), macro (K, P, Ca and Mg) elements and Si from the plant digests and extracts were measured by inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES Jobin-Yvon, Ultima-C).

II.2.6 Localization of Si and Cu by µXRF

Three root samples from Si 1mM + 0 Cu, Cu 30µM + 0 Si and Cu 30µM + Si 1mM treatments, were selected for chemical micro-analyses by µXRF. Frozen root samples were freeze dried at -55°C for about 72 hrs and then embedded in an epoxy resin. The embedded samples were then cut transversely with a diamond wire saw at about 5 cm distances from the bottom of shoots to get the comparable root cross-sections. The thickness of the obtained cross-sections was about 200 µm. The root diameter was about 450 µm. The measurements were carried out on a microscope (HORIBA XGT^{7000}) equipped with an x-ray guide tube producing a finely focused and high-intensity beam with a 10 µm spot size (Rh X-ray tube, accelerating voltage of 30 kV and a current of 1mA). X-rays emitted from the irradiated sample were detected with energy-dispersive X-ray (EDX) spectrometer equipped with a liquid nitrogen cooled high-purity Si detector. Only elements with an atomic number $Z>11$ (Z_{Na}), can be detected. Analyses were performed in sample chamber vacuum mode.

To identify the distribution of Cu and Si in the root sections, spot analyses (counting time of 1000 s/spectrum) were performed along a profile crossing the root sections (from epidermis to epidermis through the central cylinder) with a step of 10 µm (along x-axis). The counting time per step (i.e. spectrum) is then sufficient to detect trace element as Cu (detection limit of about 50-100 mg kg^{-1}).

Kα emission line intensities of Cu, Si, K, Ca and Mn were reported for each step (i.e. spectrum) and profiles have been ploted (Figures 12, 13 and 14). The intensities have not been calibrated so obtained profiles are only semi-quantitative. We cannot compare directly the intensities obtained from different profiles as the thickness of the sections can vary.
Ca and K are constituents of the roots that can be detected by µXRF. These elements can be used as tracers to localize the epidermis (Ep), the cortex (C), the endodermis (En) and the central cylinder (CC) of the roots. S, P and Zn are also detected in the roots.

II.2.7 Statistical analysis

All the data were statistically analyzed using one-way ANOVA at a significance level of $P \leq 0.05$ with SPSS 16.0 software for windows. Single step multiple comparisons of means was performed via Tukey’s post hoc test. Different letters on the histograms indicate that the means were statistically different at $P \leq 0.05$ level. Data presented is from eight treatments and with 3 or 5 replicates as described above.

II.3 Results

II.3.1 Wheat growth

In this study we considered 0.7 µM Cu in the nutrient solution as a control. Higher Cu (7.0 and 30 µM) concentrations affected the length of primary seminal roots (Figure 1 and 2). Roots treated with 7.0 µM Cu were significantly shorter and less branched when compared with 0.7 µM Cu (control). Similarly, the length of lateral roots was shorter when compared with the control plants. Roots treated with the highest Cu (30 µM) concentration were significantly shorter and also had shorter lateral roots than 7.0 µM Cu treated roots (Figure 1 and 2). Addition of Si partially mitigated the negative effects of Cu in roots. Primary seminal roots treated with Cu7.0 + Si and Cu30 + Si were longer and also had longer lateral roots when compared with 7.0 and 30 µM Cu-treated roots, respectively. Addition of Si enhanced the branching of seminal roots when compared with Cu-only treated roots (Figure 1). However, Cu addition in the nutrient solution did not significantly affect the shoot length. The highest Cu concentrations slightly reduced shoot length and addition of Si slightly increased the shoot length as compared to the same Cu treatment without Si (Figure 2).

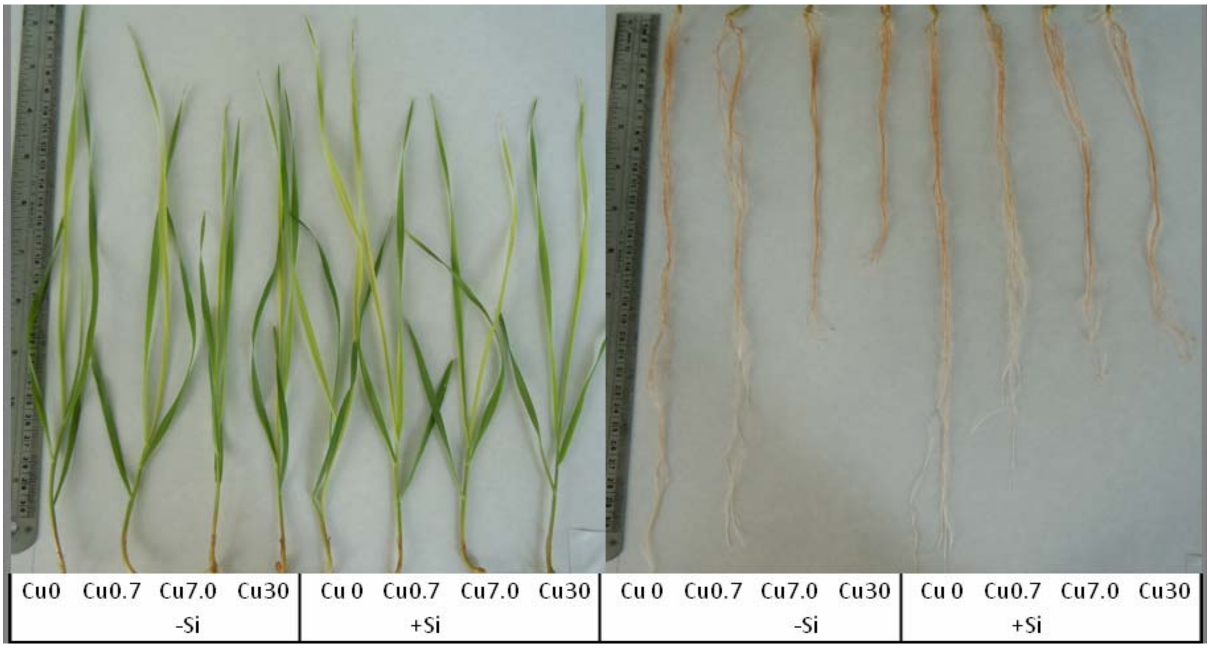

Figure 1. Appearance of shoots and roots of young wheat plants grown hydroponically with increasing Cu (0, 0.7, 7.0 and 30 µM) concentrations treated or not with 1mM Si.

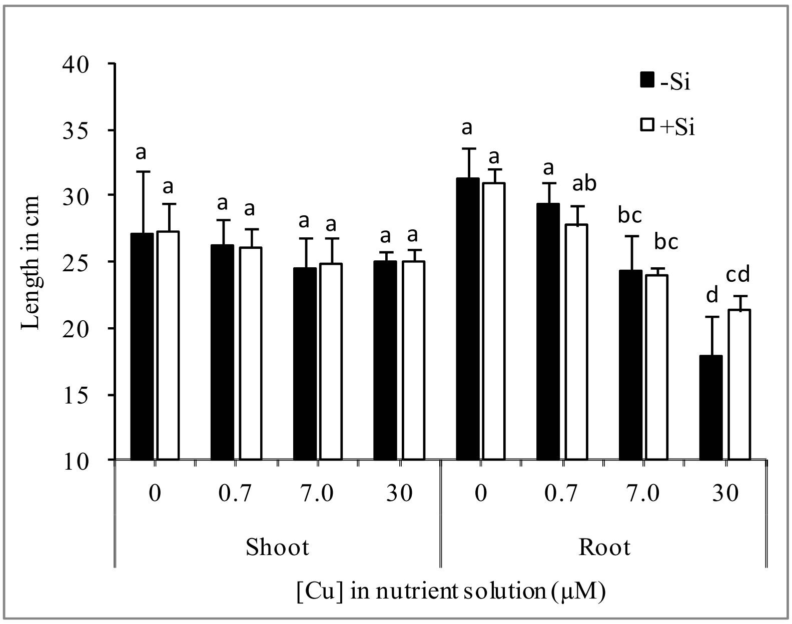

Figure 2. Length of shoots and primary seminal roots of young wheat plants grown hydroponically exposed to various Cu concentrations without and with 1mM Si addition. Bars represent SD of five replicates. Different letters indicate significant differences among the treatments at a $P < 0.05\%$ for shoot and roots separately.

Differences were observed in the fresh and dry weights of shoots and roots among the treatments (Figure 3). The shoots fresh and dry weights were not affected by Cu treatments but roots fresh and dry weights were only affected at 7.0 and 30 µM Cu concentrations. The addition of Si especially when Cu was applied at higher concentrations had a positive effect on the weights of plants grown without Si. Si applied to plants grown without Cu also increased fresh as well as dry weight of both shoots and roots when compared to Cu–only treated plants.

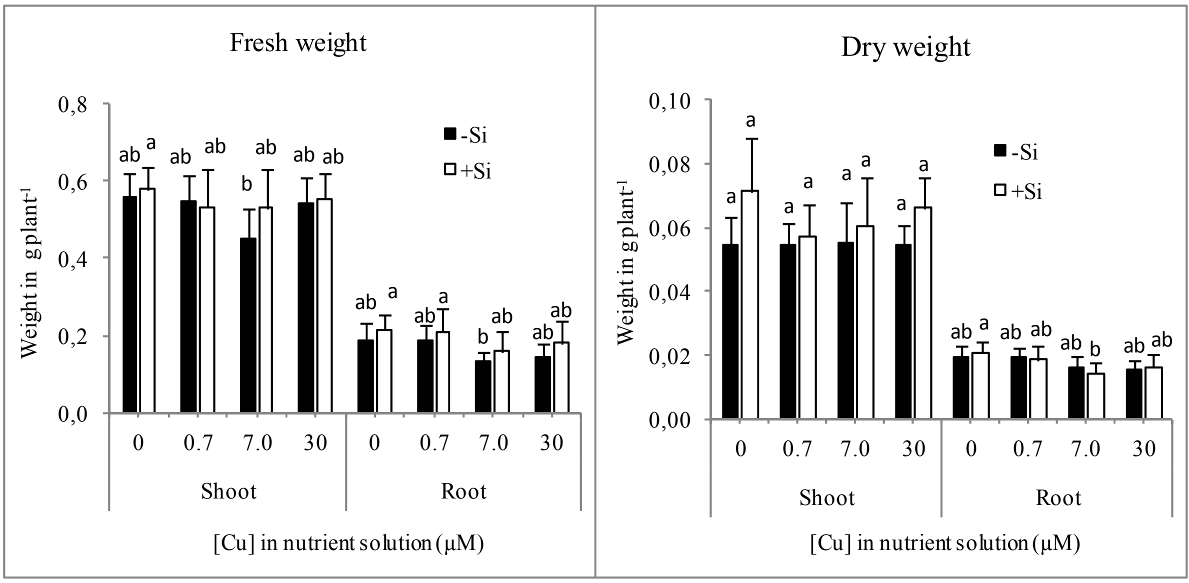

Figure 3. Fresh and dry weight of wheat plant shoots and roots grown in hydroponic conditions with increasing Cu concentrations (0, 0.7, 7.0 and 30 µM) treated or not with 1mM Si. Bars represent SD of seven replicates. Different letters indicate significant differences among the treatments at a $P < 0.05\%$ for shoot and roots separately.

II.3.2 Chlorophylls and carotenoids concentrations

No trend on chlorophyll a (Chl-a) and chlorophyll b (Chl-b) concentrations was observed with Cu treatments (Figure 4). Application of 1 mM Si significantly increased Chl-a, and carotenoids concentrations compared to respective Cu concentrations in all treatments except in Cu7.0 + Si treatment. Chl-a and Chl-b ratios increased in Cu treatments up to 7.0 µM Cu treated or not with Si and then decreased in 30 µM Cu treated plants both with and without Si. Interestingly, Chl-a/b ratios increased in all Cu + Si treated plant leaves as compared to Cu-only plants.

II.3.3 Silicon concentration in shoots and roots

Si-treated plants accumulated approximately 7 times more Si in the above-ground parts than in the below-ground parts and decreased up to 3 times with increasing Cu levels in solution

(Figure 5). Si concentration in shoots significantly decreased with increasing Cu levels in solution. In contrast to shoots, Si concentrations significantly increased in roots in all Cu levels except in the 0.7 µM Cu treatment. We also analyzed Si from shoots and roots without Si supply and the results showed that Si concentration was below quantification limit in all Cu-only treatments (data not shown).

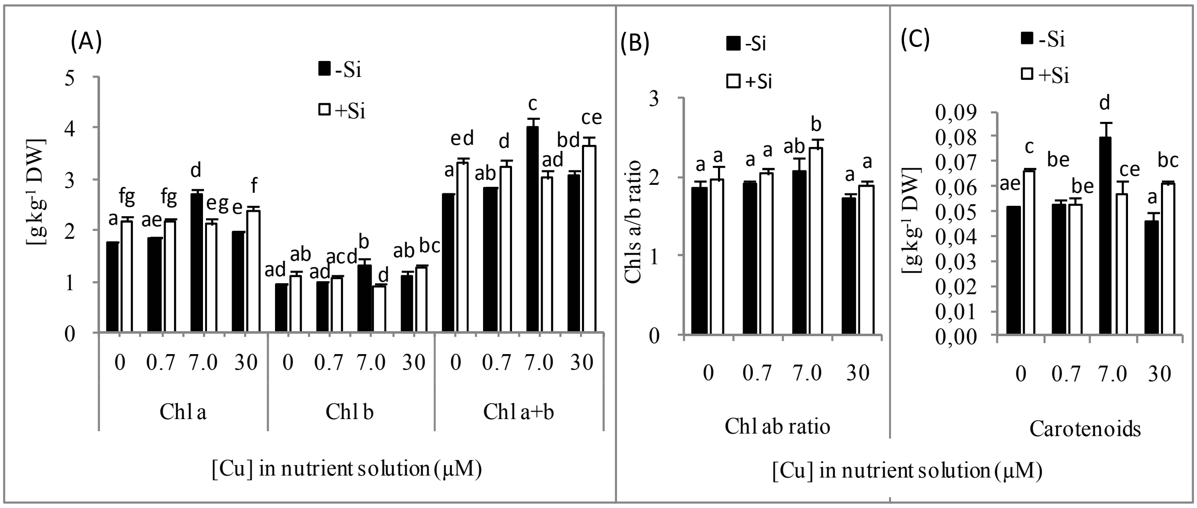

Figure 4. (A) Chlorophyll a and b concentrations in plant shoots. (B) Chlorophyll a/b ratio in plant shoots and (C) Carotenoids concentration in shoots. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments at a P < 0.05%.

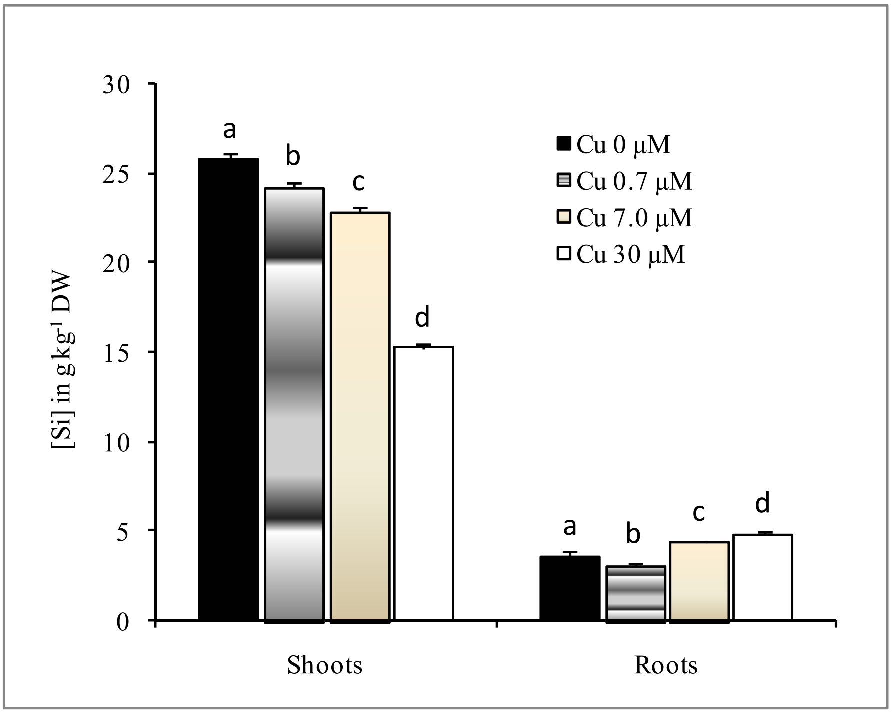

Figure 5. Silicon concentration in wheat plant shoots and roots (Si treated plants). Bars represent SD of three replicates. Different letters indicate significant differences among the treatments at a P < 0.05% for shoots and roots separately.

II.3.4 Micronutrient (Cu, Mn, Zn) concentrations in shoots and roots

Copper concentrations significantly increased in shoots with increasing Cu treatments (Figure 6). Si application significantly decreased Cu concentration in shoots in Cu30 + Si treatment compared with the same Cu treatment without Si. However, in lower Cu treatments Si only slightly increased or decreased Cu concentrations in shoots. As compared to control (0.7 µM Cu) treatment, increasing Cu concentration in the nutrient solution increased shoot Cu concentration from 16 and 53 percent in 7.0 and 30 µM Cu treatments respectively in non Si treated plants. In Si treated plants this increase in shoot was only about 9 and 5 percent in 7.0 and 30 µM Cu treatments respectively. Manganese and Zn concentrations increased up to 0.7 µM Cu treatments then significantly decreased in higher Cu (7.0 and 30 µM) treatments irrespective of Si application. Mn and Zn concentrations significantly decreased in shoots treated with Si as compared to shoots without Si except for the 7.0 µM Cu treatment (Figure 6).

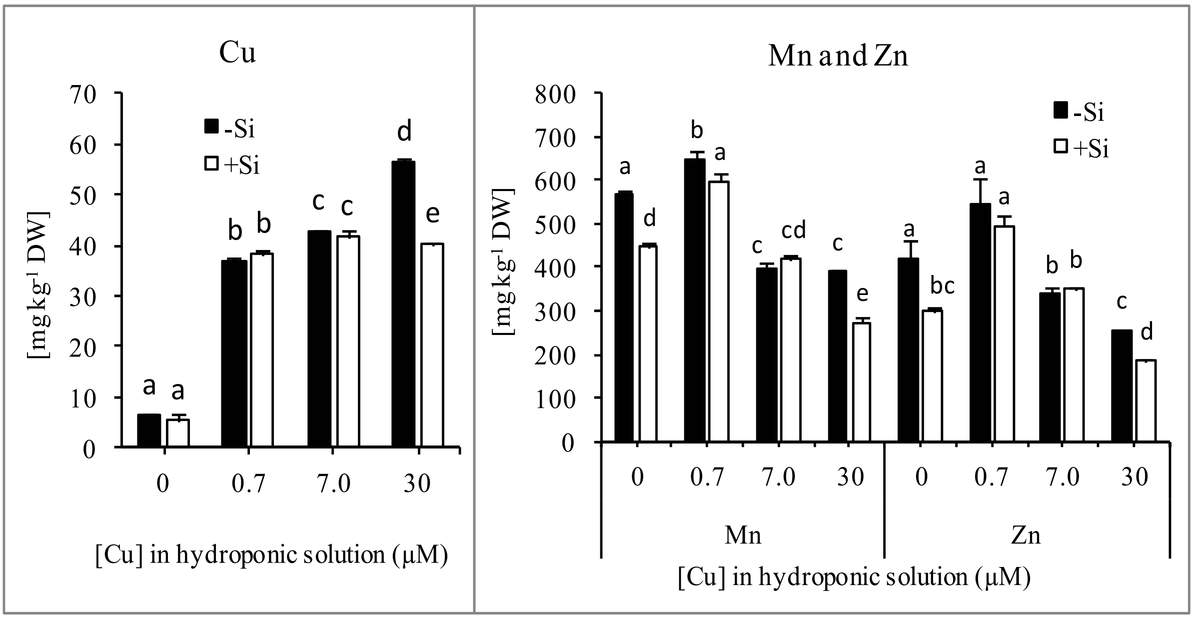

Figure 6. Cu, Mn and Zn concentrations in mg kg^{-1} DW in wheat shoots. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments at a $P < 0.05\%$.

As for roots, Cu concentrations significantly increased in acid wash (symplasmic Cu) roots with increased Cu levels in solution treated or not with Si (Figure 7). Si application significantly decreased Cu uptake by the roots when Cu was (30 µM) the highest in the nutrient solution as compared to the same treatment without Si supply. Except for the 7.0 µM Cu treatment, Cu concentration significantly decreased in roots treated with Si as compared to roots without Si. Mn and Zn concentrations significantly decreased in roots with increased Cu treatments irrespective of Si application. Zn concentration was lower in the +Si treatments except for the 0.7 µM Cu treatment. Silicon addition increased Mn concentrations than without Si addition in all treatments except for the 0 µM Cu treatment.

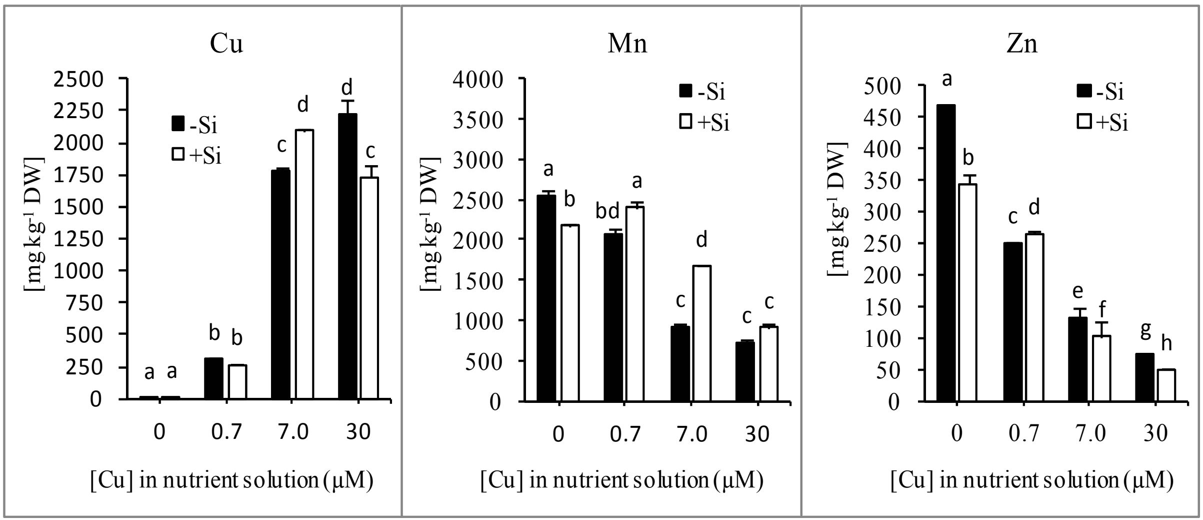

Figure 7. Cu, Mn and Zn concentrations in mg kg^{-1} DW in acid washed roots. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments at a $P < 0.05\%$.

Copper adsorption on the root surface increased with increasing Cu treatments (Figure 8). Copper adsorption was about 1.4, 21 and 37 percent of the total Cu uptake by the roots in 0.7, 7.0 and 30 µM Cu-only treatments respectively, while it increased to about 3, 14 and 42 percent in 0.7, 7.0 and 30 µM Cu + Si treatments respectively. This indicated that total proportion of adsorbed Cu increased of about 2 and 5 percent in 0.7 and 30 µM Cu + Si treatments as compared to the same treatments without Si while it decreased of about 7 percent in 7 µM Cu + Si treatment. This indicated that total Cu adsorption increased in the presence of Si. Silicon supply decreased Zn and Mn adsorption on root surface than Cu-only treatments except in roots with no Cu application in which we found opposite results (Figure 8).

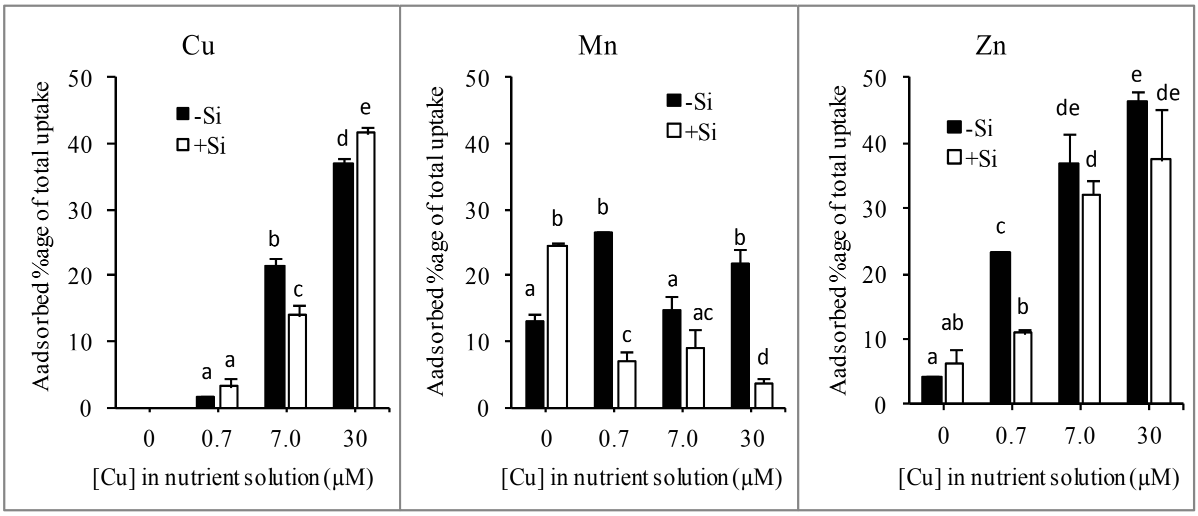

Figure 8. Apoplasmic adsorbed Cu, Mn and Zn concentrations in %age of the total uptake on dry weight bases. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments at a P < 0.05%.

II.3.5 Other minerals and organic compounds

Increasing Cu concentrations in the nutrient solution did not impact K, Ca, Mg and P concentrations in shoots, irrespective of Si addition (Figure 9). However, K, Mg and P concentrations in roots decreased with increasing Cu concentrations while Ca concentrations increased. Silicon neither modified these trends nor significantly changed macronutrient concentrations in both shoots and roots.

Concentrations of inorganic anions were larger in shoots than in roots (Figure 10). The general trend was a slight decrease in shoots and a significant decrease in roots of anions with increasing Cu concentration, except for chloride in roots, which showed an opposite trend, irrespective of Si addition. However, addition of Si increased anions concentrations in roots.

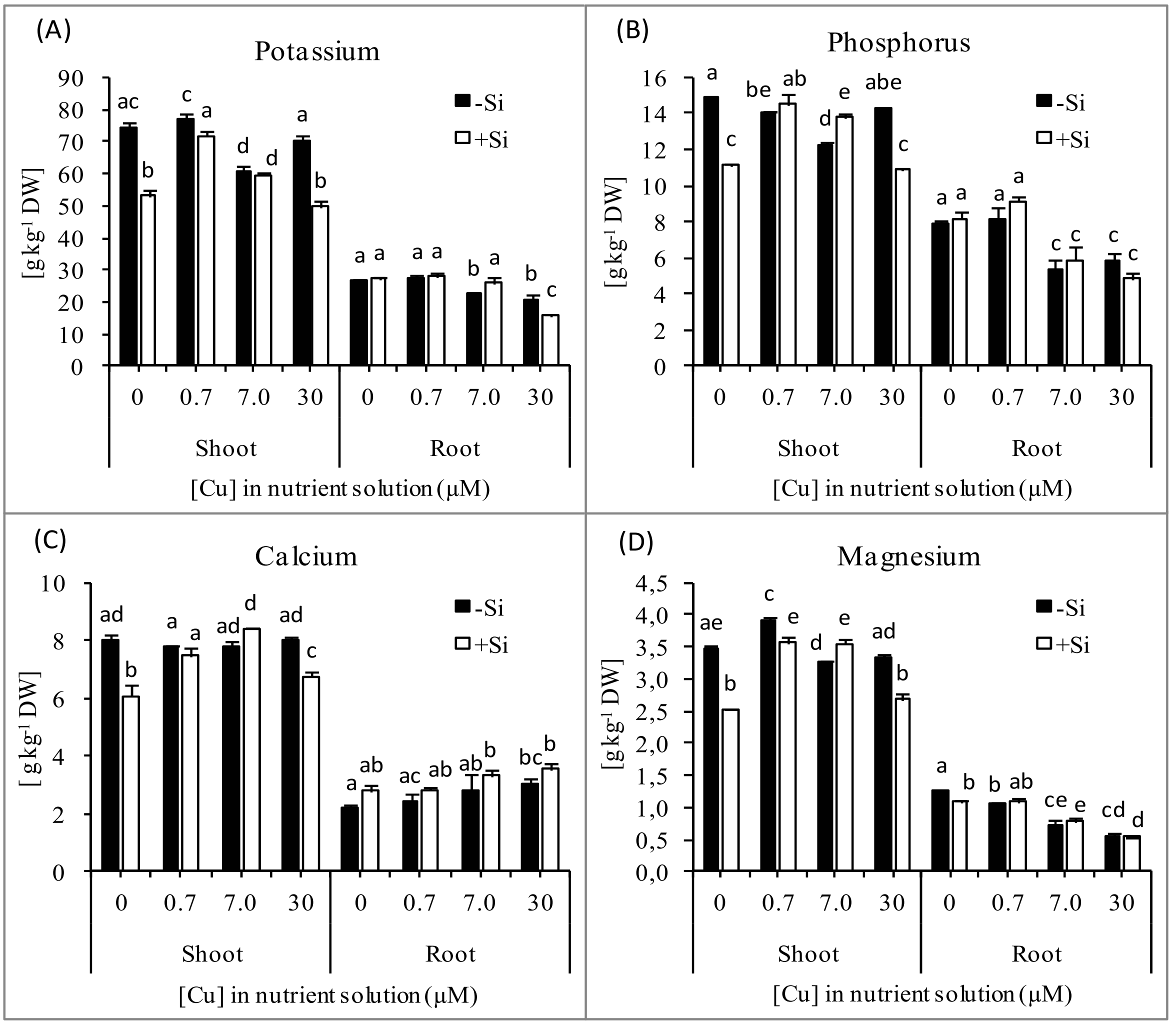

Figure 9. K, P, Ca and Mg concentrations (g kg^{-1}) in shoots and roots of wheat plants grown hydroponically with increasing Cu concentrations and treated or not with 1mM Si. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments at a $P < 0.05\%$ for shoots and roots separately.

Concentrations of malate, citrate and aconitate were larger in shoots than in roots and increased significantly in response to increasing Cu levels in solution only for citrate in roots and irrespective of Si addition (Figure 11). At highest Cu treatment, Si addition increased the concentration of these anions as compared to same treatment without Si. The most obvious effect of Si addition was an increase of aconitate concentration in roots as compared to the same treatments without Si.

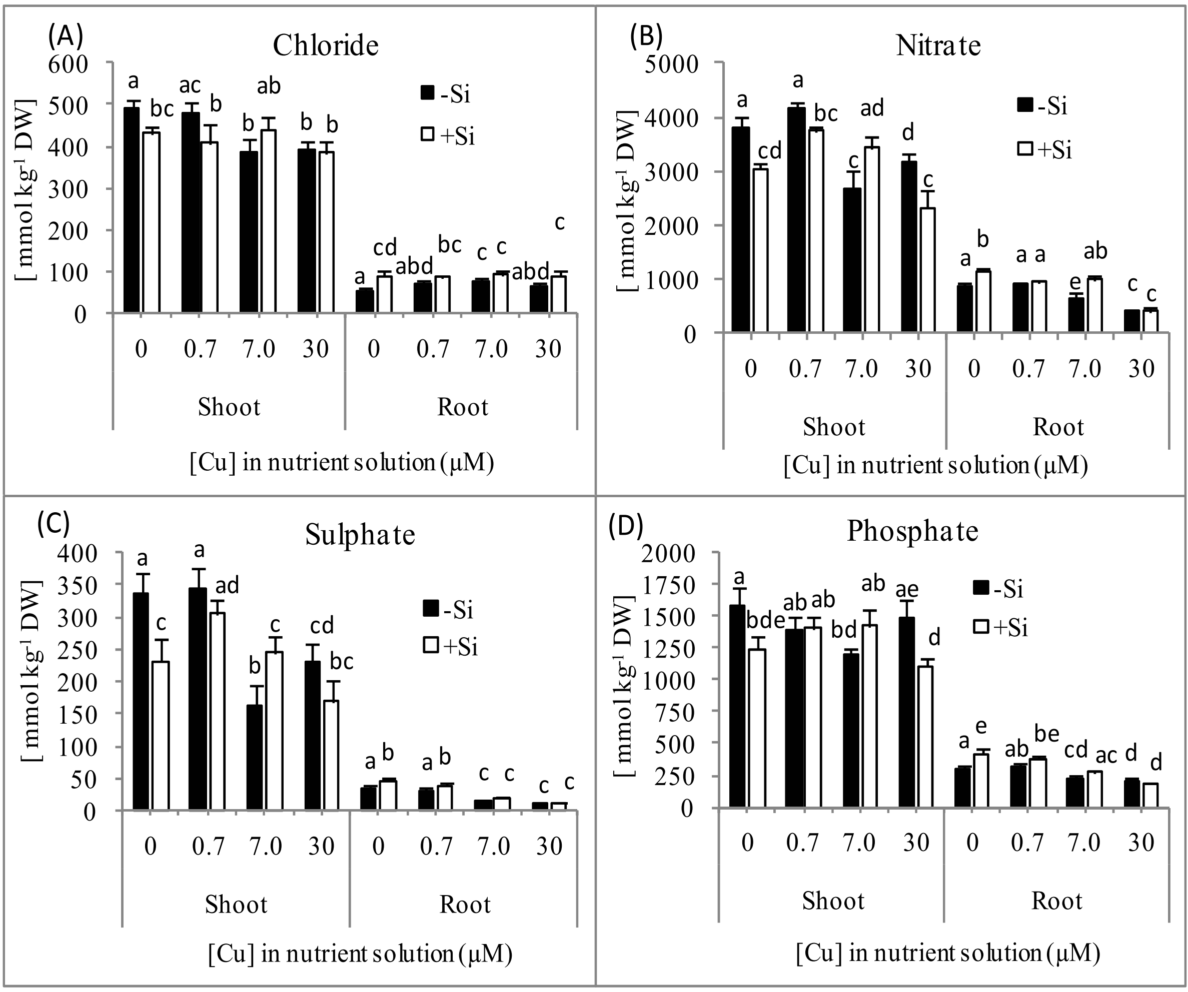

Figure 10. Inorganic anions (Cl, NO_3, SO_4 and PO_4) concentrations in plant shoots and roots. Bars represent SD of four replicates. Different letters indicate significant differences among the treatments at a $P < 0.05\%$ for shoots and roots separately.

II.3.6 Localization of elements in roots

Results of the 30 µM Cu + 0 mM Si treatment indicate that Cu is mainly accumulated in the central cylinder of the root together with K and Ca (Figure 12). Mn accumulates in the root epidermis but in lower extend. As expected Si concentration falls below the detection limit. Analysis of this root also shows that element distribution across a root is not symmetrical except K and Ca.

For the 30 µM Cu + 1 mM Si treatment, so by adding Si, the Cu distribution is modified (Figure 13). Cu is then found in the epidermis of the root but also in the center of the root with almost constant intensity. Si is found in the endodermis of the root. Mn presents the same pattern of distribution as Cu while in the absence of both Cu and Si, Mn was mainly found in the endodermis (Figure 15). The presence of Cu does not influence the distribution of Si, as for a wheat root grown in 0 µM Cu + 1 mM Si solution, Si is also found in the endodermis (Figure 14).

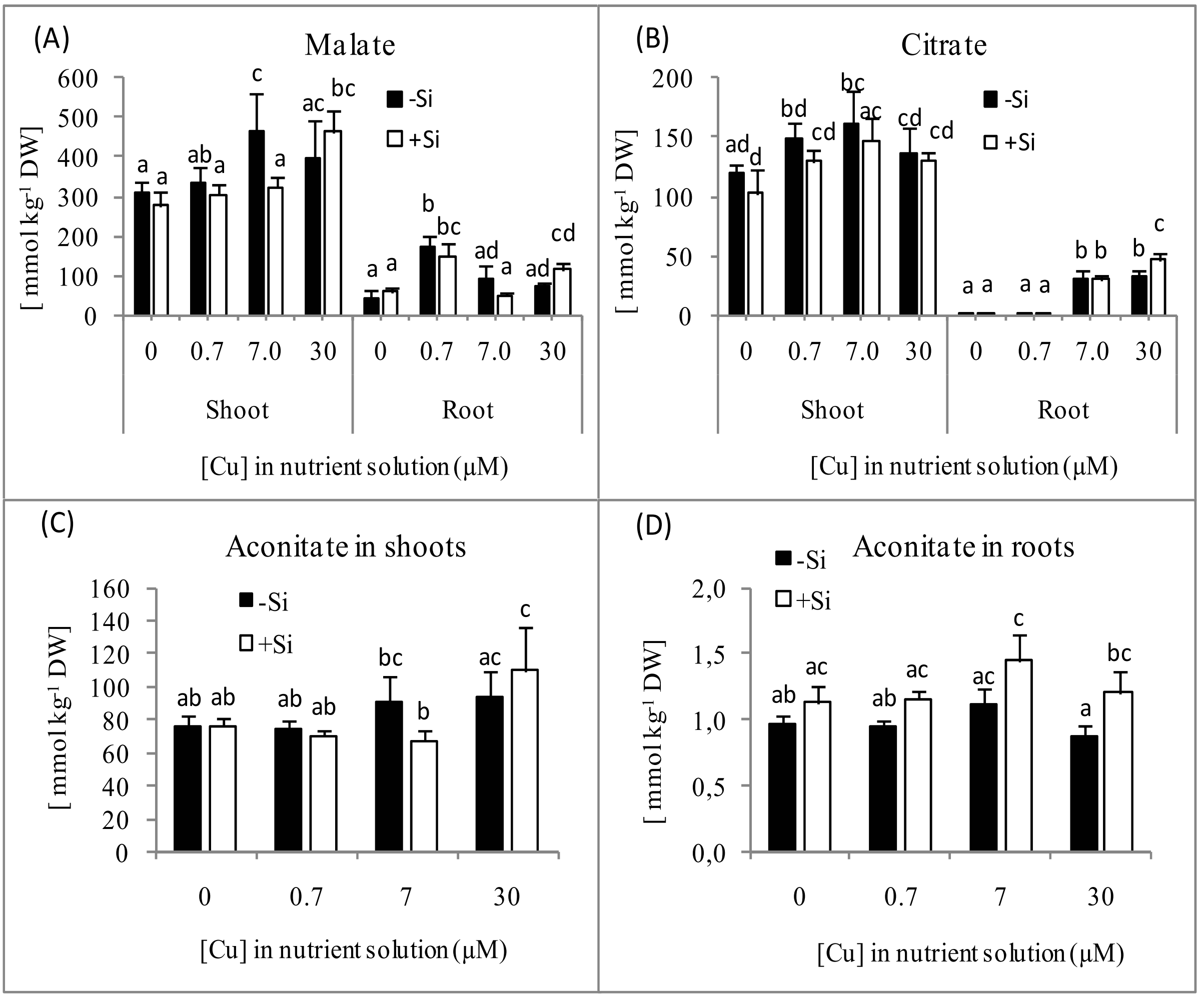

Figure 11. Organic anions (malate, citrate and aconitate) concentration in plant shoots and roots. Bars represent SD of four replicates. Different letters indicate significant differences among the treatments at a P < 0.05% for shoots and roots separately.

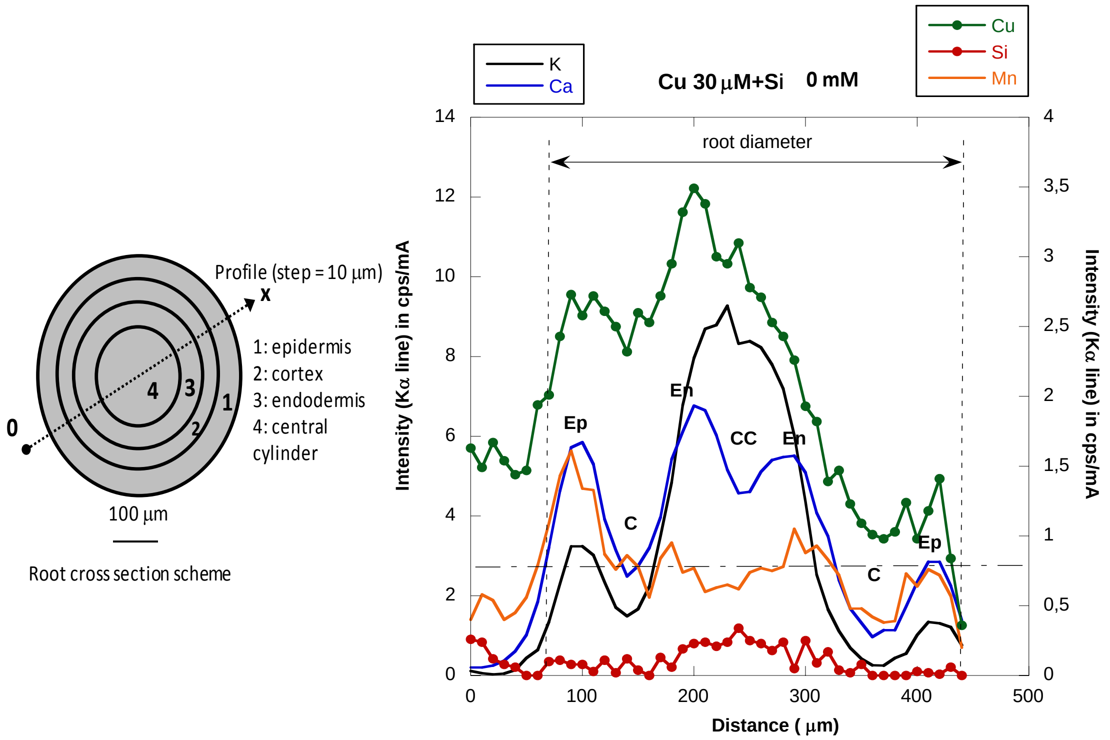

Figure 12. Distribution (µXRF) of elements in root containing 30µM Cu and 0mM Si in the nutrient solution. Ep : Epidermis, C: Cortex, En : Endodermis and CC for central cylinder.

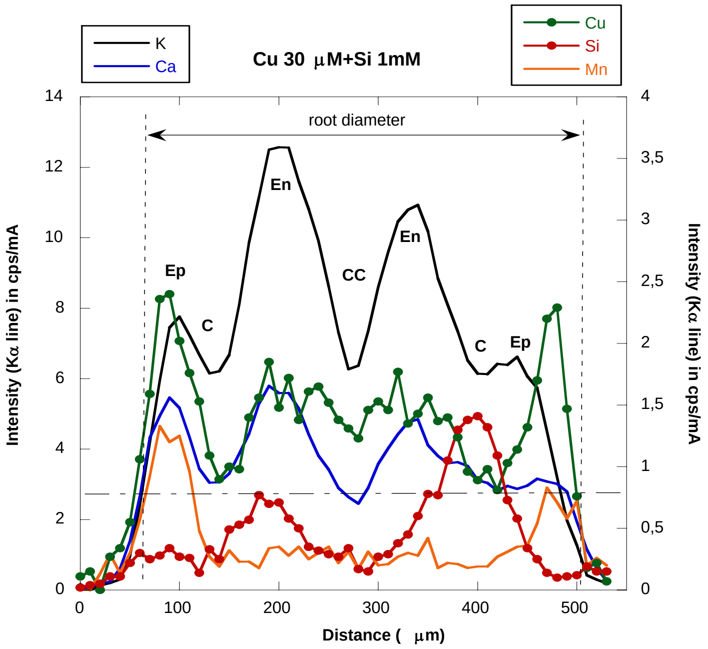

Figure 13. Distribution (µXRF) of elements in root containing 30 µM Cu and 1 mM Si in the nutrient solution. Ep : Epidermis, C: Cortex, En : Endodermis and CC for central cylinder.

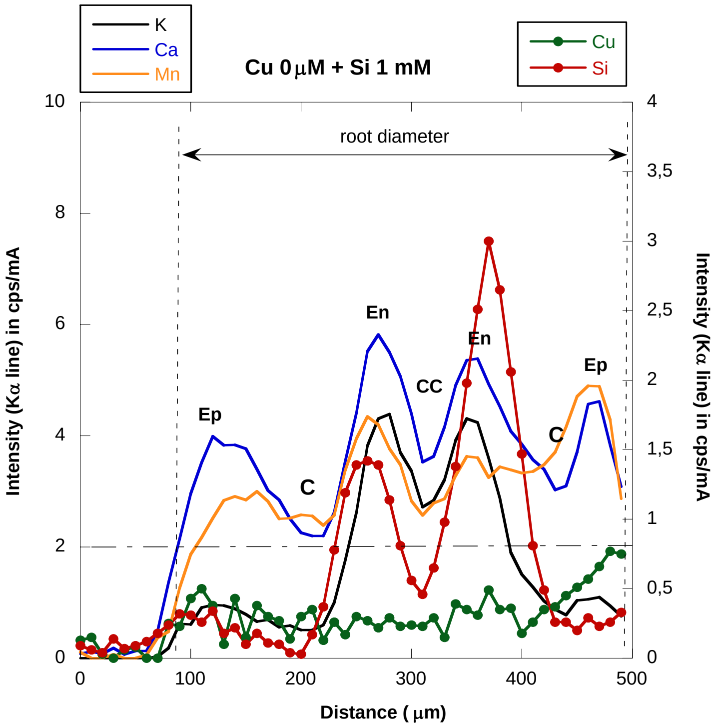

Figure 14. Distribution (µXRF) of elements in root containing 0 µM Cu and 1 mM Si in the nutrient solution. Ep : Epidermis, C: Cortex, En : Endodermis and CC for central cylinder.

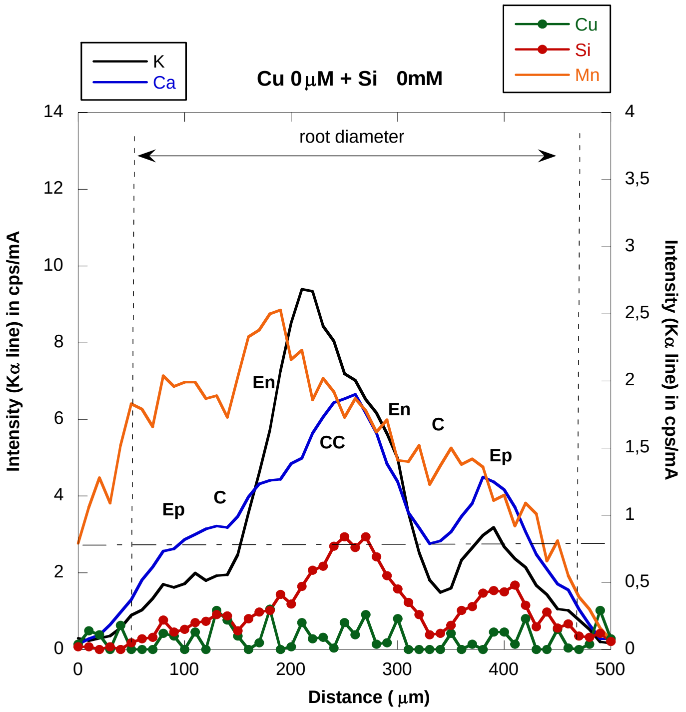

Figure 15. Distribution (µXRF) of elements in root containing 0µM Cu and 0mM Si in the nutrient solution. Ep : Epidermis, C: Cortex, En : Endodermis and CC for central cylinder.

II.4 Discussion

In our study, there was no reduction of fresh and dry weights as well as no visible symptoms of necrosis or chlorosis on the leaves under Cu deficiency or excess either with or without Si supplementation (Figures 1 and 3). This is supported by Quartacci, et al. (2001) who also found no necrosis or chlorosis symptoms on the leaves and roots of wheat plants grown in deficiency or excess Cu (50 µM) treatments. We found no visible symptoms of Cu deficiency in leaves when Cu was not applied in the nutrient solution. This may be due to the presence of small amount of Cu in leaves when plants were grown under Cu deficient conditions (Figure 6). Cu deficiency in plants normally occurs when plant Cu concentration is lower than 5 mg kg^{-1} dry weight of plant (Marschner, 1995). Cu in plants under deficient conditions may be due to the impurity in salts used as nutrients or due to the presence of Cu in seeds that plant efficiently utilized and as a result no toxicity symptoms were seen at this stage and may occurred in later stages of growth. We selected a maximum of 30 µM Cu on the basis of recent studies showing that 30 µM Cu in the nutrient solution was toxic to *Arabidopsis thaliana* showing reduced shoot fresh weight and chlorosis at the tips of older leaves (Li, et al., 2008). Similarly, Frantz, et al. (2011) found that 50 µM Cu in the nutrient solution significantly reduced stem and leaf dry weight and also displayed intervinal chlorosis in *Zinnia elegans* and *Antirrhinum majus* species. In addition, Ouzounidou et al. (1995) reported that fifty percent inhibition of maize root biomass occurred at 7.5 µM Cu in the nutrient solution. Bravin et al. (2010) also observed reduction in shoot biomass and intervinal chlorosis in durum wheat for $\{Cu^{2+}\}$ in solution equal or larger than 550 nM.

However, toxicity was primarily observed on roots. In our study, although fresh and dry weights of wheat plants did not significantly decreased, root length significantly decreased as Cu concentration increased in the nutrient solution (Figures 1, 2 and 3). These results are in agreement with previous studies indicating that Cu inhibited root length elongation of durum wheat (Quartacci et al., 2001; Michaud et al., 2008; Bravin et al., 2010) and maize (Ouzounidou et al., 1995), and with reports showing that roots are more sensitive to Cu stress than other plant parts (Nowakowski and Nowakowska, 1997; Yruela, 2005; Xu et al., 2006). This inhibitory effect of high Cu concentration on root length may be due to increased leakage of K^+ ions from roots and/or decrease in total lipid content of plasma membrane (Quartacci, et al., 2001). This reduction in root elongation may also be due to reduction in cell division and damage to root epidermal cells (Kahle, 1993; Ouzounidou et al., 1995) and/or due to increased rigidity of root cell wall in which Cu was accumulated as suggested by Kopittke and Menzies, (2006) for cowpea that may result in the increased number of poorly developed lateral roots (Kopittke and Menzies, 2006; Kopittke et al., 2007; Bravin et al., 2010).

As expected and in relation with the reduction of root length, Cu concentration in shoots and roots increased with Cu supply (Figures 6 and 7). Total Cu uptake (concentration × biomass) by the shoots and roots also increased with Cu treatments (data not shown). Larger Cu concentration was stored or immobilized in wheat roots and only a little fraction was translocated from roots to shoots. With increasing Cu concentration in the nutrient solution root to shoot translocation of Cu decreased as compared to control plants. These results confirmed previous studies showing that plants restrict Cu translocation towards their shoots as a possible mechanism of toxicity control. It also indicated that total root Cu concentration was more responsive to Cu contamination than shoot Cu concentration (Kopittke and Menzies, 2006; Michaud et al., 2007, 2008; Bravin et al., 2010).

Si supplementation partially increased the root length especially at the highest Cu (30 µM) treatment (Figures 1 and 2) as compared to the treatments without Si, indicating that Si partially alleviated Cu toxicity symptoms. This response was similar to that observed for other plant species (Li et al., 2008; Frantz et al., 2011; Khandekar and Leisner, 2011; Oliva et al., 2011). Si supply also slightly increased shoot biomass as compared to measured biomass in the treatments without Si (Figure 3). This increase in biomass may be partially associated with lower Cu concentrations in shoots in the presence of Si (Figure 6). Indeed, interestingly, Si supply significantly decreased Cu concentrations in both shoots and roots especially when Cu was highest in the nutrient solution. These results agree with the study of Nowakowski and Nowakowska, (1997), which showed that the addition of 0.4 mmol L^{-1} Si significantly decreased Cu content in both shoots and roots of 7-day-old wheat seedlings. Our results, however, do not agree with the results of Li et al. (2008) who found no effect of Si on Cu concentrations of *Arabidopsis thaliana* shoots grown in 30 µM Cu + 1.5 mM Si solution for three days. Similarly, Collin et al. (2012) reported that Cu concentration in the aerial parts of bamboo plant was not affected by Si supplementation. This contradiction might result from differences among plant species in response to metal stress and also because *Arabidopsis* is not a Si-accumulating plant.

Copper treatments did not affect the chlorophyll a and chlorophyll b concentrations while slightly decreased chlorophyll a/b ratio and carotenoid concentration in durum wheat leaves (Figure 4), although Cu concentrations in shoots did not increase much and no visible symptoms were observed indicating that these parameters may give early signal of Cu toxicity in wheat. This decrease in photosynthetic pigments may be due to Cu induced changes in fluidity of PSII-enriched membranes in wheat plants (Quartacci et al., 2000). Similarly, Vinit-Dunand et al. (2002) suggested that in cucumber plants decline in photosynthesis is probably a consequence of an altered source-sink relationship, rather than due to a toxic effect of copper on photosynthesis. This decrease in photosynthetic pigments can also be explained by the fact that the structure of chlorophylls contains a central magnesium ion and this magnesium ion may be replaced by Cu (Küpper et al., 1998). The addition of Si significantly increased the concentration of these photosynthetic pigments compared to –Si under Cu stress (Figure 4) that may be due to Si-mediated protection of chloroplast ultra-structure as proposed for cucumber plants (Feng et al., 2010). These higher values of photosynthetic pigments with Si application under Cu stress indicated that Si might be linked to protective mechanisms that help in the maintenance of photosynthetic machinery. The increase in leaf carotenoid concentration in the presence of Si may also be helpful for plants. In general, carotenoids have two important functions in plant body first, they can contribute in photosynthesis by transferring a part of the light energy absorbed by the carotenoids to chlorophylls and secondly they also provide additional protection to photosynthetic apparatus from oxidative damage (Young et al., 1991; Choudhry and Behera, 2001).

Cu treatments changed the distribution of Si in wheat plants (Figure 5). Shoots were the main sites for Si accumulation as expected for a Si-accumulating plant. Oliva et al. (2011) found that Si was accumulated in the roots of *E. andevalensis*, whereas in the Si-accumulating bamboo, Si was primarily accumulated in the leaves (Collin et al., submitted). However, Si concentration significantly decreased in shoots and significantly increased in roots with increasing Cu treatments. These results are in agreement with the results of Frantz et al. (2011) who found that Si concentration in leaves decreased in Si-accumulator species as Cu supply increased. But contrary to us, they found no significant change in root Si concentration. Although Si is able to alleviate Cu stress, the simultaneous effect of Cu on Si accumulation may thus limit Si efficiency if soluble Si in plant supposed to be the active Si pool in plant and able to modify Cu homeostasis is proportional to total Si in plant. Si accumulation differs between plant species and also between Si-accumulating and non-accumulating plants (Ma and Yamaji, 2006; Frantz et al., 2011), which may explain that our results diverge from the literature.

Our results indicated that Si concentration significantly increased in roots in the presence of Cu as compared to controls (Figure 5). We thus made the hypothesis that this higher Si deposition in roots may sequestrate Cu in roots and decrease translocation into shoots: Si supply increased the root adsorbed portion of the total Cu uptake especially when Cu concentration was the highest in the nutrient solution (Figures 7 and 8). This indicates that the effect of Si occurred very early in the process of Cu uptake, translocation and accumulation in the plants. In that case Si might increase Cu binding to cell wall, thereby lowering symplasmic Cu concentration. This mechanism has been proposed for the alleviation of Mn toxicity in cucumber plants (Rogalla and Romheld, 2002). Collin et al. (submitted) also found this type of mechanism in bamboo roots under Cu stress but contrary to our results they found that Cu content in bamboo plants was not affected by Si supplementation in the nutrient solution. By contrast, Vaculık et al., (2012) reported that in maize plants treated with low (34 nM) level of Cd, Si did not affect the distribution of apoplasmic and symplasmic Cd in roots.

Localization of elements in the roots cross-section provides further understanding of metal tolerance mechanism. We analyzed all the root cross-sections at about the same distance from the bottom of shoots. µXRF results showed that in the presence of Si, Cu and Mn were mainly localized in the vicinity of root epidermis and Si was deposited in the vicinity of root endodermis (Figure 13). On the contrary, in absence of Si, Cu was mainly localized in the central cylinder of root (Figure 12) and in the absence of Cu, Si was localized in the endodermis of root (Figure 14). These results strongly suggested that Si is decreasing Cu toxicity in wheat plant by inducing Cu deposition in the epidermis of roots. This is in accordance with the desorption results which indicated a larger percentage of adsorbed Cu in the Si-treated plants. Cu toxicity did not change the Si distribution as it is present in the vicinity of endodermis without or with Cu addition. Larger Si concentration was not detected in the vicinity of Cu, dismissing for wheat and Cu Wang et al. (2004)'s suggestion that the formation of Al-Si complexes was a tolerance mechanism in maize roots. These results also disagree with the view of Wang et al. (2000) who suggested that Si may reduce Cd in symplasm and promote Cd and Si co-precipitation in the root cell walls of rice plants.

However, this heavy deposition of Si in the vicinity of endodermis in the presence of Cu may reduce the cell wall porosity of inner root tissues particularly that of endodermis as suggested for Cd tolerance in rice (Shi et al., 2005) and maize (da Cunha and do Nascimento, 2009). Si may block the apoplastic transport of Cu by inducing larger Cu deposition in the vicinity of root epidermis.

While low level of Cu (0.7 μM) increased Mn and Zn concentrations in wheat shoots indicating a beneficial effect of Cu on essential microelements when Cu concentration in the nutrient medium was optimum (Figure 6). Increase in Mn and Zn concentrations in shoots was also observed in *Vigna radiata* under 50 mg Cu kg^{-1} soil as compared to plants without Cu treatment (Manivasagaperumal et al., 2011). Kumar et al. (2009) also reported that in wheat shoots, Mn concentration increased while Zn concentration was not affected under 1 mg Cu kg^{-1} soil as compared to lower Cu treatments. Mn and Zn concentrations significantly decreased in wheat shoots and roots at larger Cu treatments (Figures 6 and 7). Excess Cu induced Mn and Zn deficiency has also been reported in *Vigna radiata* (Manivasagaperumal et al., 2011) and in wheat (Kumar et al., 2009). This decrease in metal ions may be due to competition for the same membrane transports during the uptake and translocation in plants (Loneragon and Webb, 1993; Pearson et al., 1996). Addition of Si differentially affected the concentration of these elements. Adsorbed Mn and Zn concentrations on roots were lower in the presence of Si than without Si in all Cu treatments except in treatment without Cu in the nutrient solution (Figure 8). These results indicated that Si may alleviate the excess of Cu by selectively increasing the absorption of other essential metals.

In present study, low level of Cu (0.7 μM) slightly increased the K and Mg concentrations while P and Ca concentrations was not much affected in wheat shoots but excess Cu reduced the uptake and transport of macro nutrient like K, P and Mg and increased uptake of Ca in roots (Figure 9). Manivasagaperumal et al. (2011) also reported that lower Cu concentrations in the soil increased the macronutrients concentrations in plant shoots. Decline in the concentration of K, P and Mg at higher Cu treatments is in agreement with the previous reports that excess Cu decreased these nutrients in *Vigna radiata* (Manivasagaperumal et al., 2011), cowpea (*Vigna unguiculata*) (Kopittke and Menzies, 2006) and maize (Ouzounidou et al., 1995). However, increase in root Ca concentration disagrees with the above reports. This reduced uptake of these elements may be due to roots ruptured and due to the tearing and separation of the epidermis and outer cortex from the underlying tissues under Cu stress (Kopittke et al., 2009). Si application differentially influenced the concentration of these elements under Cu deficiency or stress. Interestingly, addition of 1mM Si supply slightly increased the root Ca concentration in all Cu treatments as compared to Cu treatments without Si supply. Ca is an element with an antagonistic effect on Cu uptake (Marschner, 1995). Recently, Zorrig et al. (2012) reported that presence of Ca in the culture medium reduced the Cd uptake in lettuce plants. Therefore, larger Ca concentration in roots in the presence of Si might contribute to reduce Cu in shoots.

Our results showed that Cu treatments generally decreased the concentration of inorganic anions in roots except chloride concentration in roots and application of Si, in general, increased the concentration of these anions in roots (Figure 10) that may complex Cu and sequestrate it into metabolically inactive parts such as vacuole. Si supply also generally decreased the concentration of organic anions in shoots while it increased organic anions in roots at highest Cu stress (Figure 11). This decrease in organic anions concentration in shoots may be due to compartmentalization of Cu into metabolically inactive parts of shoots. Vaculik et al. (2012) reported that Si application considerably decreased symplasmic and increased apoplasmic concentration of Cd in maize shoots. This decrease in anions concentration in shoots in the presence of Si may also be due to Si deposition, as phytoliths, in cell walls resulting additional Cu binding sites. In this way, phytoliths may reduce the high level of Cu in living cell which may reduce Cu stress in shoots consequently reducing the anions production in shoots. This hypothesis is based on the results of Buján, (2012) who suggested that phytoliths contain heavy metals including Cu and functions as heavy metal alleviation in plants. The co-deposition of Si-Zn in leaf epidermal cell walls has also been suggested as Zn tolerance mechanism in *Cardaminopsis halleri* (Neumann and zur Nieden, 2001). Similarly, Oliva et al. (2011) proposed that in *E. andevalensis* shoots, formation of phytoliths containing Cu might sequestrate the metal. Collin et al., (2011) suggested that in roots of bamboo plants higher concentration of malate under Cu stress may form coordinate with Cu. Similarly, Callahan et al. (2006) reviewed that carboxylic acids may also bind Cu and thus contribute to its sequestration into vacuoles or act as long distance transport. In the present study, higher concentration of carboxylic acids in roots in the presence of Si under Cu stress may sequestrate Cu to metabolically inactive parts in roots such as cell walls.

II.5 Conclusion

In conclusion, this study provides evidences for Si mediated alleviation of Cu toxicity in durum wheat. It reduces Cu toxicity by decreasing Cu uptake and translocation, by increasing uptake and translocation of Mn and Zn and by regulating Cu binding molecules such as malate, citrate and aconitate thereby reducing the amount of active Cu. As a consequence, Si supplementation has a positive effect on root length and photosynthetic pigments that seem to be an early signal for Cu toxicity.

It is demonstrated that Si limits Cu toxicity in wheat plants by acting or inducing mechanisms mainly at the root level. We propose a two-step mechanism at the root level: increased Cu adsorption onto the root and immobilization in the vicinity of root epidermis and increased Cu complexation by both inorganic and organic anions such as aconitate and, a resulting reduced root-shoot Cu translocation. The present results suggest that Si might be useful to enhance Cu tolerance in durum wheat grown in Cu contaminated soils.

III. Effect of various sources of silicon on alleviation of cadmium and copper toxicity in durum wheat (*Triticum turgidum* L. cv. Claudio W.)

Abstract

A pot experiment was conducted to evaluate the capacity of different minerals to release bioavailable silicon for decreasing cadmium (Cd) and copper (Cu) toxicity in wheat plants. For this purpose we used three types of minerals: quartz, clay (vermiculite) and amorphous silica (diatomite). Minerals were mixed according to different proportions in weight. Wheat plants were grown for 60 days in these mineral mixtures without and with 2 and 20 ppm Cd and Cu respectively. After harvesting, mineral mixtures were analysed for Cd, Cu and pH and wheat plants were analyzed for Cd, Cu and silicon. Plant Si concentrations increased with increasing amorphous silica and clay proportions which positively affected plant biomass and reduced the Cd and Cu toxicity in wheat plants. Increase of plant Si with increasing clay proportions demonstrated that clays can also be a source of Si to plants in soils containing high clay contents. We conclude that significant plant-available Si and immobilization of Cd and Cu in mineral mixtures and sequestration of metals in roots seems to reduce heavy metal toxicity in durum wheat. Moreover, increase in pH in Q+C (Quartz + Clay) group seems to play role in immobilization of metals in soil.

Key words: wheat, cadmium, copper, silicon, bioavailability, amorphous silica, clay

The results presented in this section have been prepared for submission as a short communication in “Chemosphere”

III.1 Introduction

Cadmium (Cd) in agricultural soils is generally derived from industrial processes and mining activities and mainly from the use of sewage sludge and phosphate fertilizers (Nagajyoti et al., 2010; Wuana and Okieimen, 2011). This Cd is extremely toxic to plants and inhibits plant growth and even causes plant death by disturbing the uptake of nutrients and inhibiting photosynthesis via degradation of chlorophyll (Gussarson et al., 1996; Sandalio et al., 2001). Cd concentrations in grains correlate both with Cd concentrations in shoots during the vegetative phase and translocation rate from roots to shoots both in wheat and rice (Greger and Löfstedt, 2004; Liu et al., 2007). On the other hand, Copper (Cu) is a micronutrient but larger concentration causes contamination to agricultural soils at large scale due to repeated application of Cu-containing fertilizers, fungicides, and farm manures as well as waste disposal in agricultural soil. Higher concentrations of Cu in soils adversely affect crop yield, and in severe cases lead to soil degradation and erosion (Adriano, 2001).

Heavy metals, including Cu and Cd, absorption by plants is reduced in a number of ways such as selecting metal-excluding plants (Wei et al., 2005; Wenzel et al., 2003), using soil amendments (e.g. Keller et al., 2005) and/or by proper plant fertilisation (Sarwar et al., 2010). However, these actions are costly and not environment friendly. Therefore adoption of new technologies which resolve both environment and economic problems are necessary for the reduction of metal toxicity in plants.

Silicon, however, has not been considered as an essential element for higher plants, but has been proved to be beneficial for the growth and development of many plants (Epstein, 1994). The use of Si to reduce the toxic effects of heavy metals has increased in various parts of the world and there has been increasing interest in the use of silicon for most of agricultural crops. Various beneficial effects of Si have been identified in higher plants (Epstein, 1999). Silicon significantly reduced the toxic effect of Cu in wheat seedlings by reducing Cu absorption from solution (Nowakowski and Nowakowska, 1997). Si application also decreased the toxic effects of Cd in some plants species (Vaculik et al., 2009; Feng et al., 2010). Man ignored exogenous application of Si with the belief that the soil itself can sustain its supply but most recently Guntzer et al. (2012) showed that continuous cropping and removal of wheat straw reduced the bioavailable Si from the soil. On the other hand, the silica that occurs in soil is in an unavailable polymerized form and for its absorption by plants it has to be depolymerized for plant availability.

The question of Si bioavailability in soil is still not well understood. Silicon is absorbed in the form of silicic acid (H_4SiO_4) by plants (Ding et al., 2005), but most sources of silica are not available for plants (Savant et al., 1997). External sources of Si include calcium and magnesium silicates, silicate slag, dolomite and rock phosphate but these contain only a small portion of available silicon (Savant et al., 1999). An important consideration is that these silicate sources which are derived from industrial by-product often contain high level of heavy metals (Berthelsen et al., 2001) those are highly toxic to plants. Based upon the above consideration we need a pure source of silicon with high solubility.

Both under a tropical and a temperate forest, phytoliths have been shown to be the first pool of Si for plants (Alexandre et al., 1997; Ding et al., 2008). Indeed, Fraysse et al. (2006; 2009) demonstrated that phytoliths dissolve faster than other silicates at pH greater than 4. Therefore, in natural ecosystems, soil phytoliths can be a major source of bioavailable Si for plants (Farmer et al., 2005). However, there is no source of easily accessible phytoliths that could be used as fertilizer and little is known about the ability of different minerals to release plant available silicon. In addition, its effect on Cd and Cu toxicity in durum wheat plant despite the fact that durum wheat cultivars accumulate more Cd in grains than that of bread wheat which may be due to larger root to shoot translocation of Cd in durum wheat (Meyer et al., 1982; Greger and Lofstedt, 2004).

As we have seen in 1st and 2nd part of this chapter that Si could alleviate the toxic effects of Cd and Cu in durum wheat. Therefore, we need to evaluate the efficacy of different minerals to release plant available silicon so that successful soil experiments can be formed. The purpose of the trial presented here is to understand the efficacy of different minerals to release the plant available silicon and the effect of this soluble silicon on reducing Cd and Cu toxicity in durum wheat plant. For this purpose we chose tree type of minerals, quartz, clay (vermiculite) and amorphous silica under the form of diatomite as they are known to represent low, medium and high weathering rates (Fraysse, et al., 2009). Diatomite can be mined and may be considered as an analog for phytoliths. Minerals were mixed with different proportions by weight and durum wheat (*Triticum turgidum* L.) was grown without and with addition of Cd and Cu.

III.2 Materials and methods

III.2.a Materials

In this study three types of minerals were selected: quartz, clay (natural ground vermiculite) and diatomite (Clarcel 78, CECA S.A. from Saint-Bauzile France). Diatomite was used because it contains mostly amorphous silica as diatom skeletons. Vermiculite represents a common Si bearing mineral in temperate soils. Quartz is supposed to dissolve slowly enough not to be able to release significant amount of phytoavailable Si. Plant species tested was durum wheat (*Triticum turgidum* L. cv. Claudio W.) as it is cultivated in Southern France. X-ray diffraction (start angle=4°, end angle=80°, step size=0.033°, time of step=4s, PANalytical X' pert PRO MPD) performed on initial mineral samples ground to fine powder confirms that the quartz has a high degree of purity, while smectite and quartz was detected in the diatomite sample, and other clay minerals have been detected in the vermiculite sample (See annex 3 figure 1).

III.2.b Experimental Protocol

Durum wheat was grown in a growth room under a short-day cycle (8/16 h 23°C/20°C day/night), 70% humidity and 450 µmol photons $m^{-2}S^{-1}$ light intensity. There were two main sets of pots, one set was quartz (Q) mixed with clay (C) and second was quartz mixed with diatomite (D). Minerals were mixed according to different proportions in weight: [75% (Q) + 25% (C or D)], [85% + 15%], and [95% + 5%]. For each proportion of either D or C, there were three different groups for metal treatments: first group as a control (pots without adding heavy metals), second group was treated with 2 mg kg^{-1} Cd as $Cd(NO_3)_2$. $4H_2O$ and the third group was treated with 20 mg kg^{-1} Cu as $CuSO_4$. $5H_2O$. In order to avoid heterogenous distribution of the heavy metals added at such a small rate, $Cd(NO_3)_2.4H_2O$ and $CuSO_4$. $5H_2O$ were first dissolved in 50 ml water separately and then mixed thoroughly with the material. Initial pH of these groups and proportions of minerals was about 8.8 and 5.5 for Q+C and Q+D groups respectively (See annex 3 Table 1). 400 ml-plastic pots were prepared each with 300 g of the mineral mixtures and were tested with three replicates with plants and two replicates without plant growth. Initially pots were seeded (immediately after mixing with Cd and Cu) at a density of six seeds per pot and were thinned to four individuals per pot, five days after germination. Each pot was fertilized with a 20 ml mixture solution containing 130 mg L^{-1} N (as NH_4NO_3), 130 mg L^{-1} P (as Ca $(H_2PO_4)_2$, 180 mg L^{-1} K (as 50% KCl and 50% K_2SO_4) and 40 mg L^{-1} Mg (as $MgSO_4$. $7H_2O$). Pots were regularly watered at about 80% of field capacity and randomly rotated. Weeds were removed regularly when present. All plastic and glass wares were rinsed with 10% HNO_3.

III.2.c Plant sampling and analysis

Plants were harvested after 60 days by cutting the shoots approximately one centimeter above the soil surface to remove the roots more easily. Plant samples were washed with distilled water and oven dried at 70°C till constant weight. Roots were separated from soil and first rinsed with distilled water then with 0.02 M ethylenediaminetetraacetic acid (EDTA) solution then again three times with distilled water and oven dried at 80°C till constant weight. Total dry weights of the roots and shoots were measured. All shoots and roots samples were ground to smaller size. For the determination of Cd and Cu, finely ground plant (above or underground parts) samples (0.25g) were digested in 4 mL of concentrated HNO_3 70% (Fluka, Buchs, Switzerland) in a block of mineralization with a rise in temperature up to 95°C for 6 hours then filtered with 0.22 µm membrane before analysis (modified from Keller at al., 2003). Si concentration in plant shoots and roots was extracted using the tiron extraction method (Guntzer et al., 2010).

III.2.d Substrate sampling and analysis

Substrate samples were collected at the beginning (just after mixing) and at the end of the experiment. The samples were oven dried at 40°C until constant weight. All substrate samples (initial and final) were analyzed for pH (1:2.5 soil to water ratio). DTPA-TEA extraction (0.005 M DTPA, 0.1 M TEA and 0.01 M $CaCl_2$ at pH 7.3; substrate/solution ratio was 2/20; horizontal shaker 120 min, centrifugation and filtration by a filter of porosity 0.22 µm cellulose acetate membrane), as proposed by Lindsay and Norvell (1978) modified, was performed on samples to assess the metal DTPA-extractable fraction from the substrate samples.

Cadmium, copper and Si in the substrate extractions and plant digests were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES Jobin-Yvon, Ultima-C).

Statistical treatment

Student's t-test was performed to test whether the average concentrations in the treated pots differed from those of controlled pots. Results that were significantly different were marked as (*) for $p \leq 0.05$, (**) for $p \leq 0.01$ and (***) for $p \leq 0.001$.

III.3 Results

III.3.a Effect of substrate on plant parameters

Plant biomass

Dry weights of both shoots and roots in quartz + diatomite (Q+D) group were larger than those of quartz + clay (Q+C) group and increased with increasing diatomite proportion (Figure 1 and 2). However, dry weights in Q+C group almost remained constant or slightly increased. Addition of Cd and Cu in the substrate slightly decreased the shoots and roots dry weight compared to control especially in Q+C group with the least amount of clay. With increasing the proportion of clay or diatomite the roots dry weight significantly increased in Cd and Cu treatments but not in control.

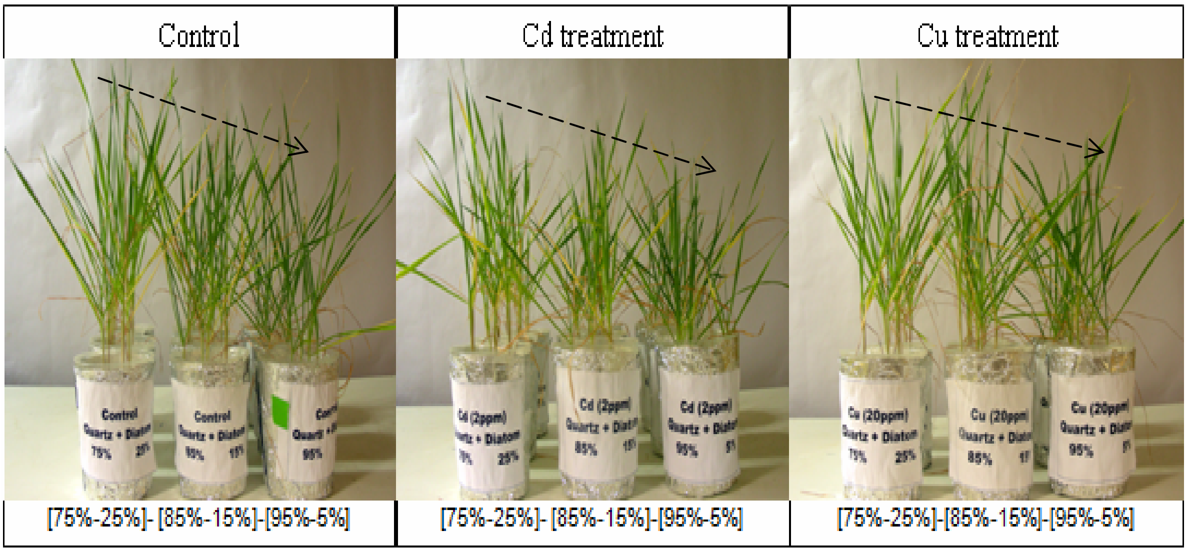

Figure 1. Wheat plants grown in pots from Q+D set of minerals. The effect of increasing doses of ASi on wheat growth can be seen in differences of the length of the above ground parts in each treatment, especially between the 75%Q+25%D and 95%Q+5%D treatment.

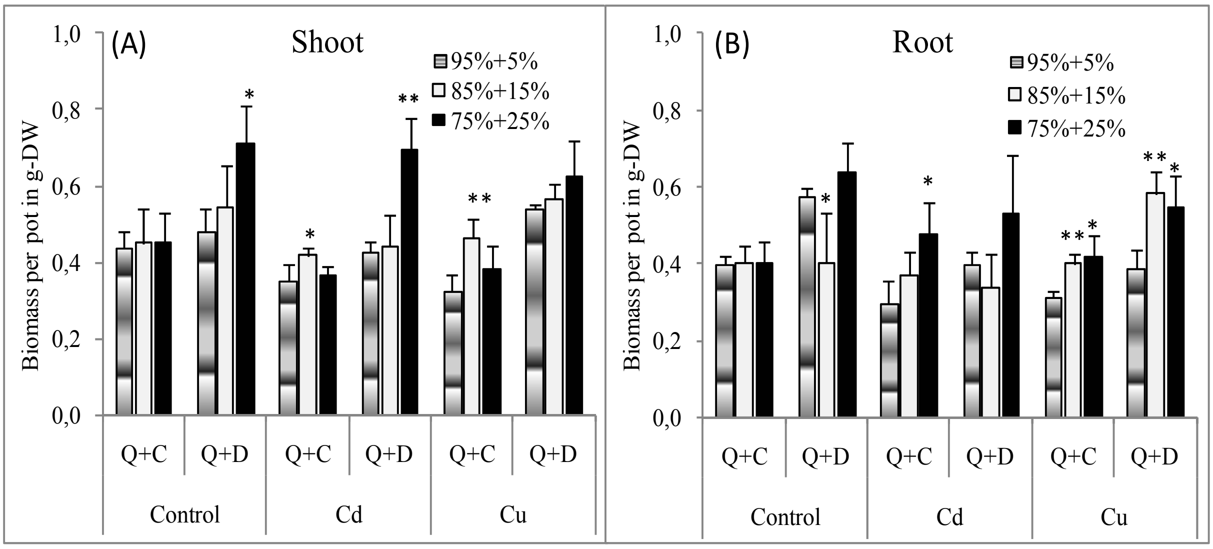

Figure 2. Dry weight of shoot and root of wheat plant grown in a mixture of minerals and treated without and with 2 and 20 ppm Cd and Cu respectively. Bars represent SD of three replicates. *means significance with relative to respective 95%+5% weight ratio of quartz and diatomite or clay of Student's t-test at P<0.05 and (**) at p<0.01.

Silicon uptake and accumulation

The Si concentrations of shoots were larger than that of roots in all proportions and mineral groups (Figure 3). The Si concentrations in both shoots and roots in Q+D group were larger as compared to Q+C group. Si concentration in shoots significantly increased with increasing proportion of clay or diatomite except in case of Cd treatment where increase in Si concentration was not significant in both groups (Figure 3A). Application of both Cd and Cu decreased Si concentration in shoots compared to control especially in Q+D group with least proportion of diatomite in the substrate and the reduction in the presence of Cd was more important than with Cu. Si concentration in roots of Q+C group increased significantly by increasing the proportion of clay in all treatments but in the case of the Q+D groups there was only a slight increase in Si concentration with increasing proportion of diatomite in the substrate (Fig. 3B). Total Si uptake was larger in Q+D groups than in Q+C groups and significantly increased both in shoots and roots with increasing proportion of clay or diatomite in mineral mixtures (Table 1).

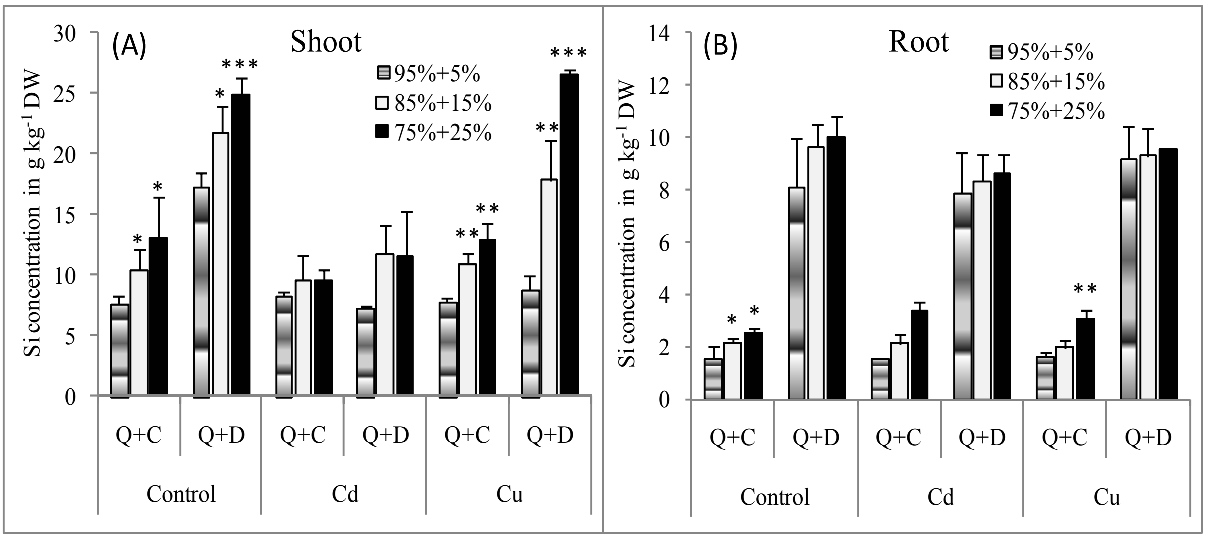

Figure 3. Si concentration (g kg^{-1} DW) in shoots and roots of wheat plants grown in a mixture of minerals and treated without and with 2 and 20ppm Cd and Cu respectively. Bars represent SD of three replicates. *means significance with relative to respective 95%+5% weight ratio of quartz and diatomite or clay of Student’s t-test at P<0.05 and (**) at p<0.01, and (***) at p<0.001.

Table 1: Total Si uptake in mg pot^{-1} of wheat shoots and roots grown in a mixture of minerals and treated without and with 2 and 20ppm Cd and Cu respectively. Bars represent SD of three replicates. *means significance with relative to respective 95%+5% weight ratio of quartz and diatomite or clay of Student’s t-test at P<0.05 and (**) at p<0.01, and (***) at p<0.001.

Si uptake mg pot^{-1}		95%+5%		85%+15%		75%+25%	
Treatments		Shoot	Root	Shoot	Root	Shoot	Root
Control	Q+C	3.36 ± 0.13	0.6 ±0.13	4.61**±0.4	0.88*±0.14	5.81 **± 1.13	1.01*±0.22
	Q+D	8.28 ± 0.91	4.67±0.87	11.88*±2.4	3.86 ± 1.15	17.71 ***± 2.25	6.34*±0.20
Cd	Q+C	2.92 ± 0.43	0.93±0.83	3.97*±0.72	0.79 ± 0.25	3.48 ± 0.19	1.61 ± 0.18
	Q+D	3.18 ± 1.57	3.39±0.45	5.31±1.87	2.78 ± 0.40	7.97*± 2.83	4.51**±1.14
Cu	Q+C	2.56 ± 0.32	0.52±0.05	5.03***±0.23	0.79* ± 0.16	4.98**±0.99	1.29 ± 0.29
	Q+D	4.73 ± 0.67	3.63±0.86	10.04***±1.26	5.39* ± 0.26	16.65***±2.71	5.22*±0.78

Cd and Cu distribution in plants

Cd and Cu concentrations were larger in roots compared to shoots and significantly decreased in both shoots and roots with increasing proportions of diatomite and clay except for Cd in shoots of the Q+D group (Figure 4). For the same amount of clay and diatomite, Cd concentration in shoots was larger with diatomite than with clay while Cu concentration in shoot was larger with clay than with diatomite except for the 95%Q+5%C group (Figure 4A). In roots both metals concentrations significantly decreased in both groups with increasing clay and diatomite proportions (Figure 4B). But both metals concentrations were larger in the Q+C group than in the Q+D group except for the 95%Q+5%C group. Although Cd and Cu concentrations were larger both in shoot and roots of the Q+C group as compared to the Q+D group they were negatively correlated with Si concentration while it was not the case for the

Q+D group except for Cu and Si concentrations in roots (See Annex 3 figure 2). Total Cd and Cu uptake was larger in roots than in shoots (Table 2). Cd uptake decreased in shoots and increased in roots with increasing clay proportions in Q+C groups while there was opposite trend for Q+D groups. Total Cu uptake decreased both in shoots and roots with increasing clay or diatomite proportions in mineral mixtures.

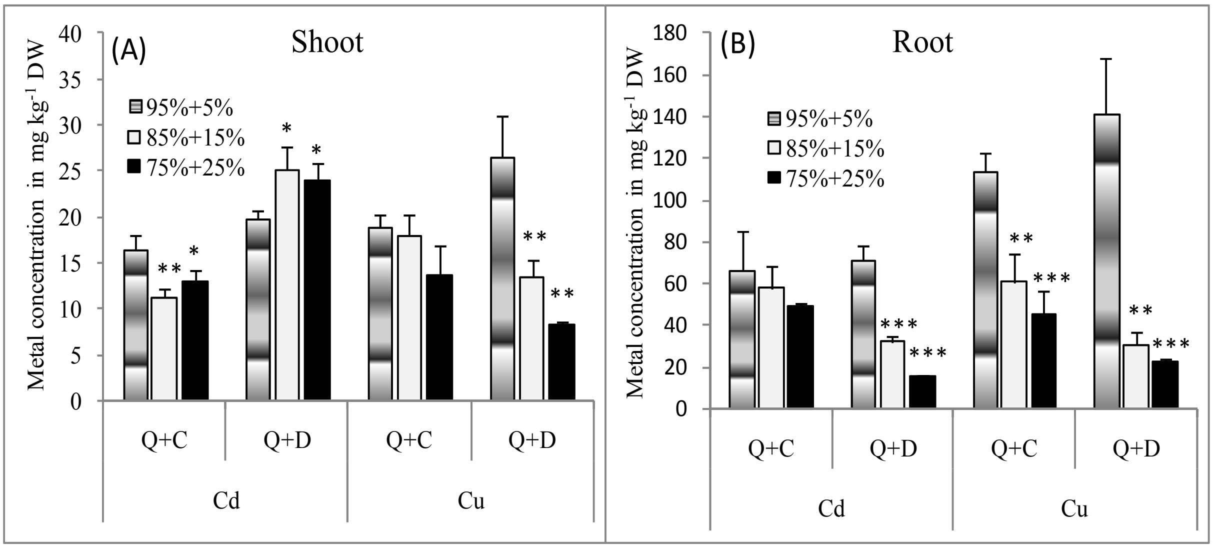

Figure 4. Cd and Cu concentrations (mg kg^{-1} DW) in shoot and root of wheat plants grown in a mixture of minerals and treated without and with 2 and 20 ppm Cd and Cu respectively. Bars represent SD of three replicates. *means significance with relative to respective 95%+5% weight ratio of quartz and diatomite or clay of Student's t-test at P<0.05, (**) at p<0.01 and (***) at p<0.001.

Table 2: Total Cd and Cu uptake in µg pot^{-1} of wheat shoots and roots grown in a mixture of minerals and treated with 2 and 20ppm Cd and Cu respectively. Bars represent SD of three replicates. *means significance with relative to respective 95%+5% weight ratio of quartz and diatomite or clay of Student's t-test at P<0.05 and (**) at p<0.01, and (***) at p<0.001.

Metal uptake µg pot^{-1}		95%+5%		85%+15%		75%+25%	
Treatments		Shoot	Root	Shoot	Root	Shoot	Root
Cd	Q+C	5.76 ± 1.08	18.88 ± 2.08	4.66 ± 0.44	21.17 ± 4.19	4.75 ± 0.70	23.32 ± 4.04
	Q+D	8.45 ± 0.45	28.78 ± 4.82	10.99*± 1.41	10.86**±2.5	16.77**± 3.35	8.49**±2.35
Cu	Q+C	8.94 ± 4.01	35.5 ± 4.16	10.28 ± 3.0	24.66*± 6.82	6.65 ± 2.62	18.68**±3.28
	Q+D	14.31 ± 2.72	54.51 ± 6.72	7.66**±1.44	18.05***±3.8	6.55**± 1.59	12.48***±1.47

III.3.b Effect of mineral mixture on substrate characteristics

Substrate pH

The pH values of final mixture of minerals were larger in Q+C group as compared to Q+D group (Figure 5). Final pH values of Q+C group were above 8.5 and of Q+D group were almost below 6.0. The pH did not change significantly within each Q+C group while it decreased in Q+D group with plant compared to without plant and all pH values significantly decreased with increasing proportions of diatomite irrespective of the presence of plant.

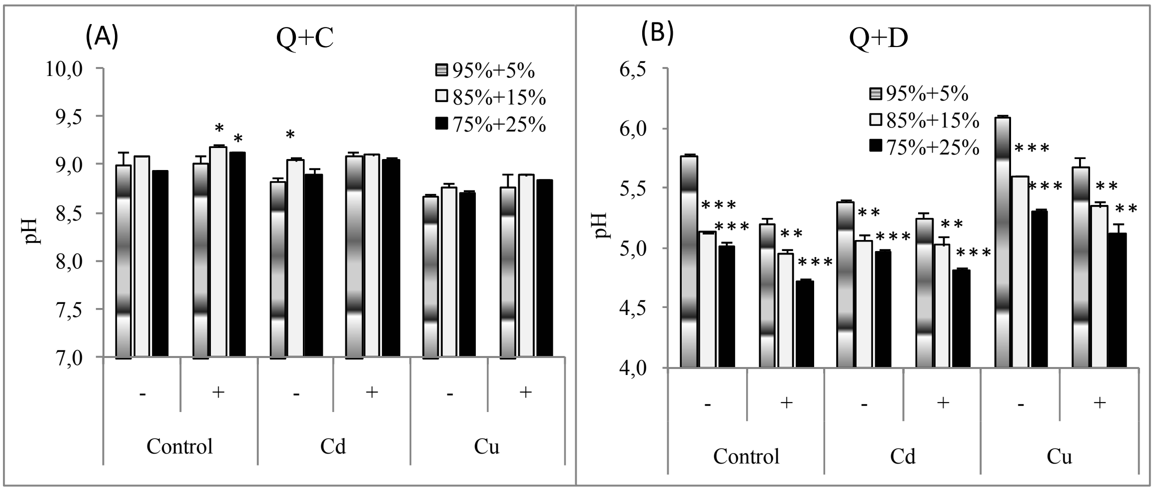

Figure 5. Substrate pH measured after harvesting of crop. Signs + and – indicate soil with and without plant respectively. Bars represent SD of three replicates with plants and two without plants. *means significance with relative to respective 95%+5% weight ratio of quartz and diatomite or clay of Student's t-test at P<0.05 and (**) at p<0.01 and (***) at p<0.001.

Heavy metals concentrations in DTPA-TEA extracts

DTPA-TEA extractable Cd concentration was larger in the Q+C group than in the Q+D group of minerals (Figure 6A) while Cu concentration was lower in Q+C group compared to Q+D group (Figure 6B). Cd and Cu concentrations were slightly lower in pots with plant compared to pots without plant and significantly decreased by increasing the proportion of clay or diatomite in the substrate irrespective of the presence of plant.

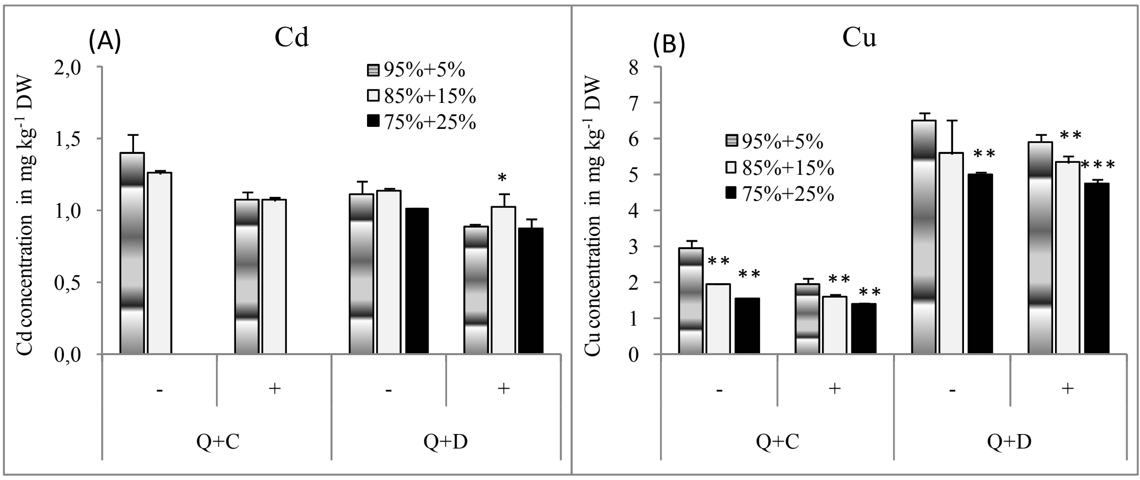

Figure 6. Effect of mineral mixtures on DTPA-TEA extractable Cu and Cd concentrations planted (+) or not (-) with wheat plants. Concentration in mg kg^{-1} is measured after harvest. Bars represent SD of three replicates with plants and two without plants. *means significance with relative to respective 95%+5%

weight ratio of quartz and diatomite or clay of Student’s t-test at $P<0.05$ and (**) at $p<0.01$ and (***) at $p<0.001$.

III.4 Discussion

Our results indicated that plant biomass (shoot and root) increased with increasing Si concentrations in plant shoots and roots and even under heavy metals stress, the total biomass of the Q+D group remained at a steady level (Figure 1 and 2). This gives evidence that Si is beneficial for the growth and development of durum wheat plants especially under heavy metal stress. This positive effect of Si on plant growth and biomass under metal stress has also been observed in *Arabidopsis* (Li et al., 2008), maize (Liang et al., 2005; Vaculik et al., 2009), rice (Ma et al., 2001), strawberries (Treder and Cieslinki, 2005) and wheat (Rizwan et al., 2012). In our results, increase in plant biomass was correlated to decrease in Cd and Cu concentrations and increase in Si concentrations in shoots and roots. This increase in plant biomass with Si under Cd and Cu toxicity has also been observed in the previous parts of this chapter.

The shoot and root Si concentrations increased with increasing diatomite and vermiculite proportions in substrate (Figure 3). The higher values of plant Si with increasing proportion of diatomite clearly indicated that amorphous silica is rapidly soluble and is an important pool of bioavailable silicon (Jones and Handreck, 1967; Fraysse et al., 2009). This higher absorption of Si by wheat coincides with the report of Rafi and Epstein (1999) that Si is rapidly absorbed by wheat (*Triticum aestivum* L.) plants from solution culture. Increasing the proportions of quartz decreased Si concentrations in plants which is in agreement with Savant et al. (1997) stating that most sources of silica are not available for plants and the solubility of quartz is very low compared to amorphous silica (Savant et al., 1999). Interestingly, in the present study, increasing vermiculite proportion in the substrate increased the Si concentration in shoots and roots (Figure 3). This indicated that in soils with large clay content, clay may be a significant source of Si for plants.

Cadmium and copper concentrations in shoots and roots decreased with increasing diatomite or clay proportions in mineral mixtures (Figure 4) while Si concentrations increased in shoots and roots with increasing diatomite or clay proportions in mineral mixtures (Figure 3). This decreased Cd and Cu concentrations in shoots and roots may be due to increased Si concentrations in shoots and roots or decreased roots to shoots translocation of metals. The increased retention of metals in roots which may be due to Si deposition in the roots has also been reported by Zhang et al. (2008) in rice plant. They observed that Cd was mainly deposited in the vicinity of the endodermis and epidermis in rice roots, while Shi et al. (2005) observed that Si was mainly deposited in the cell walls of the root endodermis in rice. In the present study the decreased uptake by the roots may be due to increased metal adsorption on the roots surface as we observed in Si and Cd and Cu hydroponic studies (See 1st and 2nd parts of this chapter) that Si increased the Cd and Cu adsorption on the roots surface. Total Si uptake increased and Cu uptake decreased in shoots and roots with increasing diatomite or clay proportions in mineral mixtures (Tables 1 and 2). This decrease in Cu uptake lead to less toxicity and thus healthier plants (Figure 1). We have also observed decrease in total Cu uptake in wheat plant grown hydroponically (See 2nd part of this chap.). Total Cd uptake decreased in shoots and increased in roots with increasing clay proportions in mineral mixtures while increased in shoots and decreased in roots with increasing diatomite proportions in mineral mixtures (Table 2). These differences, however, were mainly due to the increase in shoots biomass of the Q+D group as compared to the Q+C group.

After the plant harvest, the bioavailable heavy metal (Cd and Cu) concentrations in all substrates decreased irrespective of the plant growth (Figure 6). This decrease could be attributed to uptake by plants through roots or due to immobilization in the substrate, as observed in pots without plant (Figure 6). In almost all cases Cd and Cu concentrations decreased with increasing percentage of diatomite or clay in the mixed minerals in both pots without and with plant. In pots with plants, plant uptake could have accounted for a decrease in available Cd and Cu as Cd concentrations decreased of about 22.7 % and 19.9 % in 95% Q + 5% C and 95% Q + 5% D as compared to without plants respectively and Cu concentrations decreased about 34.2% and 9.2% in 95% Q + 5% C and 95% Q + 5% D as compared to without plants respectively. However, this mechanism is ruled out in the pot without plants. This clearly indicated that amorphous silica or clay prevented the heavy metals absorption by the roots. This may be related to the cation exchange capacity of clay (Suganya and Sivasamy, 2006) and diatomite (Elden et al., 2010). DTPA-extractable Cu concentration was lower in the Q+C mixtures than in the Q+D mixture despite the fact that the Q+C group has lower ASi concentration than the Q+D group (Figure 6). This reduction in metal concentration in Q+C substrate could be explained by an additional pH effect (Figure 5A). Indeed, the larger pH measured in the Q+C group may be responsible for a reduction in the metal uptake by the plants through immobilisation in the soil (Bravin et al., 2009; Liang et al., 2005) and a decreased availability to plants. Gu et al. (2011) suggested that, in a contaminated acidic soil amended with FA (fly ash) and SS (steel slag), the reduction in Cd, Zn, Cu and Pb uptake by rice was attributed to in situ immobilisation of heavy metals in soil and this mechanism was associated to an increase in pH. Silicon concentration in the soil solution was not measured, but pH of Q+D group was always slightly acidic, which may have prevented a fast release of amorphous silica in solution as Fraysse et al. (2006, 2009) have shown that phytoliths (amorphous silica) dissolve more slowly at lower pH.

III.5 Conclusion

In conclusion this study demonstrated that amorphous silica is 2.2 times more available than vermiculite in the conditions of the experiment. Si present as amorphous Si (diatomite) is a significant source for plant uptake. However part of Si brought by vermiculite is also available: in soils with large clay content, clay may be a significant source of Si for plants. Si alleviates metal stress in wheat induced by toxic amounts of Cd or Cu in soils by immobilizing in the substrate and sequestrating in roots and thus increased wheat tolerance to Cd and Cu. We propose that Si has both a soil and a plant effect by immobilizing Cd and Cu in soil and reduced the metal uptake and translocation to shoots. This also indicates that amorphous silica that can be found as diatomite could be used as an easily available source of Si for soil remediation.

Chapter 5: Silicon-mediated enhancement of heavy metals tolerance in durum wheat grown in soil

As we saw in the previous chapters, diatomite containing amorphous silica is an important pool of available silicon for plants. Repeated croppings of durum wheat resulted in the decrease of bioavailable Si which was mostly due to Si plant uptake along with other factors. Silicon application in the nutrient solution decreased the toxic effects of Cd and Cu in durum wheat in a number of ways such as Si supplementation modify the uptake and translocation of Cd and Cu as well as other metals and mineral nutrients. Si application in the hydroponics also positively affected wheat physiology and enhanced the chlorophyll contents in leaves. Based upon our previous results we needed to develop soil based studies to evaluate the practical implications of Si-mediated Cd and Cu tolerance in durum wheat. Therefore, we performed a soil based study to evaluate the effects of Si in metal detoxification in long term experiment as compared to usual hydroponic studies. In this study emphasis was given to Cd as compared to other toxic metals.

This chapter is devoted to evaluate the role of Si in metal tolerance in wheat plant grown in soils. In this study we selected two texturally and historically different soils to perform a soil experiment to test increasing doses of diatomite on wheat plant heavy metal tolerance. We used two soils; a soil contaminated due to sewage sludge application (First part) and a non contaminated soil was spiked with soluble Cd (Second part).

I. Effect of silicon on reducing cadmium toxicity in durum wheat (*Triticum turgidum* L. cv. Claudio W.) grown in a soil with aged contamination

Abstract

Agricultural soil contamination and subsequently crops still requires alternative solutions to reduce associated environmental risks. The effects of silica application on alleviating cadmium (Cd) phytotoxicity in wheat plants were investigated in a 71-day pot experiment conducted with a historically contaminated agricultural soil. We used amorphous silica (ASi) that had been extracted from a diatomite mine for Si distribution at 0, 1, 10 and 15 ton ASi ha^{-1} ASi applications increased plant biomass and plant Si concentrations, reduced the available Cd in the soil and the Cd translocation to shoots, while Cd was more efficiently sequestrated in roots. But ASi is limiting for Si uptake by plants. We conclude that significant plant-available Si in soil contributes to decreased Cd concentrations in wheat shoots and could be implemented in a general scheme aiming at controlling Cd concentrations in wheat.

Key words: Metals, uptake, silicon, wheat, remediation

This part has already been published in Journal of hazardous materials, 2012, 209-210: 326-334.

I.1 Introduction

The productivity and biological efficiency of plants are limited by the presence of toxic elements in the soil. Among these toxins, cadmium (Cd) is highly toxic for both plants and animals (Das et al., 1997; Wagner, 1993). It is widely used in household and industrial appliances, and domestic and industrial sewage sludge contains higher concentrations of Cd than other metals or metalloids. Cd enters the soil, whether intentionally or not, through atmospheric fallout, sewage sludge and fertiliser application (Kirkham, 2006). Once in the soil, the bioavailability of Cd depends on a number of soil characteristics, soil-solution characteristics (Lehoczky et al., 2000; Sauvé et al., 2000), interactions with other elements such as iron (Krishnamurti et al., 2000) and on plant species or populations that may have various capabilities of taking up metals.

Due to its high mobility and assimilability, Cd readily accumulates in the different plant parts and organs depending on the plant species (Kabata-Pendias and Pendias, 2001). Cadmium uptake by the roots and translocation to shoots has been characterised in a number of plant species including wheat (Jalil et al., 1994b) and maize (Florijn and Van Beusichem, 1993). After root absorption, Cd is translocated to the shoots mainly in an ionic form in xylem and phloem (Tudoreanu and Phillips, 2004) either passively by transpiration rate (Salt et al., 1995) or actively through Fe transporters (Nakanishi et al., 2006). Cadmium accumulation in durum wheat grains may be due to the increase in Cd translocation from leaves and stalks to maturing grains (Harris and Taylor, 2001) and may be related to phloem movement (Hart et al., 1998). Similarly, Cd concentrations in grains correlate with Cd concentrations in shoots during the vegetative phase and the translocation rate from roots to shoots of plants both in wheat and rice (*Oryza sativa*) (Greger and Löfstedt, 2004; Liu et al., 2007), indicating a direct effect of Cd uptake by plant and food chain contamination.

Concurrently, heavy metals contamination reduction or elimination from the soil and/or metal transfer reduction from soil to crops and further up the food chain has become a growing concern worldwide. Soil contamination is indeed considered one of the main threats to soil as identified in the EU soil communication (CEC, 2002), Cd being of great concern to human health along with mercury and lead. Foodstuffs are the main source of cadmium exposure for the non-smoking general population and Cd is the only metal for which guide values have been published for food (EFSA, 2009). Different actions can be undertaken to reduce the absorption of Cd by plants, such as selecting metal-excluding plants (Poschenrieder et al., 2001; Seregin et al., 2003; Wei et al., 2005; Wenzel et al., 2003), using soil amendments (e.g. Keller et al., 2005) and/or applying an effective plant fertilisation (Sarwar et al., 2010). However, these actions may not be efficient in all cases. There is still a need for a range of more efficient and economical approaches for coping with metal toxicity in plants that may occur in large areas.

Silicon, however, is not considered to be an essential element for higher plants but it has been proved to be beneficial for the growth and development of many plants, particularly graminaceous plants such as rice and sugarcane (Epstein, 1994; Ma et al., 2006). Like most of the Poaceae, wheat is a Si accumulator which implies that Si concentrations in shoots are generally over 10 mg g^{-1} and that the [Si] / [Ca] ratio is less than 1 (Ma and Takahashi, 2002). A number of studies show that Si is actively absorbed by wheat plants and transported to different plant organs (Rafi and Epstein, 1999). Indeed, Si influx transporters have been identified in rice (Ma et al., 2001) and in maize (Mitani et al., 2009) but not yet in wheat, although it has been suggested by Rains et al. (2006). The beneficial effects of Si are particularly distinct in plants exposed to biotic or abiotic stresses (Epstein, 1999; Feng et al., 2010; Guntzer et al., 2011; Liang et al., 2007; Maksimović et al., 2007; Rogalla and Römheld, 2002; Shi et al., 2005a), and Si has been shown to alleviate the deleterious effects of metals in many plants (Chen et al., 2000; Doncheva et al., 2009; Liang et al., 2005; Sommer et al., 2006). There is an increasing body of literature showing that Si application to Cd-contaminated soils in hydroponic conditions enhances biomass production of many plant species such as maize (da Cunha et al., 2008; da Cunha and do Nascimento, 2009; Vaculik et al., 2009) and strawberry (Treder and Cieslinki, 2005). Similarly, Si reduced toxicity symptoms in rice plants (Liu et al., 2009; Nwugo and Huerta, 2008; Shi et al., 2005b). However, the role of Si in heavy metals tolerance in wheat and durum wheat has rarely been studied, despite the fact that wheat is the most produced cereal crop in Europe and the second most produced in the world, with 228 and 686 MT produced in 2009, respectively (FAOSTAT, May 2011). France is the second largest durum wheat producer in Europe. In addition, most of the studies related to the Si-mediated reduction of metal stress in plants are conducted on a short-term basis and in hydroponic cultures. In the few experiments conducted in soils, it was very difficult to distinguish between the soil factors and the root effects on Cd uptake (Kirkham, 2006) and thus to determine the consequences of Si addition to soil on Cd uptake. The possible mechanisms involved in minimising Cd accumulation in crops by improving silicon nutrition have been summarised by Sarwar et al. (2010) and it appears that the mechanisms of the alleviating effects remain unclear and/or are probably multifaceted. Thus, we need long-term (as compared to hydroponic cultures), more realistic soil-based studies to fully understand the practical implications of Si-mediated metal tolerance so that successful field experiments can be conducted.

We conducted a greenhouse experiment to investigate the beneficial effects of Si on durum wheat plants grown in pots using a soil with aged contamination. The main objectives of the study were as follows: (1) to investigate the effect of amorphous silica (ASi) application on metal-stressed wheat plants grown in contaminated soil; (2) to highlight the effect of amorphous silica application on soil available metal pool and on metal transfer to wheat plants with an emphasis on Cd; and (3) to identify the possible mechanisms responsible for the reduction of metal toxicity and accumulation in plants.

I.2 Materials and Methods

I.2.a Materials

The soil was collected in Rafz (414m above sea level, Switzerland) from the surface (0-20 cm) of an agricultural soil that had been polluted twenty years ago by municipal and industrial sewage sludge and, as a result, is contaminated by a range of heavy metals including cadmium (Cd), copper (Cu), zinc (Zn) and lead (Pb). It has been used since then for crop production. According to the FAO taxonomy, it is a sandy loam haplic luvisol (US-taxonomy: typic hapludalf). The soil is neutral to slightly alkaline and the soil is virtually free of carbonate. The carbon content is in the normal range usually for agricultural soils (Table 1). More information on the soil characteristics can be found in Krebs et al. (1999).

In France, durum wheat is cultivated on 400 000 ha, mostly in the South, and yielded a production of 2 Mton in 2009. *T. turgidum* L. cv. Claudio W. was grown in the Rafz soil with and without Si applications. We applied a natural ASi powder (siliceous fossil meal, Clarcel 78®) that had been extracted from a diatomite mine exploited by CECA (ARKEMA Group) from Saint-Bauzile (France), which contains 87 % SiO_2 and makes it interesting because it is rather concentrated. The remaining 13% include Al, Fe, Ca, Mg and K. ASi is an opal A-like mineral that was chosen because it has been shown to dissolve easily (Fraysse et al., 2009).

Table 1: Initial physicochemical properties of the Rafz soil used in the pot experiment

Physicochemical properties	
pH (1/2.5 soil to water ratio)	6.8
Organic C (mg g^{-1})	16.0
Total N (mg g^{-1})	8.0
Olsen P (mg g^{-1})	0.163
Sand (mg g^{-1})	501
Silt (mg g^{-1})	335
Clay (mg g^{-1})	164
Cation Exchange Capacity (cobaltihexamine; $cmol^{+}$ kg^{-1})	10.8
Si Tamm[a] (mg g^{-1})	0.33
Si CBD[b] (mg g^{-1})	1.25
Total Cd (mg kg^{-1})	0.7
Total Zn (mg kg^{-1})	495
Total Cu (mg kg^{-1})	48.5
Total Pb (mg kg^{-1})	309

[a] Tamm extraction (Tamm, 1922); [b] CBD citrate bicarbonate dithionite extraction Mehra and Jackson (1960).

I.2.b Protocol of the experiment

Experimental design

The pot experiment was conducted in a greenhouse at 18-25 °C and 60% humidity. Silica was added at doses of 0, 1, 10 and 15 ton ASi ha^{-1} to each soil batch treatment and then thoroughly mixed by hand. The effective doses that were applied to the pots were calculated by taking a 20-cm depth of soil with a density of 1.49 g/cm^3 ha^{-1}, assuming that the diatomite was 87% pure. Each batch was then divided to fill the replicated pots. All treatments were performed in three replicates without plants and in four replicates with plants. Pots were initially seeded (mid February 2009, immediately after mixing with ASi) at a density of six seeds per pot, then thinned to four individuals per pot after five days of germination. Each pot was fertilised with a 30-ml solution containing 130 mg L^{-1} N (as NH_4NO_3), 130 mg L^{-1} P (as $Ca\ (H_2PO_4)_2$), 180 mg L^{-1} K (as 50% KCl and 50% K_2SO_4) and 40 mg L^{-1} Mg (as $MgSO_4$. $7H_2O$). Pots were regularly watered and randomly rotated. Weeds were removed regularly when present. Two Rhizon soil moisture samplers (SMS, Rhizon®, Rhizosphere Research Products, NL) were installed per pot. They were first dipped into a 10% HNO_3 p.a. solution overnight and then washed with distilled water until the pH returned to 7. The SMS were then inserted horizontally in the wet soil two days after sowing. All plastic and glass wares were rinsed with 10% HNO_3.

Plant sampling and analysis

Plants were harvested after 71 days, just before booting stage, by cutting the shoots approximately one centimetre above the soil surface. Plant samples were washed with distilled water and oven dried at 70 °C until a constant weight was reached. Roots were separated from soil by the following procedure: rinsing with distilled water, followed by a second rinsing with 0.02 M ethylenediaminetetraacetic (EDTA) solution and three other rinsing steps with distilled water, before oven drying at 80 °C until a constant weight was reached. Total dry weights of the roots and shoots were measured. All shoot and root samples were finely ground before digesting them with concentrated HNO_3 (70%) in a water bath at 95°C for 6 h. Solutions were filtered on 0.22 µm membrane before analysis (modified from Keller et al., 2003). Silicon was extracted according to Guntzer et al. (2010).

Soil solution, soil sampling and analysis

Soil solution samples were collected at different intervals of time (47, 57, 64 and 71 days after sowing), analysed for pH, acidified with 70% sp HNO_3 and measured for Si, Cu, Zn, Cd and Pb concentrations. Soil samples were taken at the beginning (just after mixing) and at the end of the experiment. The samples were oven dried at 40 °C until constant weight and sieved at 2 mm before analysis. Soil pH was measured (soil/water ratio of 1/2.5) and DTPA-TEA extractions were performed according to a modified Lindsay and Norvell (1978) procedure. We used 0.005 M DTPA, 0.1 M TEA and 0.01 M $CaCl_2$ at pH 7.3; the soil/solution ratio was 2/20; the solutions were horizontally shaken for 120 min and centrifuged and filtrated through a 0.22 µm cellulose acetate membrane. Cadmium and the other elements in the soil extractions and plant digests were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES Jobin-Yvon, Ultima-C). Metals from soil solution samples were also measured by ICP-MS (Perkin-Elmer Elan 6000, LMTG Toulouse). Si concentrations in soil solutions were measured by molybdate blue colorimetric method (Hansen and Koroleff, 1999) with a spectrophotometer (Jasco V650)

Statistical treatment

Student’s t-test was performed to test whether the average concentrations in the treated pots differed from those of controlled pots. Results that were significantly different were marked by (*) for $p \leq 0.05$, (**) for $p \leq 0.01$ and (***) for $p \leq 0.001$. Statistics were performed using the XLStat package (Addinsoft, Paris, F).

I.3 Results

I.3.a Effect of Si treatments on plant parameters

Plant growth and biomass

The shoot length of wheat plants (Figure 1), as well as the dry weight (DW) of both shoots and roots (Figure 2), increased with increasing doses of supplemented Si. Shoot dry weights of the 10- and 15-ton ASi ha^{-1} pots were respectively 10% and 42% larger than those of the control pots. The maximum significant increase in root dry weight (approximately 43% of the control) was also observed in the 15-ton Si ha^{-1} pots.

Figure 1. Wheat plants grown in pots from a historically contaminated soil treated with increasing doses of amorphous silica (ASi): 0 (control), 1, 10 and 15 ton ASi ha^{-1}. The effect of silicon on wheat growth can be seen in differences of the length of the above-ground parts in each treatment, especially between the control and the 15 ton ha-1 ASi treatment.

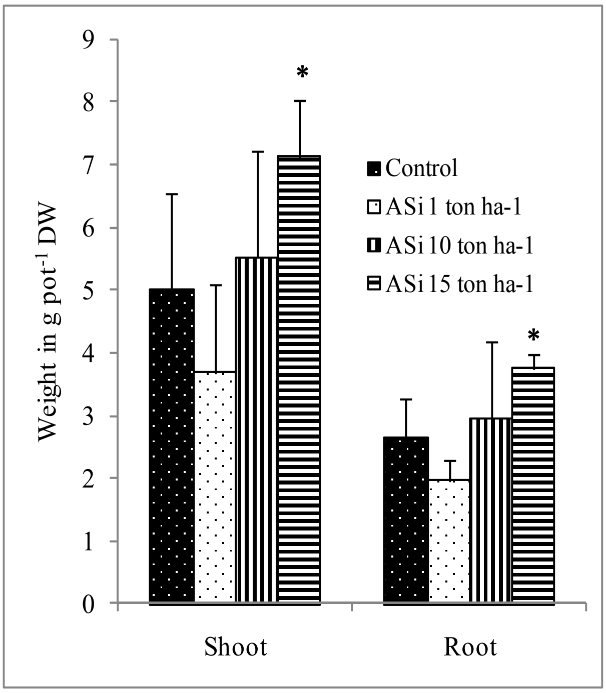

Figure 2. Dry weight of wheat plant shoots and roots grown in a historically contaminated soil treated with increasing doses of ASi (0, 1, 10 and 15 ton Si ha^{-1}). Bars represent SD of four replicates. (*) denotes significance relative to the respective control as determined by Student's t-test at a $p<0.05$ and (**) at $p<0.01$.

Silicon uptake and accumulation

The Si concentration in both shoots and roots did not increase significantly with increasing Si doses, and Si concentrations in shoots were slightly larger than those in roots (Table 2). The shoot to root ratio was not significantly different, but the 15-ton ASi ha^{-1} treatment was the largest. Plant uptake was calculated using Si concentrations multiplied by the biomass production (Figure 3). Shoot uptake increased significantly in applications greater than 10-ton ASi ha^{-1}, being 63% larger in the 15-ton Si ha^{-1} treatment than in the control pots. However, this effect was not significant for roots.

Table 2: Si concentration (g kg^{-1} DW) in shoot and root as well as the shoot-to-root ratio of wheat plants grown in a historically metal-contaminated soil treated with increasing doses of ASi. Values are mean ± SD (n = 4).

ASi application (ton ha^{-1})	Shoot	Root	Shoot/Root
		g kg^{-1} DW	
0	3.98 ± 0.81	3.21 ± 0.81	1.24
1	4.27 ± 0.66	3.27 ± 0.68	1.31
10	4.66 ± 0.69	4.65 ± 1.74	1.00
15	4.43 ± 0.25	3.19 ± 0.20	1.39

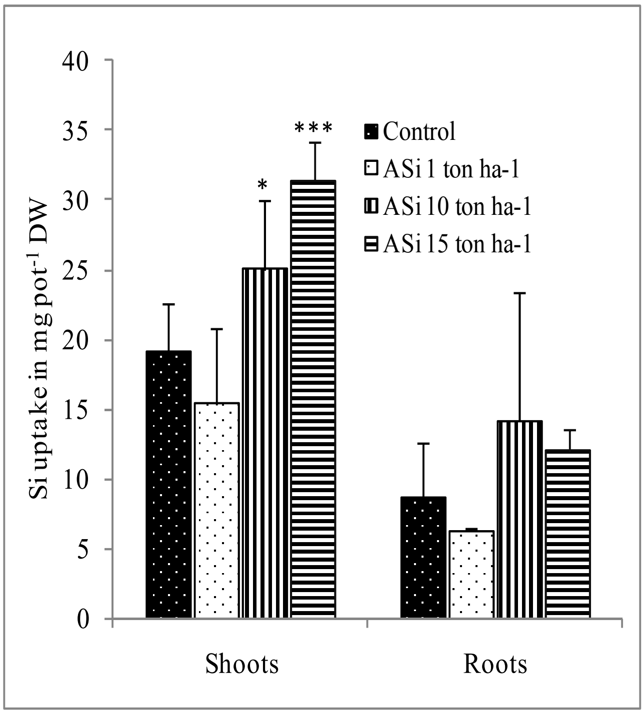

Figure 3. Total Si uptake in mg pot^{-1} of wheat shoots and roots grown in a historically contaminated soil treated with increasing doses of ASi. Bars represent SD of four replicates. (*) denotes significance relative to the respective control as determined by Student's t-test at a $p<0.05$ and (***) at $p<0.001$.

Metal distribution in plants

Cadmium concentrations in the plant shoots significantly decreased with increasing ASi supplementation (Figure 4A), while the opposite trend was observed for roots. However, the Si shoot to root ratio remained constant for all treatments (Table 2). Contrarily, when the total Cd output from both shoots and roots was calculated (Figure 4B), the Cd amount in shoots was similar to that of control plants, while the root total uptake was still significantly larger with the largest Si application. The other metal concentrations (Zn, Cu and Pb) were larger in roots than in shoots (Table 3). Zinc concentrations increased in both shoots and roots with Si treatments, while Cu and Pb concentrations did not increase significantly in both shoots and roots throughout the Si treatments.

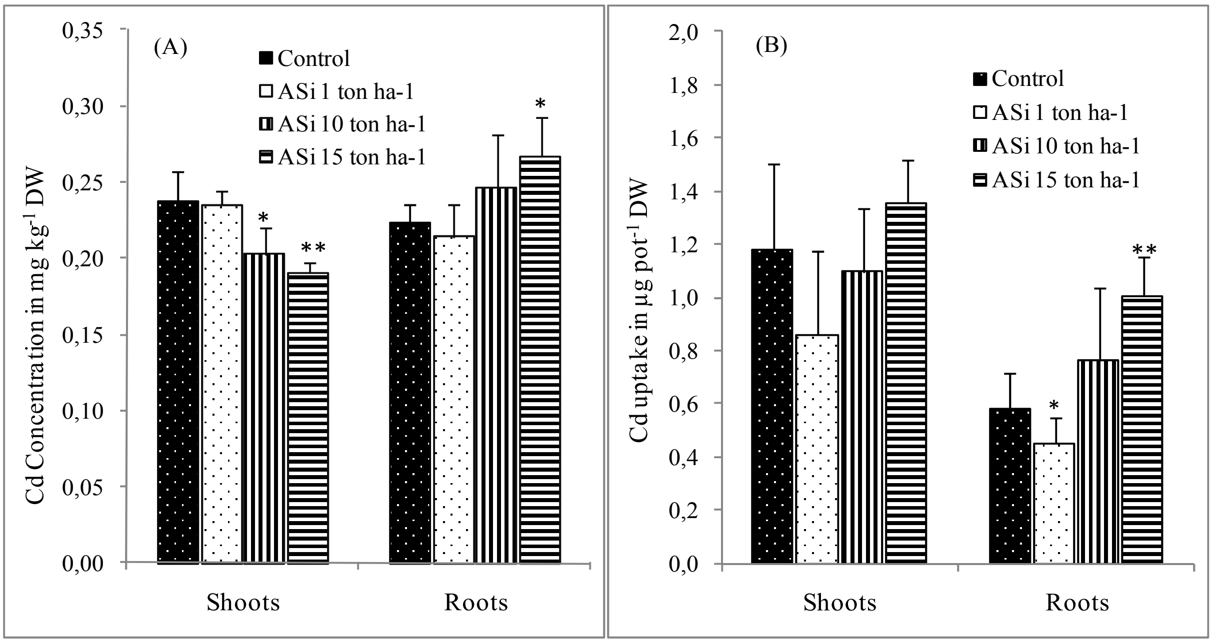

Figure 4. Cadmium concentration in mg kg^{-1} (A) and Cd total uptake in mg pot-1 (B) in shoots and roots of wheat plants grown in a historically contaminated soil treated with increasing doses of ASi. Bars represent SD of four replicates. (*) denotes significance relative to the respective control as determined by Student's t-test at a $p<0.05$ and (**) at $p<0.01$.

Table 3: Zinc, Cu and Pb concentrations (mg kg^{-1} DW) in shoots and roots of wheat plants grown in a historically metal-contaminated soil treated with increasing doses of ASi. Values are means ± SD (n=4). (*) denotes significance relative to the respective control as determined by Student's t-test at a $p<0.05$, (**) at $p<0.01$ and (***) at $p<0.001$.

ASi applications (ton ha^{-1})	Zn		Cu		Pb	
	mg kg^{-1} DW					
	Shoot	Root	Shoot	Root	Shoot	Root
0	57.5±4.2	94.8±2.3	8.1±0.3	14.0±0.6	1.1±0.1	29.7±3.5
1	77 .4±3.5***	106.7±6.6**	9.3±0.7**	13.2±0.3*	1.3±0.1**	28.1±0.9
10	60.1±13.4	108.6±17.9	7.9±1.4	14.2±1.4	1.1±0.3	30.5±5.1
15	58.7±6.3	108.7±9.7**	8.6±0.6	13.3±0.5	1.2±0.1	29.2±1.0

I.3.b Effect of Si treatments on soil characteristics

Soil pH in pots with plants was larger than in pots without plants (Table 4). The pH slightly decreased in the pots without plants treated at 10 and 15 ton ASi ha^{-1}. No pH changes were observed along the various treatments with plants. In non-sown pots, pH was slightly lower (not significantly) after 71 days in the pots that had been treated with 10 and 15 ton ASi ha^{-1} than in both the control and the 1-ton ASi ha^{-1} pots. In sown pots, pH did not change significantly with ASi treatments as compared to the control pots. Figure 5 presents the results of the DTPA-TEA extractions performed on the soil collected from the pots after wheat harvest. In pots both with and without plants, DTPA-extractable Cd concentrations slightly decreased in all ASi treatments, but this decrease was only statistically significant in sown pots. A decreasing trend was observed for Zn, but not for Cu and Pb concentrations (Table 5) for pots both with and without plants.

Table 4: Soil pH measured after crop harvest in a historically metal-contaminated soil sown or unsown with wheat plants and treated with increasing doses of ASi. Values are mean ± SD (n =3 without plants, and n= 4 with plants).

ASi applications (ton ha^{-1})	Without plant	With plant
0	6.09 ± 0.15	6.42 ± 0.10
1	6.11 ± 0.10	6.57 ± 0.13
10	5.98 ± 0.13	6.52 ± 0.15
15	6.01 ± 0.14	6.50 ± 0.09

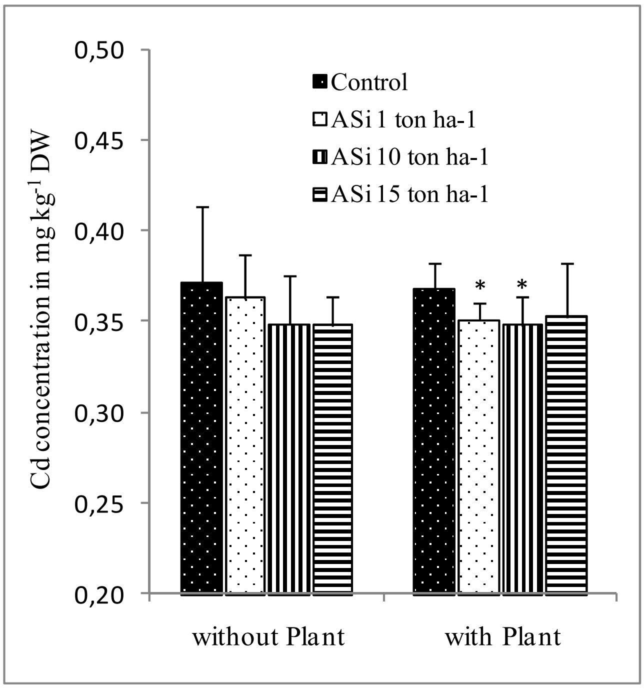

Figure 5. Effect of ASi application on DTPA-TEA extractable Cd concentration measured in a historically contaminated soil sown or not with wheat plants and treated with increasing doses of ASi. Concentration in mg kg-1 is measured after harvest. Bars represent SD of four replicates. (*) denotes significance relative to the respective control as determined by Student's t-test at a p<0.05.

Table 5: DTPA-TEA extractable Zn. Cu and Pb (mg/kg^{-1} DW) concentrations measured after crop harvest in a historically metal-contaminated soil sown or unsown with wheat plants and treated with increasing doses of ASi. Values are means ± SD (n=4). (*) denotes significance relative to the respective control as determined by Student's t-test at a p<0.05.

ASi application (ton ha^{-1})	Zn		Cu		Pb	
	mg kg^{-1} DW					
	Without plant	With plant	Without plant	With plant	Without plant	With plant
0	121.2 ± 11.5	101.1 ± 7.1	15.5 ± 0.2	15.6 ±0.3	80.5 ± 1.2	93.9 ±4.3
1	116.7 ± 2.6	98.7*± 4.2	15.5 ± 0.3	15.3 ± 0.4	76.5*±2.9	86.9*±1.9
10	116.8 ± 0.7	98.3 ± 2.3	15.2 ± 0.3	15.2*± 0.1	80.2 ±1.5	86.8*±2.6
15	113.1 ± 5.3	93.2*± 1.1	14.9*± 0.3	15.3 ± 0.7	78.2 ± 4.7	92.2 ±5.5

I.3.c Effect of Si treatment on the soil solution

pH

The pH of the soil solution collected from pots with plants was larger than the pH of soil solution of pots without plants (Figure 6). In pots without plants, pH significantly increased with each Si treatment. The pH remained almost the same in soil solutions collected from pots with plants.

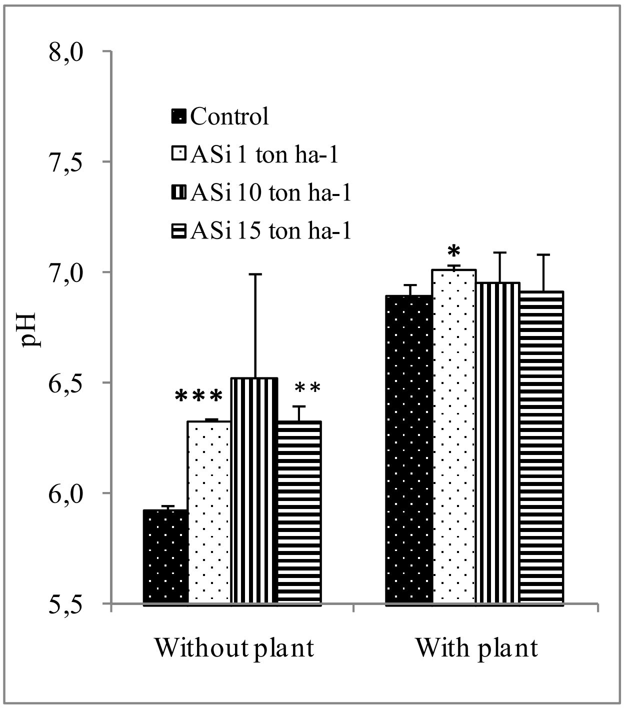

Figure 6. pH measured in the soil solution collected by Rhizon® in pots of a historically metal-contaminated soil sown or unsown with wheat plants and treated with increasing doses of ASi. Sampling was performed 71 days after sowing, just before harvesting. (*) denotes significance relative to the respective control as determined by Student's t-test at a $p<0.05$, (**) at $p<0.01$ and (***) at $p<0.001$.

Silicon concentration

The silicon concentration was always larger in the soil solutions collected from pots without plants than in soil solutions from pots with plants (Figure 7). Si concentrations changed with time: in soil solutions from pots without plants. Si concentrations increased between the 41st and the 57th day after sowing and then decreased till harvest in all Si treatments. In pots with plants, Si concentrations slightly decreased over time for all treatments, including controls.

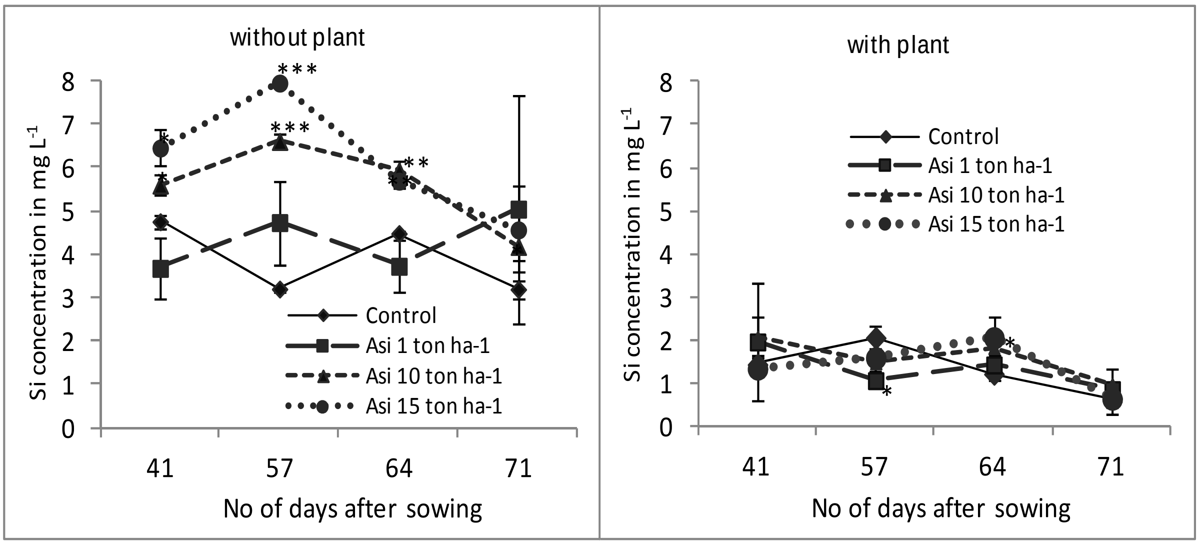

Figure 7. Evolution of Si concentration through time in the soil solution collected by Rhizon® in pots of a historically metal-contaminated soil sown or unsown with wheat plants and treated with increasing doses of ASi. Bars represent SD of two replicates. (*) denotes significance relative to the respective control as determined by Student's t-test at a $p<0.05$, (**) at $p<0.01$ and (***) at $p<0.001$.

Trace element concentrations

Cadmium, Zn, Cu and Pb concentrations were measured once after 57 days of sowing because this was when Si concentrations exhibited the largest differences between treatments, and we assumed the potential effect on trace elements would be the largest as well. Cadmium concentrations were lower in all soil solutions from sown pots than in solutions from unsown pots for all ASi treatments (Figure 8), and Si showed no observable effect.

As for Cd, differences between treatments were not significant for Zn, Pb and Cu. Zn concentrations in soil solutions increased unevenly in all pots (without and with plants), with ASi treatments (Table 6), while Cu concentrations decreased in pots without plants in all ASi treatments. Zinc, Cu and Pb concentrations were higher in both soil solutions without and with plants in the 15 ton ASi ha^{-1}.

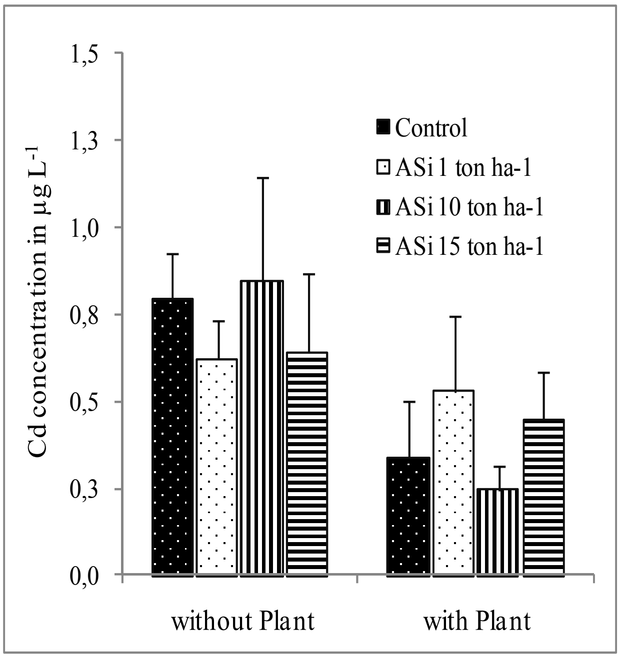

Figure 8. Cd concentration in µg L^{-1} measured in the soil solution collected by Rhizon® 57 days after sowing in pots of a historically metal-contaminated soil sown or unsown with wheat plants and treated with increasing doses of ASi. Bars represent SD of four replicates (with plant) or two replicates (without plant).

Table 6: Zinc, Cu and Pd concentration in µg L^{-1} measured in the soil solution collected 57 days after sowing. Values are means ± SD (n=2). (*) denotes significance relative to the respective control as determined by Student's t-test at a $p<0.05$, (nd) denotes no data available.

ASi applications (ton ha^{-1})	Zn		Cu		Pb	
	µg L^{-1}					
	Without plant	With plant	Without plant	With plant	Without plant	With plant
0	nd	29.1 ± 4.5	18.4 ± 2.0	20.3 ± 6.6	0.72 ± 0.01	0.74 ± 0.54
1	84.9 ± 4.8	30 .9 ± 5.8	15.8 ± 1.0	19.2 ± 7.1	0.73 ± 0.08	0.85 ± 0.28
10	88.1 ± 17.4	26.9 ± 0.2	10.2± 2.1*	19.0 ± 1.3	0.56 ± 0.12*	0.56 ± 0.07
15	120.3 ± 0.1	34.6 ± 1.2	16.2 ± 1.3	29.2 ± 2.5	0.95 ± 0.11	1.13 ± 0.12

I.4 Discussion and conclusion

This study indicates that ASi supply increased the biomass of both shoots and roots of durum wheat (Figure 2), even at low doses. This positive effect of Si on growth and biomass has also been observed in experiments with maize plants in both soil and hydroponic conditions (da cunha et al., 2009; Vaculik et al., 2009; da Cunha and do Nascimento, 2008; Liang et al., 2005) and in strawberries grown in contaminated soil (Treder and Cieslinki, 2005). Here, the increase in biomass was concomitant with a decrease in Cd concentration in shoots (Figure

4A), indicating either a dilution effect (same uptake but larger biomass) or a decrease in Cd uptake leading to less toxicity and thus healthier plants (see Figure 1).

Concurrently, plants treated with ASi accumulated significantly more Si in shoots than in roots (Figure 3). This activity where accumulation increases in shoots when plants are supplied with available Si is typical for Si-accumulating plants, such as rice (Ma et al., 2006), and indicates an active uptake (Ma et al., 2001; Takahashi et al., 1990). Active uptake has also been suggested for wheat (Rains et al., 2006). Silicon is generally taken up as silicic acid from the soil solution (Epstein, 1994, 1999). It is then precipitated primarily as amorphous silica (Opal A; $SiO_2 \cdot nH_2O$) in cell walls, lumens and in the intercellular spaces as phytoliths (Raven, 1983; Jones and Handreck, 1967) where water evaporates from the plants (Currie and Perry, 2007; Sommer et al., 2006). However, Si concentrations in shoots and roots did not increase with increased ASi supplementation in the soil, even though Si concentrations in the soil solution were initially larger (Figure 7). The increase in biomass probably contributed to a dilution effect.

Cadmium concentrations in shoots and roots of wheat plants did not follow the same trend as shoot concentrations decreased while root concentrations increased with increasing Si application doses (Figure 4A). Similar results were also observed in rice treated with foliar Si (Liu et al., 2009) or in rice grown hydroponically (Zhang et al., 2008; Shi et al., 2005b; Qin and Huang, 1997), in *Brassica chinensis* (Song et al., 2009), in peanut (Shi et al., 2010) and in cucumber (Feng et al., 2010); but our results disagree with authors who found both an increase in root and shoot concentrations following Si application (Vaculik et al., 2009) or a decrease in both roots and shoots Cd concentrations (Nwugo and Huerta, 2008). In wheat, a decrease in both root and shoot Cu concentrations was also observed in wheat grown hydroponically (Nowakowski and Nowakowska, 1997), but in our case no decrease was observed for the other metal concentrations in either roots or shoots. This increased Cd retention in roots could be due to Si deposition in the roots as found by several authors. For example, Zhang et al. (2008) reported that Cd was mainly deposited in the vicinity of the endodermis and epidermis in rice roots, while Shi et al. (2005b) observed that Si was mainly deposited in the cell walls of the endodermis. However, in our study Si concentrations were not significantly increased in roots, while Cd concentrations increased. In general, mineral nutrients that reach the endodermis must be actively taken up to be uploaded in the xylem.

The increased Cd amount measured in wheat root could have been immobilised at the endodermis as a way of reducing metal toxicity by isolating the metals into metabolically inactive parts, which is also one function of the apoplast (Rogalla and Romheld, 2002; Neumann et al., 1997). Lux et al. (2011) concluded that the development of apoplastic barriers against Cd movement to the xylem, including maturation of the endodermis was stimulated by high external Cd. However external Cd concentrations, as measured in the soil solution after 57 days, were not significantly different between the ASi treatments.

Contrarily, soluble Si concentrations in plants, especially apoplastic soluble Si, might be more representative of the “efficient” Si concentrations in plants than the total Si concentration, as suggested by Iwasaki et al. (2002). In our case, treated plants were continuously exposed to enhanced Si concentrations in the soil solution (Figure 7). Consequently, although Si concentrations in roots did not increase with Si applications, soluble apoplastic Si concentrations might have been larger than in untreated plants and constant over the whole growth period. This possibility was suggested by an increased total Si output in leaves and thus might have stimulated the maturation of the endodermis and increased Cd concentrations in roots. Vaculik et al. (2009) indeed suggested that Si stimulated the development of endodermal suberin lamellae in maize plants grown hydroponically and exposed to Cd + Si, while da Cunha et al. (2008) concluded that Si helped to immobilise Cd and Zn in the endodermis through the thickening of the Casparian strips. Corrales et al. (1997) Observed that Si decreased Al uptake by maize and suggested thar enhanced root exudation or higher pH at the root surface may both lead to Al precipitation ar the root surface.A study of the localisation of the respective Cd and Si distributions within the root tissues, as well as a monitoring of Si concentration in the apoplast, would help to conclude on this point.

In our experimental shoots, Si concentrations were similar regardless of the treatment, while Cd concentrations decreased with increasing ASi soil applications. The decrease in Cd concentrations might be related to both. a restricted root-shoot translocation ability. as indicated by a decrease in the shoot/root ratio from 1.1 to 0.7, and a dilution effect, as indicated by a similar Cd output in shoots of all treated plants, while the Si output increased. In shoots, silica precipitation occurs where water evaporates from the plants (Currie and Perry, 2007; Sommer et al., 2006), which is in the vicinity of the epidermis and mostly as phytoliths. Zhang et al. (2008) have shown that Si decreased Cd accumulation in shoots, and

Cd and Si accumulated simultaneously in phytoliths of rice shoots grown hydroponically. This mechanism cannot be excluded in our case, but it would imply that Si has an impact on Cd compartmentalisation in shoots, and thus on its toxicity, rather than on its concentration. This co-precipitation would restrict Cd translocation from shoots to the grain and would help to reduce grain contamination (Liu et al., 2009). Neumann et al. (1997) reported that zinc was co-precipitated as zinc silicate in the leaf epidermal cell wall of *Minuartia verna* plant species, which served as an explanation for the high zinc tolerance in *M. verna*. More recent studies with cucumber (*Communis sativus* L.) showed that less Mn (10%) was located in the symplast and more Mn (90%) was bound to the cell wall in the Si-treated plants than in the non-Si-treated plants (Rogalla and Romheld, 2002), indicating changes in compartmentalisation. In these cases, However, Si concentrations were increased in shoots of the treated plants. Although these mechanisms cannot be ruled out in our case, they do not seem to be the major mechanism involved in the decrease in Cd concentration in shoots.

Silicon concentrations in the soil solution of pots without plants were always larger than those measured in pots with plants. In the pots without plants, Si concentrations in 10 and 15 ton ASi ha^{-1} treated pots were larger between 41 and 64 days after sowing, indicating that application of amorphous silica released Si in the soil solution, whereas the lowest treatment did not exhibit any effect. This was probably because the amount of amorphous silica was too low. After 64 days Si concentrations decreased and returned to concentrations measured in the control pots, indicating that Si was reorganised within the soil either through adsorption of Si on soil surfaces (Iler, 1979), as a result of complexation with organic and inorganic compounds. or was taken up by the microorganisms (Currie and Perry, 2007; Sommer et al., 2006; Matichenkov and Bocharnikova, 2001; Exley and Birchall, 1993).

The lower Si concentrations measured in solutions from pots with plants, is a good demonstration of plant uptake. Concurrently, similar Si concentrations in plant shoots, regardless of the Si treatment, can be explained both by similar Si concentrations measured in the soil solution and an enhanced Si uptake due to a larger biomass production with increasing Si doses. as already mentioned. This also indicates that, in our case, soil Si release is the limiting factor for Si plant uptake. Except for copper, all metal concentrations in soil solutions were lower in pots with plants than in those without plants. As for Si, these concentration variations can be explained by plant uptake, but the slight increase in pH may also be responsible for a reduction in the metal uptake by the plants through immobilisation in the soil (Bravin et al., 2009; Liang et al., 2005) and a decreased availability to plants. as also indicated by slightly lower levels of DTPA-TEA-extractable metals in ASi-treated pots (Figure 5 and Table 5). Gu et al. (2011) suggested that, in a contaminated acidic soil amended with FA (fly ash) and SS (steel slag), the reduction in Cd, Zn, Cu and Pb uptake by rice was attributed to two processes, including the in situ immobilisation of heavy metals in soil and the Si-mediated effects on rice. The application of FA and SS decreased the heavy metal DGT pools and the fluxes from the soil solid phase to the solution by transforming the soluble metals to the less soluble and slower exchanging forms, such as metal silicates, phosphates and hydroxides. The mechanism was associated to an increase in pH. Putwattana et al. (2010) also showed that silicate fertiliser could effectively immobilise Cd in the soil and decrease its uptake and translocation in sweet basil (*Ocimum basilicum*).

Finally, most of the pot experiments performed with metals and Si used an additional soluble salt to reach high Cd concentrations in soils, and available concentrations were often unrealistic for in situ levels (Cunha and Nascimento, 2008; Liang et al., 2005). Speciation in the soil may change very quickly in the soil when soil is not equilibrated with the treated solution, which means that the effect of Si addition observed on metal availability might well be a combined effect of aging (Keller et al., 2000), higher pH levels and silicate phase addition. The soil we used had been contaminated 25 years prior, and no aging effect has been known to occur during the experiment. Although the initial Cd concentration was rather low, we observed a significant alleviating effect of Si addition to soil on Cd transfer to plants, whereas no alleviating effect was observed on Cu, Zn and Pb.

This study suggests that the alleviating effect of ASi on metal-induced stress, as evidenced by an increase of biomass with increasing doses of ASi applied to soil, is mainly due to changes in the Cd balance. As suggested by Liang et al. (2005), we observed a combined effect in both the soil and in plants; silica application induced a decrease in available Cd in the soil, while at the plant level, Cd was sequestrated in roots more efficiently when ASi had been added and Cd translocation to shoots was reduced. Biomass production was then increased either because of a Si fertilising effect or because Cd concentrations were reduced and contributed in turn to an additional diluting effect of Cd concentrations in shoots. As suggested by the results, Si release in the soil is the limiting factor for Si plant uptake. This is because Si concentrations in the soil solution of pots with plants were low regardless of the dose application and because no plateau was observed in the Si and Cd concentration trends in plants, although the highest rate applied was already larger than the Japanese recommended optimum rate for rice of 1.5-3.0 t ha^{-1} of silicate slag (Korndörfer and Lepsch, 2001). Larger ASi doses may thus provide even more significant results than what were established in this study. This would also help to better quantify the respective aspect of the various processes involved in alleviating Cd toxicity in wheat.

In conclusion, application of ASi amendment to soil may contribute to decreased Cd concentrations in wheat shoots, even if initial Cd concentrations in soil are limited or if Cd is combined with other. Adding ASi to soils may thus be part of a general protocol aiming to control Cd concentrations in wheat shoots and as a consequence in grain, along with other management practices (Gao et al., 2010; Perilli et al., 2010).

II. Effect of silicon on reducing cadmium toxicity in durum wheat (*Triticum turgidum* L. cv. Claudio W.) grown in uncontaminated and cadmium spiked soils

Abstract

A pot experiment was conducted to investigate the effects of amorphous silica (ASi) application on alleviating cadmium (Cd) phytotoxicity in durum wheat grown in uncontaminated and Cd spiked soils. For this purpose we used ASi that had been extracted from a diatomite mine at a rate of 0, 1, 10 and 15 ton ASi ha^{-1} and without and with Cd (10 mg kg^{-1}) supply. Soils were tested without and with plant growth for 71-day in the greenhouse. ASi supply increased plant Si concentrations in both without and with Cd treated soils and the effect was significant under Cd stress as compared to control. In uncontaminated soil, Si did not show any beneficial affect but in Cd spiked soil the ameliorative effect of Si may due to lower Cd uptake and translocation to shoots while larger uptake and translocation of Zn and Cu.

Key words: Metals, uptake, silicon, wheat, remediation

II.1 Introduction

Cadmium (Cd) is highly toxic metal for both plants and animals and is released into the environment largely due to mining, industrial and agricultural activities (Toppi and Gabbrielli, 1999; Das et al., 1998; Grant et al., 1998). In agricultural soils, Cd mainly enters through pesticides, sewage sludge and fertilizers application (Wagner, 1993). It readily accumulates in the different plant parts and organs depending on the plant species and cultivars (Kabata-Pendias and Pendias, 2001; Gill et al., 2011). During plant uptake, Cd ions can compete for the same trans-membrane carriers with the nutrients such as Fe, Cu and Zn (Benavides et al., 2005). Several symptoms of Cd toxicity have been observed in plant shoots and roots such as reduction in shoot and root biomass, photosynthesis and oxidative stress (Sandalio et al., 2001; Mobin and Khan, 2007; Chen et al., 2011).

Wheat (*Triticum aestivum* L.*)* is one of the World's major crops in terms of food production. Wheat can accumulate much higher Cd concentration as compared to other cereal crops (Sarwar et al., 2010). However, among wheat cultivars, durum wheat (*Triticum turgidum* L.) is more sensitive to Cd toxicity as compared to bread wheat (Koleli et al., 2004). Wheat plants can absorb Si from solution culture (Rafi and Epstein, 1999). Active uptake of Si has been reported in wheat plant (Rains et al., 2006). Although wheat is Si accumulator plant (Mayland et al., 1991), it has received little attention as compared to other crops like rice and maize. It is generally recognized that Si is beneficial for plant and plays an important role in alleviating both abiotic and biotic stress in plants (Liang et al., 2007; Guntzer et al., 2011). It has also been shown that Si can reduce Cd toxicity in many plant species such as rice (Zhang et al., 2008) cucumber (Shi et al., 2005) and maize (da Cunha and do Nascimento, 2009). Si can alleviate Cd toxicity by enhancing the capacity of the roots to trap Cd and decreasing translocation to shoots of rice (Zhang et al., 2008). In maize, decrease Cd toxicity may be due to Si deposition in the endodermis of roots (da Cunha and do Nascimento, 2009; Da Cunha et al., 2008). However, the mechanisms of Si-mediated Cd tolerance remain poorly understood particularly relating to uptake and transport of Cd and other metals like Zn and Cu and thus need further investigation.

From previous studies, it can be concluded that understanding of both external and internal effects of Si on plants will be helpful in further understanding of Si-mediated metal resistance mechanisms. In the previous chapter of this thesis we found that in hydroponics, Si is reducing Cd and Cu toxicity in wheat by enhancing Zn uptake and translocation along with other mechanisms as well (Chapter 4 part I and II). By contrast, in the previous part of this chapter we found that application of Si in the form of amorphous silica in the soil decreased Cd translocation from roots to shoots by efficiently sequestrating Cd in roots but did not affect the other metals like Zn, Cu and Pb despite the fact that this soil was contaminated with these metals prior to 25 years ago. Therefore further investigation is needed to clarify how Si ameliorates Cd toxicity in plants grown in soils only contaminated with Cd. In the present study we hypothesized that the mechanisms of Si-mediated alleviation of Cd toxicity in wheat plant may be different in soil only spiked with Cd as compared to aged contaminated soils. To test this hypothesis, we investigated the effects of amorphous silica on Cd, Zn and Cu bioavailability, uptake and translocation in durum wheat plants grown in uncontaminated and Cd spiked soils in the greenhouse.

II.2 Materials and Methods

II.2.a Materials

The agricultural soil was collected from Gréoux-les-bains (340 m above sea level) which is a municipality commune in the Alpes-de-Haute-de-Provence in Southeastern France. It is a clay loam soil and is not contaminated. It was cultivated with cereals at the time of sampling and its pH is around 8. Soil samples were taken at the surface (0-20cm depth) at several different points, mixed, homogenized, dried and sieved to 3mm. The main physicochemical characteristics of the soil are given in Table 1. Durum wheat (*Triticum turgidum* L. cv. Claudio W.) was grown in the soil treated or not with Cd and without and with Si applications. For Si we used a natural ASi powder (siliceous fossil meal, Clarcel 78®). For detail of ASi see previous part of this chapter

Table 1: Initial physicochemical properties of the Gréoux soil used in the pot experiment.

Physicochemical properties	
pH (1/2.5 soil to water ratio)	8.2
Organic C (mg g^{-1})	14.4
Total N (mg g^{-1})	1.38
Olsen P (mg g^{-1})	0.038
Sand (mg g^{-1})	199
Silt (mg g^{-1})	524
Clay (mg g^{-1})	277
Cation Exchange Capacity (cobaltihexamine; $cmol^{+}$ kg^{-1})	14.6
Si Tamm[a] (mg g^{-1})	0.59
Si CBD[b] (mg g^{-1})	1.34
Total Cd (mg kg^{-1})	0.3
Total Zn (mg kg^{-1})	81.8
Total Cu (mg kg^{-1})	24.6
Total Pb (mg kg^{-1})	21.5

[a] Tamm extraction (Tamm, 1922) ; [b] CBD citrate bicarbonate dithionite extraction (Mehra and Jackson, 1960)

II.2.b Protocol of the experiment

Experimental design

The experiment was conducted in a greenhouse at 18-25 °C and 60% humidity. Soil was divided into two portions and one was artificially spiked with 10 mg kg^{-1} Cd in the form of $Cd(NO_3)_2.4H_2O$. Cadmium was mixed one day before transferring the soil into the pots. In order to avoid heterogeneous distribution of the Cd added at such a small rate, cadmium nitrate tetra hydrate was first dissolved in 50 ml water and then thoroughly mixed in the soil. After mixing Cd, the remaining protocol of the experiment was performed according to the previous part of this chapter (I.2.b). In brief, silica was added at a rate of 0, 1, 10 and 15 ton ASi ha^{-1} to each soil batch treatment and thoroughly mixed by hand. These effective doses of Si were calculated by taking a 20-cm depth of soil with a density of 1.49 g/cm^3 ha^{-1}, assuming that the diatomite was 87% pure SiO_2. Each batch was then divided to fill the replicated pots. All treatments were performed in three replicates without plants and four replicates with plants. Pots were initially seeded (mid February 2009, immediately after mixing with ASi and Cd) at a density of six seeds per pot then thinned to four individuals per pot after five days of germination. Each pot was fertilized with 30-ml of fertilizer solution.

Plant, soil and soil solution sampling and analyses

Plants were harvested after 71 days, just before booting stage, by cutting the shoots approximately one centimetre above the soil surface. Plant harvesting and sampling and analyses were performed according to previous part of this chapter (see I.2.b Protocol of the experiment). The only difference is the soil solution sampling times which were taken at 43, 59, 64 and 71 days after sowing.

Statistical treatment

Student's t-test was performed to test whether the average concentrations in the treated pots differed from those of controlled pots. Results that were significantly different were marked by (*) for $p \leq 0.05$, (**) for $p \leq 0.01$ and (***) for $p \leq 0.001$. Statistics were performed using the XLStat Package (Addinsoft, Paris, F.).

II.3 Results

II.3.a Effect of ASi treatments on plant parameters

Plant growth and biomass

To the naked eye, Si application did not affect the shoot length in both un-amended and Cd amended soils (Figure 1). In soil without Cd treatment, a significant increase in shoot dry weight (24% that of control) was observed in 1-ton ASi ha^{-1} pots while in roots significant increase (approximately 15% of the control) was observed in 15-ton ASi ha^{-1} pots while there was no affect of Si in larger Si treatments. However, shoot dry weights decreased in all ASi treatments in the Cd treated pots while root dry weights almost remained the same (Figure 2).

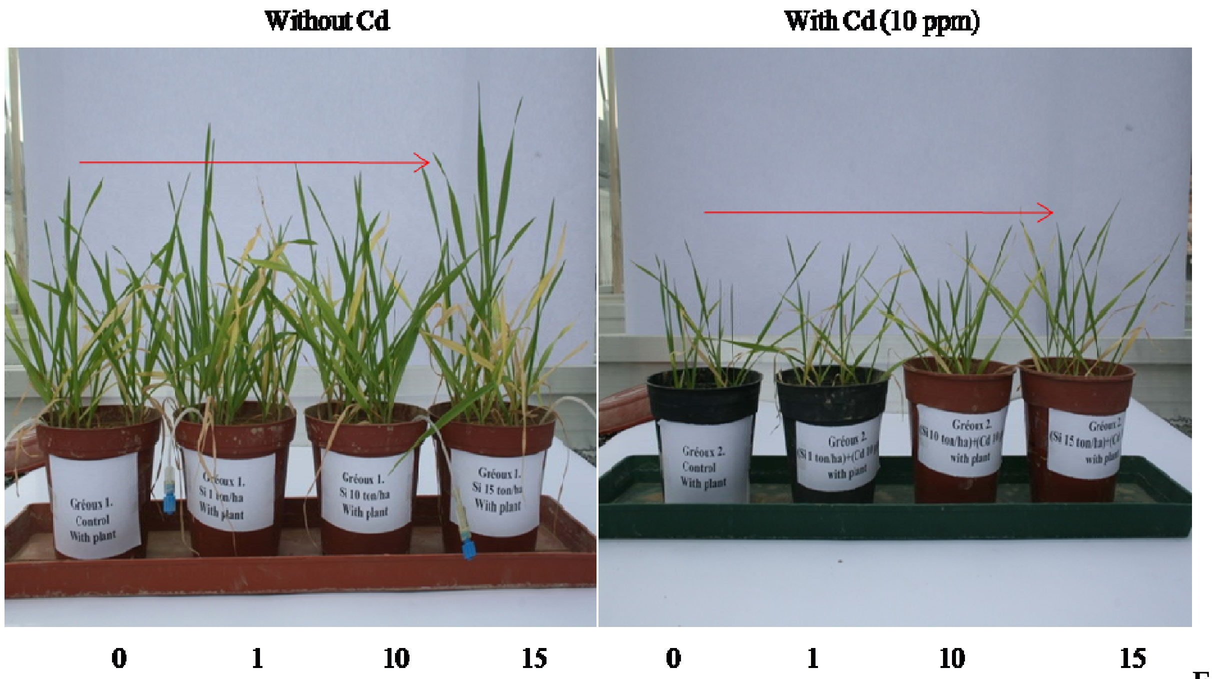

Figure 1. Wheat plants grown in pots from soil without and with Cd addition and treated with increasing doses of silicon: 0 (control), 1, 10 and 15 ton Si ha^{-1} applied as amorphous silica (ASi). The effect of silicon on growth can be seen in differences of the length of the above ground parts in each treatment.

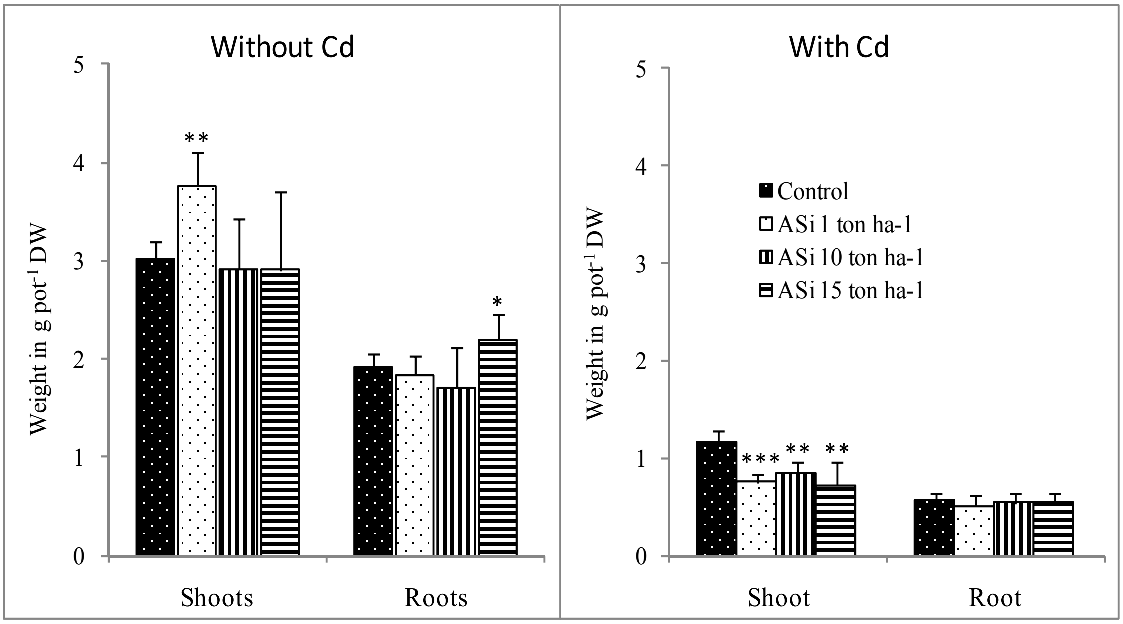

Figure 2. Dry weight of wheat plant shoots and roots grown in soil without and with Cd addition and treated with increasing doses of silicon (0, 1, 10 and 15 t Si ha^{-1}) applied as amorphous silica (ASi). Bars represent SD of four replicates. *means significance with relative to respective control of Student's t-test at $p<0.05$, (**) at $p<0.01$ and (***) at $p<0.001$.

Silicon uptake and accumulation in shoots and roots

ASi application in the soil increased, in general, the Si concentration in wheat plants as compared to control (Figure 3). The shoot Si concentrations were larger than roots in both without and with Cd treated plants. A significant increase in shoot Si concentration was observed in both without and with Cd treated plants with ASi supplementation except in shoots of 1-ton ASi ha^{-1} pots without Cd treatment. The maximum significant increase in root Si concentration was observed in 15-ton ASi ha^{-1} without Cd treatment while a highly significant decrease was observed in the same ASi treatment with Cd application but it almost remained same in other treatments. Si shoot-to-root ratios of plants without Cd are 2.5, 2.3, 3.0 and 1.8 and of Cd treated plants are 1.4, 2.1, 2.2 and 4.9 for 0, 1, 10 and 15-ton ASi ha^{-1} treatments respectively. This indicates that shoots-to-roots ratios are lower in Cd treated plants than non treated plants. Plant uptake was calculated using Si concentrations multiplied by the biomass production. In both, un-amended and Cd-amended soil, shoot and roots total Si uptake increased with increasing doses of ASi supplementation but total Si uptake is lower in Cd treated soil as compared to without Cd treated soil which is due to lower biomass production in this soil (Annex 4 table 1).

Effect of silicon on metal distribution in plants

Cadmium concentrations were below detection limit in roots of plants without Cd treatment while in shoots Cd concentrations significantly decreased with increasing doses of ASi supplementation (Figure 4). In Cd treated plants, Cd concentrations were larger than in the non Cd-treated plants. In shoots of Cd-amended plants, Cd concentrations highly significantly decreased (atleast 41% of control) with increasing doses of ASi in the pots. In roots, Cd concentration slightly increased in the roots of Cd treated plants with ASi supplementation. Total Cd uptake in shoots significantly decreased while in roots it almost remained same with ASi supplementation in both treated or not with Cd (Figure 5).

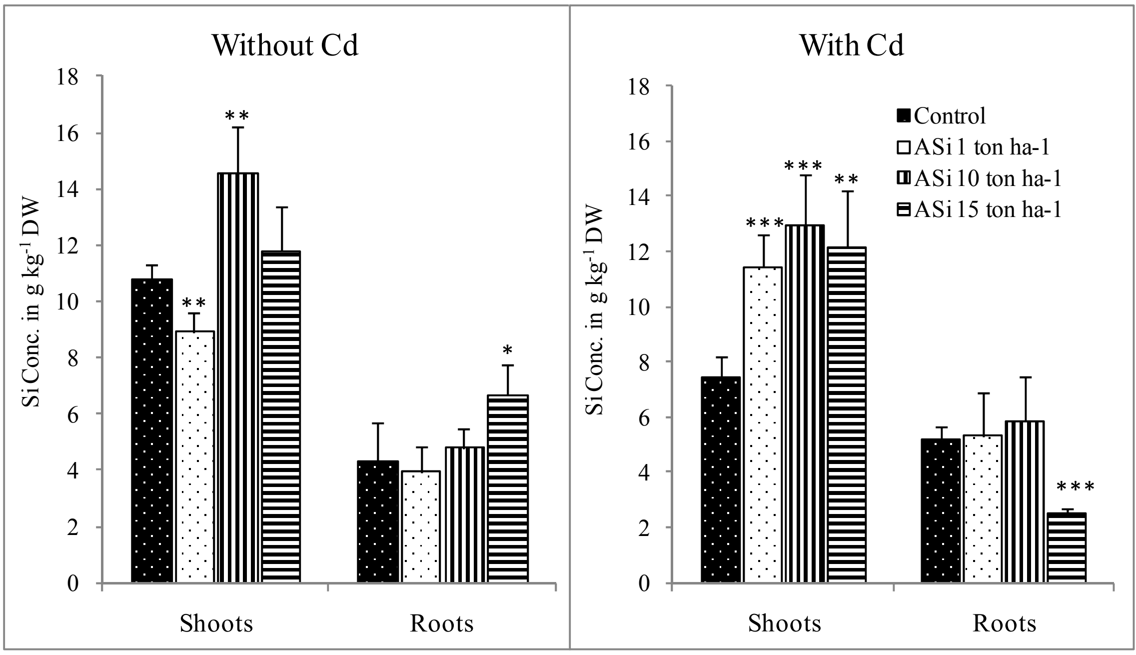

Figure 3. Si concentration (g kg^{-1} DW) in shoot and root grown in a soil without and with Cd addition and treated with increasing doses of amorphous silica (ASi). Bars represent SD of four replicates and * means significance with relative to respective control of Student's t-test at $p<0.05$, (**) at $p<0.01$ and (***) at $p<0.001$.

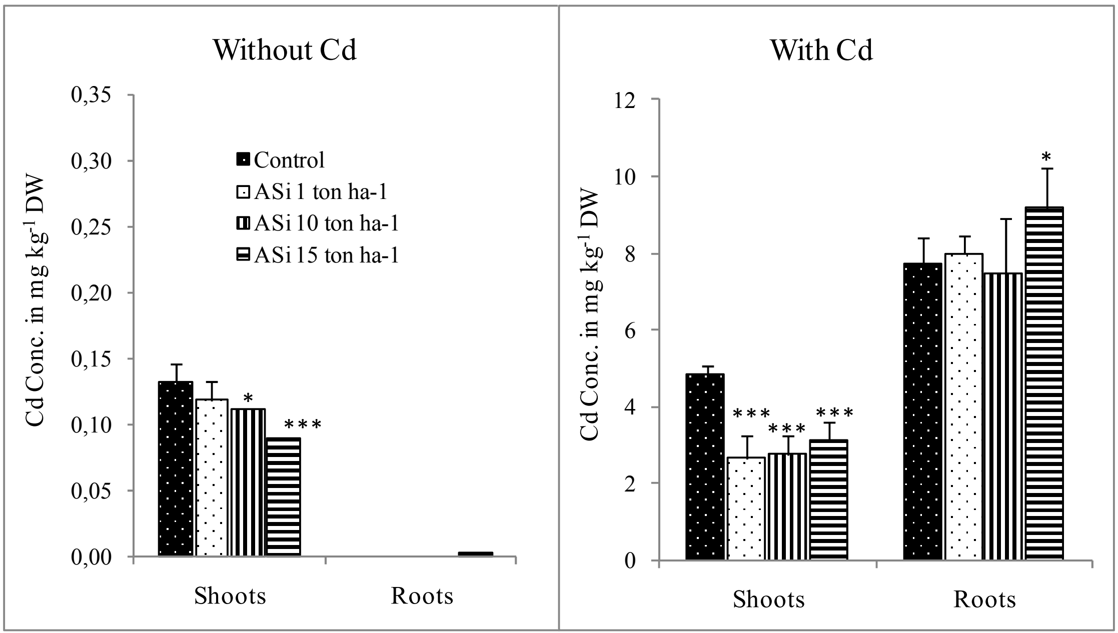

Figure 4. Cadmium concentration in mg kg^{-1} DW in shoots and roots of wheat plants grown in a soil without and with Cd addition and treated with increasing doses of amorphous silica (ASi). Bars represent SD of four replicates and * means significance relative to control plants of Student's t-test at $p<0.05$. (**) at $p<0.01$ and (***) at $p<0.001$.

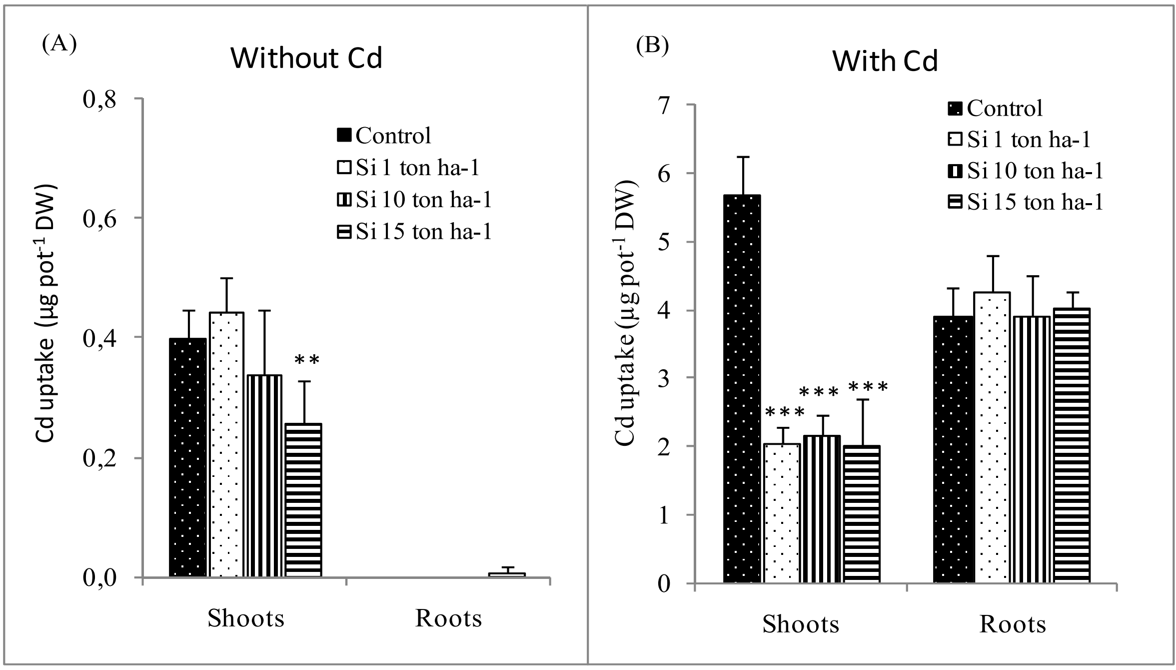

Figure 5. Cadmium uptake in µg kg^{-1} DW in shoots and roots of wheat plants grown in a soil without and with Cd addition and treated with increasing doses of amorphous silica (ASi). Bars represent SD of four replicates and * means significance relative to control plants of Student's t-test at $p<0.05$. (**) at $p<0.01$ and (***) at $p<0.001$.

In shoots of plants without Cd treatment our results showed that Zn and Cu concentrations almost remained same or slightly decreased with ASi supplementation while in roots Zn and Cu concentration slightly increased and decreased respectively with ASi treatments (Figure 5 and 6). Zn and Cu concentrations highly significantly increased in shoots of Cd treated plants with ASi supplementation. In roots of Cd treated plants, Zn concentrations slightly increased while Cu concentrations significantly decreased ASi supplementation. In plants without Cd treatment, Zn and Cu shoot-to-root ratios decreased with increasing ASi supplementation while in Cd treated plants these ratios increased with increasing ASi supplementation (Table 2).

Table 2: Zn and Cu shoot-to-root ratios of wheat plants grown in a soil without and with Cd (10 mg kg^{-1}) application and treated with increasing doses of ASi.

Si application (ton ha^{-1})	Zn		Cu	
	Without Cd	With Cd	Without Cd	With Cd
0	0.85 ± 0.14	0.98 ± 0.19	1.26 ± 0.31	0.56 ± 0.02
1	0.81 ± 0.04	1.01 ± 0.47	1.19 ± 0.04	1.82 ± 0.07
10	0.72 ± 0.12	1.39 ± 0.24	1.22 ± 0.01	1.53 ± 0.18
15	0.75 ± 0.07	1.10 ± 0.26	1.00 ± 0.03	1.31 ± 0.03

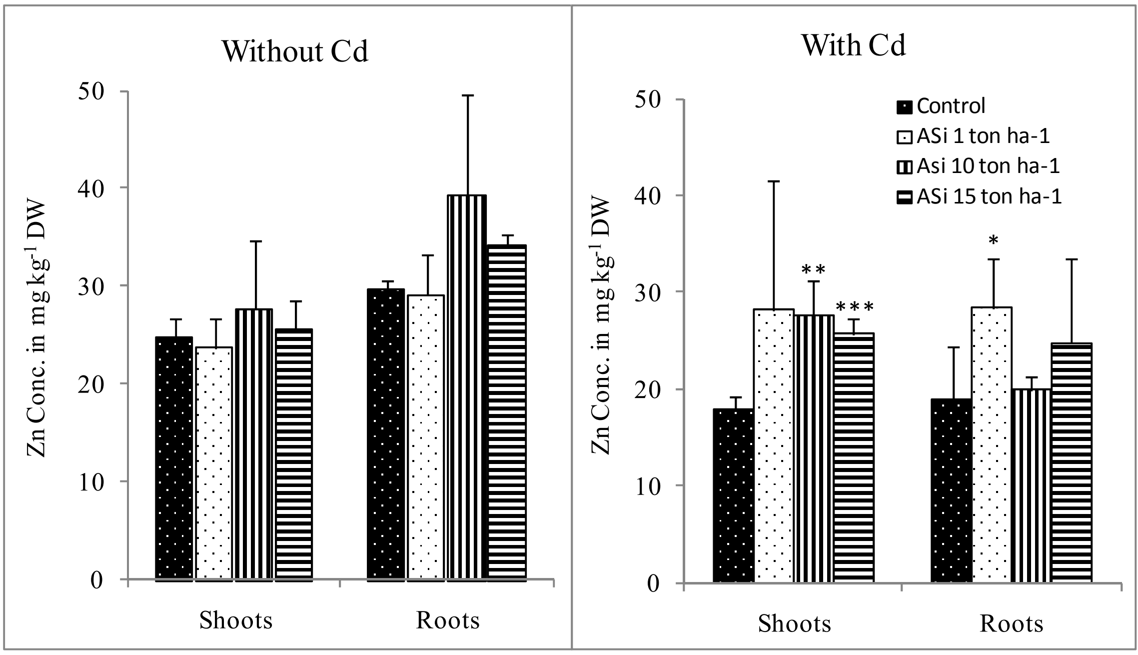

Figure 6. Zinc concentration (mg kg^{-1} DW) in shoots and roots of wheat plants grown in a soil without and with Cd addition and treated with increasing doses of silicon. Values are means ± SD (n=4). * means significance with relative to respective control of Student’s t-test at $p<0.05$ and (**) at $p<0.01$ and (***) at $p<0.001$.

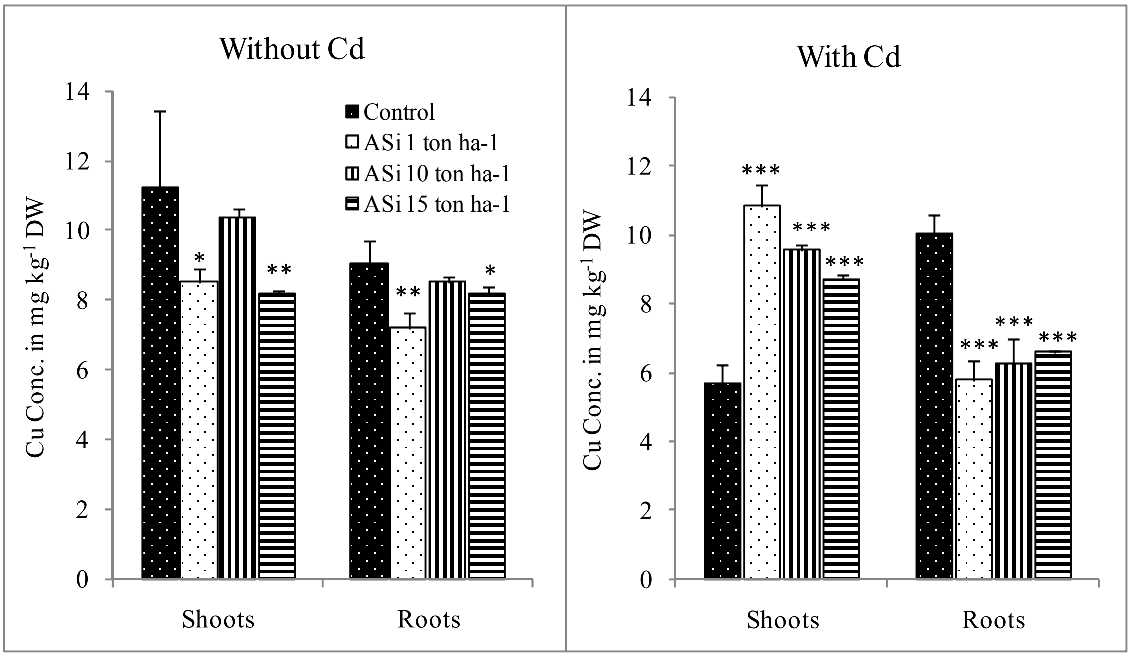

Figure 7. Copper concentration (mg kg^{-1} DW) in shoots and roots of wheat plants grown in a soil without and with Cd addition and treated with increasing doses of ASi. Values are means ± SD (n=4). * means significance with relative to respective control of Student’s t-test at $p<0.05$, (**) at $p<0.01$ and (***) at $p<0.001$.

II.3.b Effect of ASi treatments on soil characteristics

Soil pH

In both without and with Cd treated soils, pH in pots with plants was larger than pots without plants. In either soils, amended or not, pH slightly increased in pots with plants treated at 10 and 15 ton ASi ha^{-1} while in pots without plant growth there was slight increase or decrease in pH with ASi supplementation (Annex 4 table 2).

Effect of silicon on trace elements concentrations in DTPA extracts

Concentrations of trace elements (Cd, Zn and Cu) in soil were determined after wheat harvest. In pots without Cd treatment, Cd concentrations significantly decreased in pots with plant growth with ASi supplementation while in pots without plant growth Cd concentrations were slightly lower in ASi treated pots compared to control (Figure 8). In Cd treated pots, there was no significant effect of ASi supply on Cd concentrations in both without and with plant grown pots. Zn concentrations were larger in pots without Cd treatments than pots with Cd treatments and slightly decreased with ASi supplementation. Cu concentrations almost remained the same or slightly increased or decreased in both pots without and with Cd treatments sown or not with plants (Annex 4 table 3).

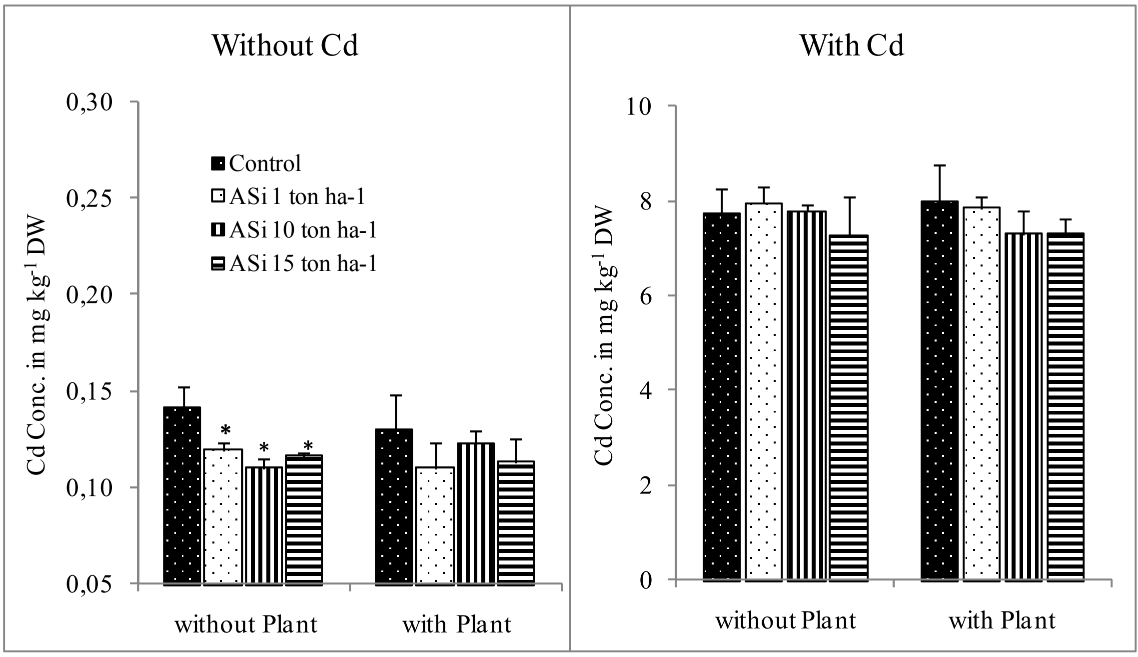

Figure 8. Effect of amorphous silica (ASi) application on DTPA-TEA extractable Cd concentration in a soil without and with Cd addition and planted or not with wheat plants. Concentration in mg kg^{-1} is measured after harvest. Bars represent SD of four replicates (with plant) or three replicates (without plant) and * means significance relative to control of Student's t-test at $p<0.05$.

II.3.c Effect of ASi treatments on the soil solution

pH and Si concentration

Soil solution pH was measured before harvesting of wheat plants. Soil solution pH of Cd treated pots was lower than pots without Cd treatment (Figure 9). In pots without Cd treatment, there was no significant effect of ASi supplementation on the pH of soil. The soil solution pH of Cd treated pots significantly decreased both without and with plant growth with ASi supplementation.

In both untreated and Cd treated soils, the silicon concentrations were always larger in the soil solutions collected from pots without plant than in soil solutions from pots with plants and decreased with time in both pots without and with plant growth. (Annex 4 figure 1 and 2).

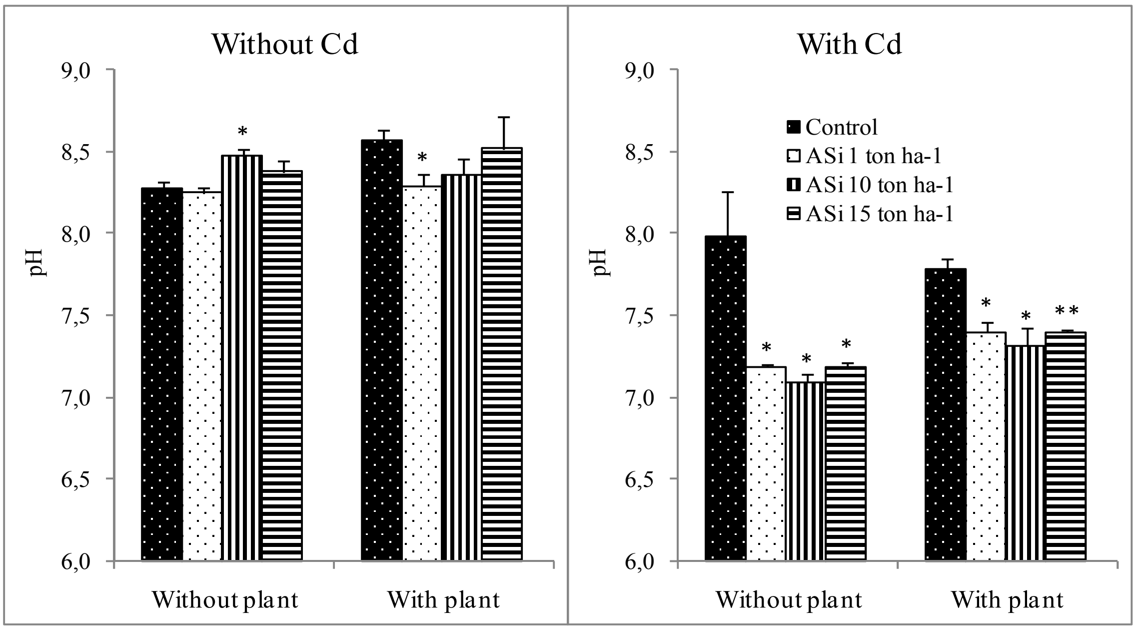

Figure 9. pH of the soil solution collected by Rhizon® from pots with a non contaminated and artificially Cd contaminated soils before harvesting of wheat plants (71 days after sowing). Values are mean ± SD (n =2). *means significance relative to control of Student's t-test at $p<0.05$ and (**) at $p<0.01$.

Trace element concentrations

In the soil solution Cd, Zn and Cu concentrations were measured once after 59 and 55 days of sowing in untreated and treated with Cd pots respectively because this was when Si concentrations in the soil solution exhibited the largest differences among treatments. We assumed that at this stage the potential effect of ASi on trace elements would be the largest as well.

In the soil without Cd treatment, there was no effect of ASi on Cd concentrations in both soil solution without and with plants (Figure 10). In Cd treated soil, Cd concentrations in soil solution with plants were significantly lower in all ASi treatments compared to control. In soil solution without Cd treatments, Zn concentrations remained almost same or slightly increased in pots both without and with plant growth in all ASi treatments except in 15 ton ASi ha^{-1} treatment (Annex 4 table 4). In Cd treated soil, Zn concentrations in soil solution were lower compared to untreated soil and slightly increased in both pots with ASi application. Cu concentrations in Cd treated soil solution were lower compared to untreated soil solution and slightly increased in both soil solutions with ASi treatments ((Annex 4 table 4).

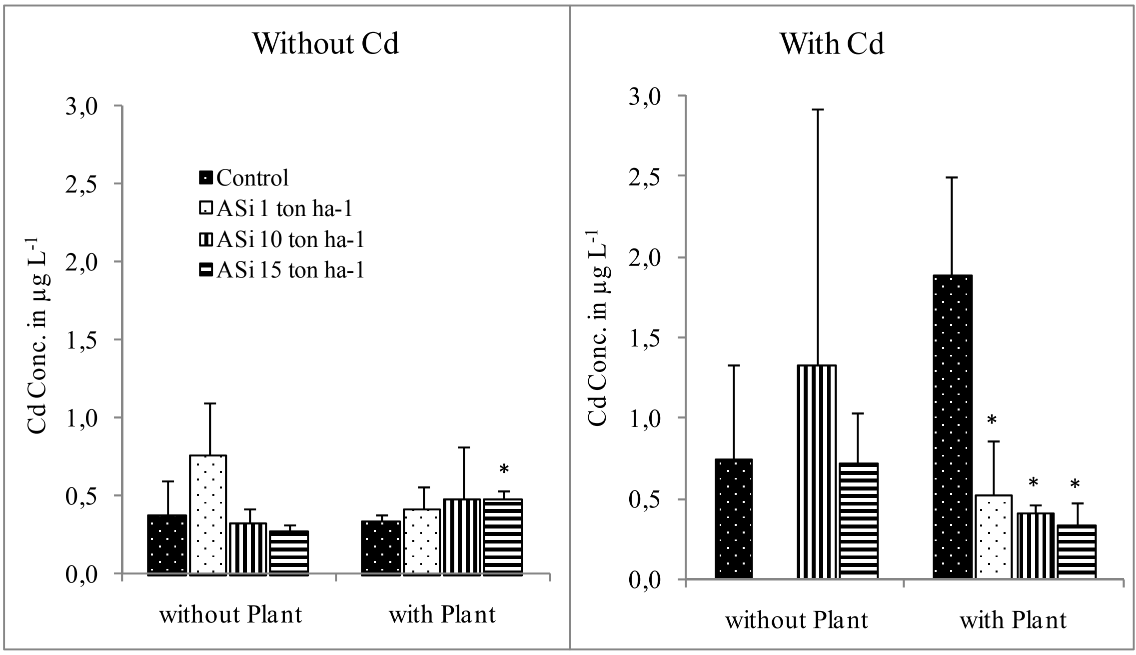

Figure 10. Cd concentration in µg L^{-1} measured in the soil solution collected by Rhizon® 59 days after sowing in pots of a soil without and with Cd addition and planted or not with wheat. Bars represent SD of two replicates and * means significance relative to control of Student's t-test at $p<0.05$.

II.4 Discussion and conclusion

In present study, the Si supply did not increase the shoots and roots biomass of Cd amended plant. By contrast, in the previous part of this chapter we found that Si supply increased shoot and root biomass in aged contaminated soil with Cd, Zn, Pb and Cu. The reduction in growth in the present study can be explained in the way that this dose of Cd (10 mg kg^{-1}) may be highly toxic to wheat plants and/or the Si is able to reduce Cd toxicity in soils with slight contamination. However, in the present study Si is still significantly reducing Cd translocation to shoots as compared to control (Figure 4) and increasing Zn and Cu uptake and translocation to shoots (Figure 6 and 7) which can imply that the positive effects of Si on plants may be seen at later stages of plant growth.

In Cd treated plants Si concentration significantly increased in shoots with increasing doses of ASi compared to control while in roots it almost remained same or decreased (Figure 3). Similarly, Si supply significantly decreased Cd concentrations in shoot and increased in roots (Figure 4). These results are similar to our previous results of this chapter indicating that ASi addition reduced Cd in shoots of wheat plant grown on contaminated soil but disagree with authors who found Si-mediated increase in both shoots and roots Cd concentration in maize (Vaculik et al., 2009) or decrease in both shoots and roots Cd concentrations in rice plant (Shi et al., 2005; Nwugo and Huerta, 2008). In the present study, it is obvious that ASi supplementation significantly decreased Cd concentration in shoots of Cd spiked soil but these Cd concentrations in shoots may still toxic to plants which may be observed by the lower biomass production from plants grown in this soil compared to biomass of plants grown in un-spiked soil.

Copper concentrations, in general, slightly decreased and Zn concentrations not affected by ASi supplementation in soil without Cd addition but in control of Cd amended soil, shoot Zn and Cu concentrations are in a deficiency range (Figure 6 and 7), with Zn concentrations in shoots below 20 mg kg^{-1} DW and Cu concentration below 5 mg kg^{-1} DW (Kabata-Pendias and Pendias 2001). By contrast, Zn and Cu concentrations significantly increased in shoots unlike in roots with ASi supply in Cd spiked soil (Figure 6 and 7) but these were still lower than in untreated soil. Zn and Cu are micronutrients are needed for better growth and development (Kabata-Pendias and Pendias, 2001). In our study Si is regulating the shoot concentration of these metals by increasing them when plant uptake is lower especially under Cd stress. As a result Si reduced Cd translocation from root to shoot (Table 2) by increasing Zn and Cu translocation in Cd spiked soil (Figure 6 and 7). These results disagree with the authors who found decrease in both Zn (Song et al., 2011; Gu et al., 2012) and Cu (Frantz et al., 2011) translocations with Si supply or increase in Zn and Cd concentrations with Si addition (da Cunha et al., 2008; da Cunha and do Nascimento, 2009). Da Cunha et al. (2008) and Da Cunha and do Nascimento, (2009) reported that Si application up to 150 mg kg^{-1} of soil increased the Cd and Zn concentrations both in shoots and roots of maize grown in soils spiked with 10 and 100 mg kg^{-1} Cd and Zn respectively. However, in our study initial Zn concentrations were slightly lower (81.8 mg kg^{-1}) than above studies but ASi supply decreased Cd and increased Zn and Cu concentrations in shoots under Cd stress. These higher concentrations of Zn and Cu in shoots may be helpful in reducing Cd toxicity. For example Cakmak et al., (2000) reported that in mature leafs of durum wheat increased Zn supply (0.5µM) reduced Cd re-translocation in shoots. They argued that this may be due to inhibitory action of Zn in shoot tissues on phloem mediated Cd transport. Similarly, Welch et al., (1999) showed that in durum wheat Zn reduced Cd phloem loading into wheat grain even when net Cd uptake is slightly enhanced due to the presence of Zn. Cd toxicity in durum wheat become more sever under Zn deficient conditions and decreased Zn concentrations in Zn deficient plants (Koleli et al., 2004).

In our study ASi supply increased Zn and Cu translocation under Cd stress but not without Cd stress. This suggests that Si may change the membrane permeability only under Cd stress and is involved in nutritional changes in plants grown without and with Cd stress. It has also been found in our previous study (Chap. 4 part I) that under Cd stress, Si application increased Zn uptake by roots while less was immobilized as apoplasmic Zn. Similarly, under Cu stress (Chap. 4 part II), Si is reducing Cu translocation to shoots by decreasing Cu uptake and increasing apoplasmic adsorption of Cu while increasing Mn and Zn uptake. Moreover, in the previous part of this chapter, ASi application in Cd and Zn aged contaminated soil slightly increased Zn concentrations in both shoots and roots of wheat plants but not affected the Cu concentration. However, in present study ASi supply in the soil is differently modifying Zn and Cu translocation in uncontaminated and Cd contaminated soils to alleviate metal toxicity. This clearly indicates that without and with Cd stress, presence of Si changed the uptake and distribution of metals within plants to alleviate the toxicity of metals. Under Cd stress, Cu concentrations are larger translocated to shoots when ASi supply is 1-ton ha^{-1} (Table 2). This indicates that Si is having an effect even at its low concentrations. It is thus clear that the mechanisms of Si-mediated alleviation of metal toxicity in plants may change with metal, soil and experimental (growth room vs green house) conditions along with other factors as well.

In conclusion, our results in this study showed that the alleviating effect of ASi on Cd-induced stress, as evidenced by decrease in Cd concentrations in shoots of Cd spiked soil, is mainly due to changes in the Cd, Zn and Cu balance. We observed a combined effect in both the soil and in plants; silica application immobilized Cd in soil while at the plant level, Cd was sequestrated in roots more efficiently when ASi had been added and Cd translocation to shoots was reduced and Zn and Cu uptake and translocation to shoots increased as compared to control. However, in un-amended soil there was no effect of ASi addition on plants nutrient uptake and growth parameters.

III Conclusion chapter 5

Results of the above two studies showed that Si concentration in wheat shoots and roots increased with increasing doses of ASi in the soil. ASi application also decreased Cd concentration in plant shoots and increased the pH of soils and the effect was clear in aged contaminated soil as compared to artificially contaminated soil but in non contaminated soil there was no effect of silicon. Based on our results we conclude that the application of ASi amendment to soil may decrease Cd concentrations in wheat shoots which may be due to increase in biomass or decrease in Cd uptake and translocation and increase in Zn and Cu uptake and translocation.

Chapter 6: General conclusion

Plant productivity is seriously limited by heavy metal toxicity. Studies suggest that Si can alleviate the metal toxicity in plants but its detoxification mechanisms and bioavailability in soil is not well defined. A major objective of this thesis was to identify the physiological effects of Si on reducing Cd and Cu toxicity in durum wheat. For this purpose we launched two hydroponic experiments with increasing Cd and Cu treatments with or without 1mM Si. Results related to both Cd and Cu studies showed that **Si supply in solution decreased Cd and Cu uptake, translocation and toxicity symptoms in durum wheat seedlings by increasing root apoplasmic bound metal and Zn uptake.** Increasing metal concentrations increased the physiological stress in wheat roots and shoots while Si-mediated lower uptake and translocation of metal decreased physiological stress in plants that resulted in a larger production of photosynthetic pigments and lower production of organic acids especially citrate and aconitate in shoots and roots

Co-precipitation of Cd together with Si was not found to be a major mechanism, neither at the shoot nor at the root level because SEM-EDX analyses showed that there was no correlation between Si and Cd in roots and phytoliths EDX spectra showed that there was no Cd in phytoliths. µXRF results confirmed that Cu and Si was not co-deposited in roots under Cu stress but in the presence of Si, Cu and Mn were located in the epidermis while in the absence of Si, Cu was located in the central cylinder of roots which clearly indicates that Si is changing the distribution of Cu in roots. In addition, presence of Cu did not change the distribution of Si in roots. X-ray Photoelectron Spectroscopy analyses performed on freeze dried root surfaces demonstrated that most of the surface (< 100 Å) root layer was enriched by both Si and Cd in solution containing 50 µM Cd and 1 mM Si compared to without Si addition but the distribution of these Cd-retention sites was not homogeneous over the root surface.

One focus of the thesis work was to evaluate the efficiency of different minerals to release bioavailable Si and the effect of this Si on reducing Cd and Cu toxicity in durum wheat. We used clay and diatomite minerals, mixed them in different proportions with quartz by weight and wheat was sown with 2 ppm Cd and 20 ppm Cu separately. Results showed that Si present as amorphous Si (diatomite) was about 2.2 times more available for plant uptake as compared to vermiculite. However part of Si brought by vermiculite was available so the soils with large clay content, clay may be a significant source of Si for plants. Amorphous Si alleviates metal stress induced by toxic amounts of Cd or Cu in soils and thus increases wheat tolerance to metals in soil. To our knowledge, this is the **first study which indicates the importance of clays with plant available silicon and their role in metal detoxification in plants.**

In the pot experiment with different soils, ASi_{Na} was well correlated with the Si in plants shoots which indicated that ASi_{Na} can also be used for a good proxy of Si bioavailable to plants. Because ASi is more available to plants, we used it for pot experiment in order to evaluate its potentiality for soil remediation. Two types of soils were used; one was historically contaminated with heavy metals while other was not contaminated. Half portion of uncontaminated soil was artificially spiked with 10 ppm Cd. Results showed that in historically contaminated soil, Si supply increased shoots and roots biomass and reduced the Cd uptake by wheat plants by immobilizing Cd in soil which may be due to increase in pH with ASi application. Si supply decreased DTPA-TEA extractable Cd in both soils with and without plant. Si supply efficiently sequestrated the Cd in roots and decreased translocation to shoots. Although this soil was also contaminated with Zn and Pb, Si addition did not influence the uptake and translocation of these metals. **This showed that under mixed contamination Si effect on reducing Cd toxicity is more efficient as compared to other metals**. When we spiked Cd in the uncontaminated soil, Si changed the balance of Cd and other metals like Zn and Cu both in soil and plant. Zn and Cu uptake and root-to-shoot translocation increased under Cd stress while without Cd stress there was no significant effect of Si on these metals. However, both Zn and Cu concentrations in shoots were in normal range of plants. This indicated that when there is only Cd toxicity, Si is reducing Cd uptake and translocation by increasing the uptake and translocation of other metals. In un-spiked soil there was no effect of Si on plants nutrient uptake and growth parameters which indicates that Si is not performing better functions under normal condition.

The results of this thesis work clearly indicate that Si could be used to remediate metal contaminated soils associated with durum wheat production. Overall positive effects of Si application under metal stress which were observed in this thesis work are presented in figure 1.

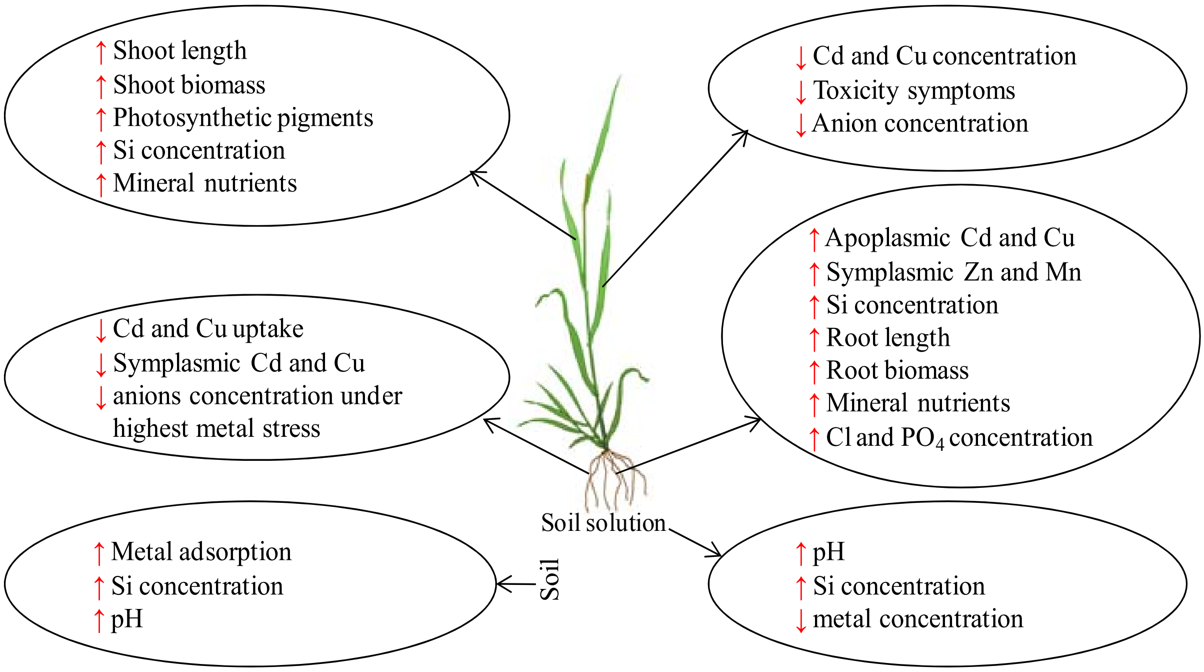

Figure 1. Positive effects of Si on the wheat plants both at the soil and plant levels. (↑) indicates the increase in value and (↓) indicates decrease in the values.

Conclusion générale

La productivité des plantes peut être fortement impactée par la présence de métaux dans leur milieu de croissance. Le silicium peut augmenter la tolérance au stress métallique des plantes, mais les mécanismes impliqués sont encore plus connus.

Nos résultats confirment ces constatations et permettent de mettre en évidence certains points supplémentaires. Le silicium « actif » dans le sol, et donc à même d'être prélevé par les plantes, n'est pas mesuré de manière standard, or son estimation est un pré-requis afin de vérifier la disponibilité de Si pour les plantes. Nous montrons que ASi extrait par Na_2CO_3 est bien corrélé avec les concentrations en Si dans les plantes et nous permet de proposer cet extractant comme un bon estimateur du silicium phytodisponible. Les sources de silicium ont par ailleurs une disponibilité variable : la silice amorphe constitutive de la diatomite est par exemple 2.2 fois plus disponible pour les plantes que celle de la vermiculite. Cependant les argiles semblent pouvoir être une source non négligeable de Si.

En conditions hydroponiques, des concentrations croissantes en Cd ou Cu dans le milieu induisent un stress physiologique croissant observable au niveau des parties aériennes et des racines des plantes de blé dur. L'absorption et la translocation racines-tiges réduites de Cd et Cu induites par l'apport de silicium diminuent le stress physiologique des plantes, ce qui résulte en une plus grande production de pigments photosynthétiques, une synthèse plus faible d'acides organiques, et plus particulièrement du citrate et de l'aconitate, à la fois dans les parties aériennes et les racines. Cet effet peut être attribué à une compartimentation accrue des métaux dans des parties métaboliquement moins actives. Cependant, la co-précipitation de Cd ou Cu avec Si dans les phytolithes des feuilles ou au niveau des racines ne semble pas être un mécanisme majeur de détoxification.

Dans le sol, l'apport de silice amorphe sous forme de diatomite diminue le stress métallique des plants de blé dur induit par la présence de Cd ou Cu, ainsi que les concentrations plus faibles de ces éléments dans les plantes l'attestent. Ceci semble être essentiellement dû des modifications de la balance de Cd, Cu et Zn dans la plante. En particulier, Cd est séquestré de manière plus importante dans les racines et sa translocation racines-tiges est réduite, tandis que l'absorption de Cu et Zn est accrue. Cependant, l'effet de l'ajout de ASi est aussi effectif dans le sol où il contribue à l’immobilisation des métaux. L’effet de l’ajout de ASi n’est pas visible lorsqu’il n’y a pas de stress métallique observé et nos résultats confirment donc que le silicium est bien un élément bénéfique pour les plantes car il n’a un effet qu’en présence de stress métallique. Les résultats obtenus permettent de proposer l’ajout de silicium comme une solution alternative et/ou complémentaire pour la diminution du stress métallique induit par Cd ou Cu et augmentation de la tolérance du blé dur.

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Annexes

Annexe 1. Correction of the contribution of silicate minerals to the extent of the amorphous silica by the method of sodium carbonate (See chapter 3).

Demaster (1981) used sodium carbonate to measure biogenic silica in marine sediments and calculated a correction accounting for the crystalline silicates present in the sample based on experimental data that showed that amorphous silica phases dissolve faster than crystalline silicate minerals. He assumed that most of the amorphous silica dissolved in first two hours of extraction. Aliquots of the extracting solution are taken at different times during the course of the extraction when all amorphous silica is supposed to have already dissolved. This should allow recording the dissolution of minerals, as illustrated on figure 1. The different aliquots form a line in a time versus extracted-Si graph. The equation of this line, $y = ax + b$, gives the rate of dissolution of the minerals (a) and the amorphous silica content (b) of the sample.

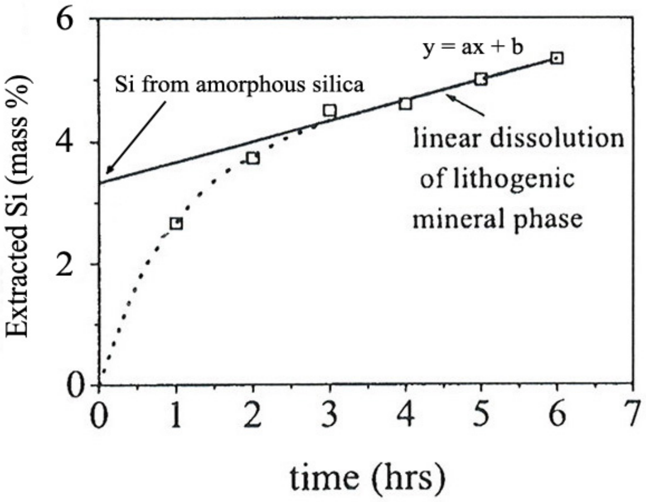

Figure 1. Theoritical dissolution curve over time of a marine sediment in sodium carbonate. Extrapolation of the mineral line to the y-axis substracts the contribution of silica from the lithogenic or crystalline fraction. The Y-intercept gives the concentration of Si coming from amorphous silica.

Table 1: Comparison of Na_2CO_3-extracted Si and Si from amorphous silica particles extracted by the gravimetry method (w.t. %) on selected calcisol pots. (See chapter 3).

	Na_2CO_3-extracted Si	Si from physically-extracted amorphous silica particles
Calcisol (control) after first crop	0.06 ± 0.05	0.09
Calcisol (control) after fourth crop	0.27 ± 0.19	0.17
Calcisol (planted) after fourth crop	0.27 ± 0.24	0.32

Annexe 2: Effects of silicon on growth and physiology of wheat seedlings (Triticum turgidum L. cv. Claudio under Cd stress: a hydroponic study (See chapter 4 part 1).

Table 1: Silicon and Cd concentrations in the nutrition solution.

(A)	Si concentration (mM)				**(B)** Cd (µM) concentration in 6th renewal			
Cd (µM)	3rd renewal		6th renewal		(-Si)		(+Si)	
	input	output	input	output	input	output	input	output
0	0.5	0.8	0.2	0.3	0.1	0.1	0.2	0.1
0.5	0.6	0.6	0.2	0.2	1.0	0.03	0.3	< 0.001
5.0	0.5	0.6	0.2	0.3	5.2	3.1	4.1	3.3
50	0.7	0.6	0.2	0.3	35.4	35.2	35.1	33.7

Table 2: Dissolved organic carbon (DOC) in the nutrient solution. (A) Presents the DOC in the third 3rd renewal before adding Cd treatments and (B) presents last renewal before harvesting.

DOC (mg L^{-1})	(A) 3rd renewal (without Cd) nutritive solution				(B) 6th renewal (with Cd) nutritive solution			
	(-Si)		(+Si)		(-Si)		(+Si)	
Cd (µM)	input	output	input	output	input	output	input	output
0	134.6	105.2	181.1	199.0	113.2	130.7	197.0	139.2
0.5	72.5	135.9	182.0	181.5	135.8	129.0	169.4	138.2
5.0	244.9	111.8	185.0	185.5	147.9	127.1	203.1	170.8
50	113.7	104.4	197.8	148.6	145.4	110.3	174.8	172.5

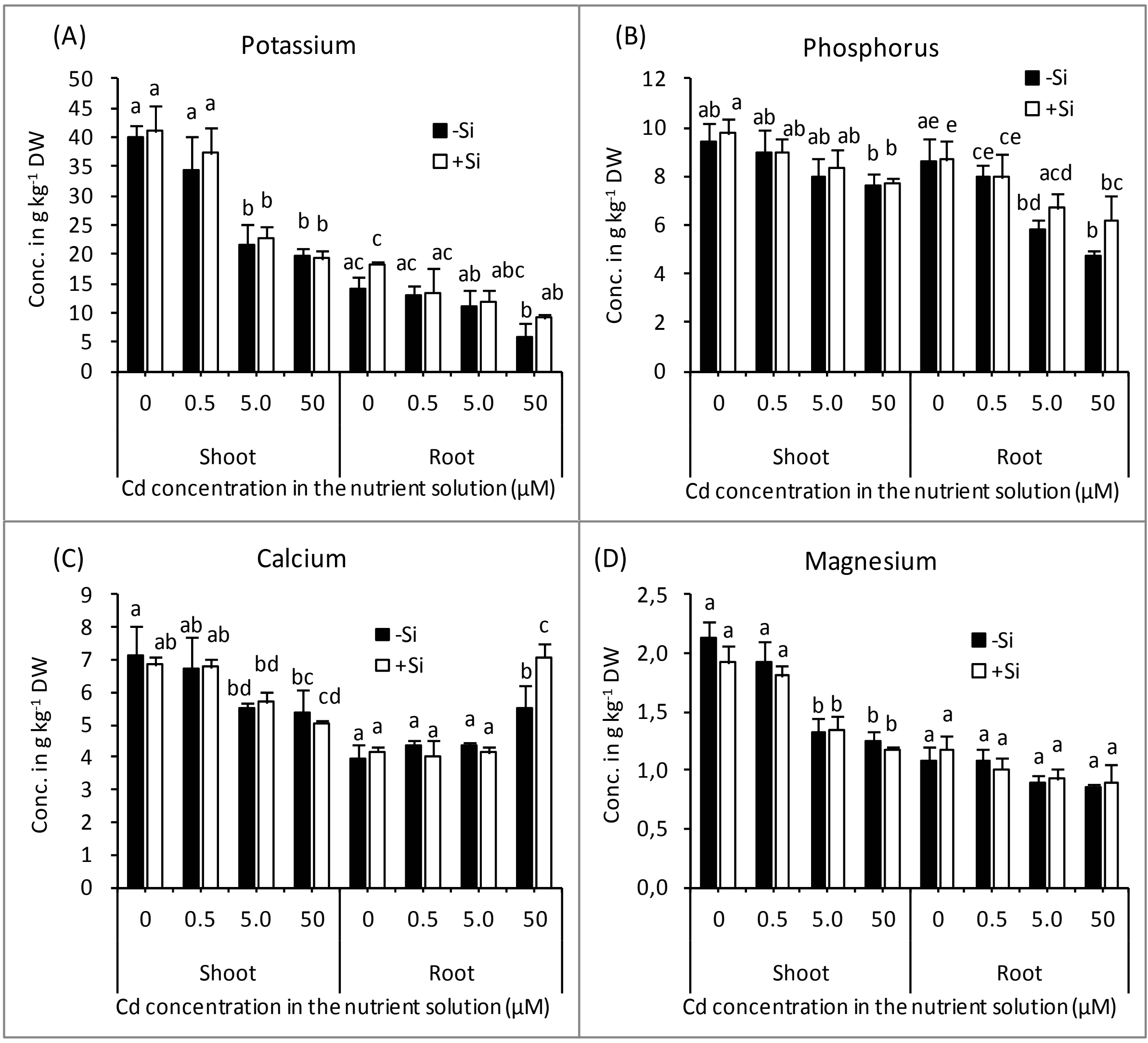

Figure 1. K. P. Ca and Mg concentrations (g kg^{-1}) in shoots and roots of wheat plants grown hydroponically with increasing concentrations of Cd and treated or not with 1mM Si. Bars represent SD of three replicates. Different letters indicate significant differences among the treatments at a $P < 0.05\%$ for shoots and roots separately.

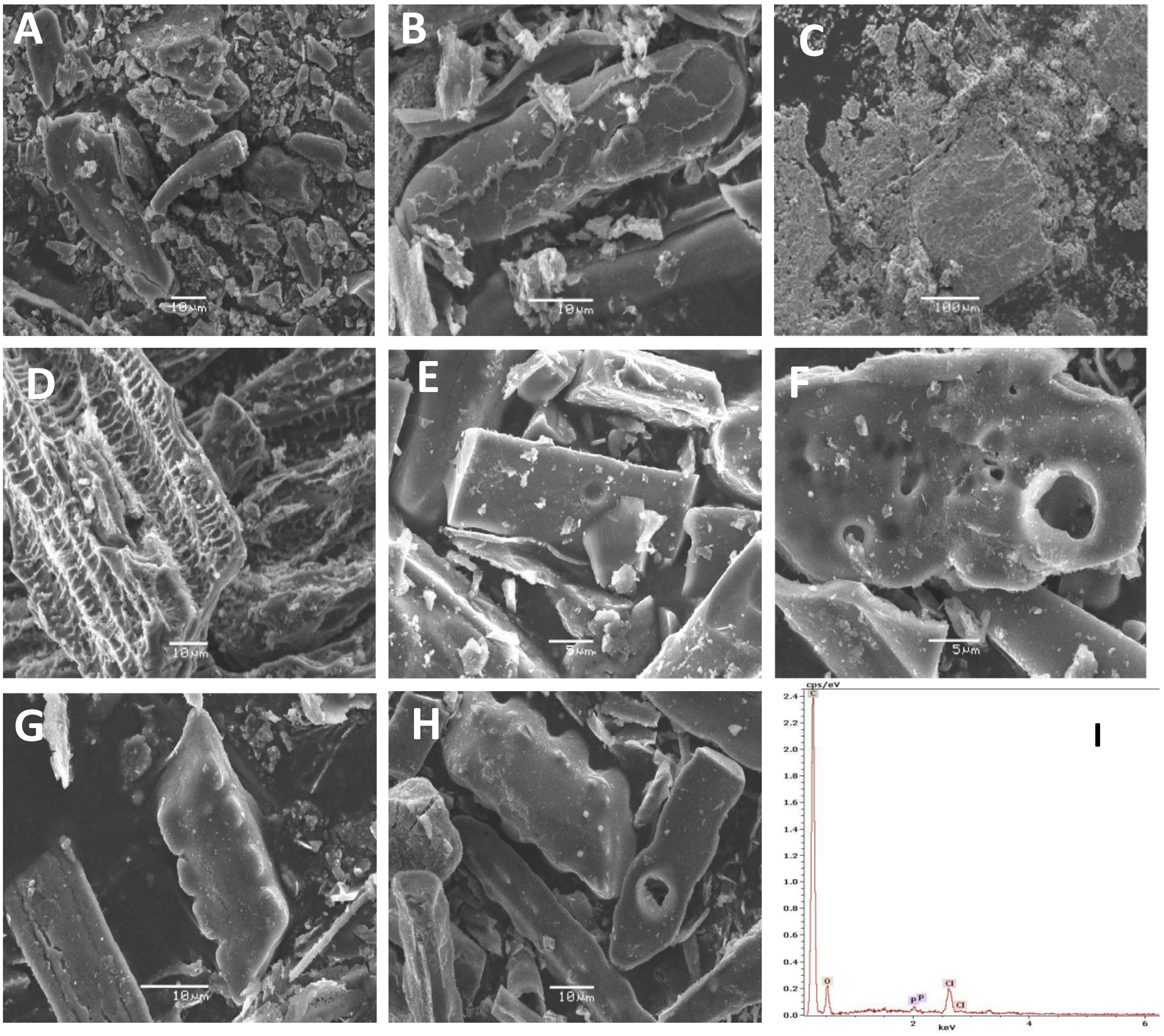

Figure 2. Scanning electron microscopy (SEM) microphotograph of phytoliths from ashed leaves of wheat plant. A, B, C, D, E, F, G, H represents the treatment with 0 mM Si+0µM Cd, 0 mM Si+0.5µM Cd, 0 mM Si+5.0µM Cd, 0 mM Si+50µM Cd, 1 mM Si+0µM Cd, 1 mM Si+0.5µM Cd, 1 mM Si+5.0µM Cd and 1 mM Si+50µM Cd respectively. C and D microphotographs contain no Si as shown in EDX spectrum (I).

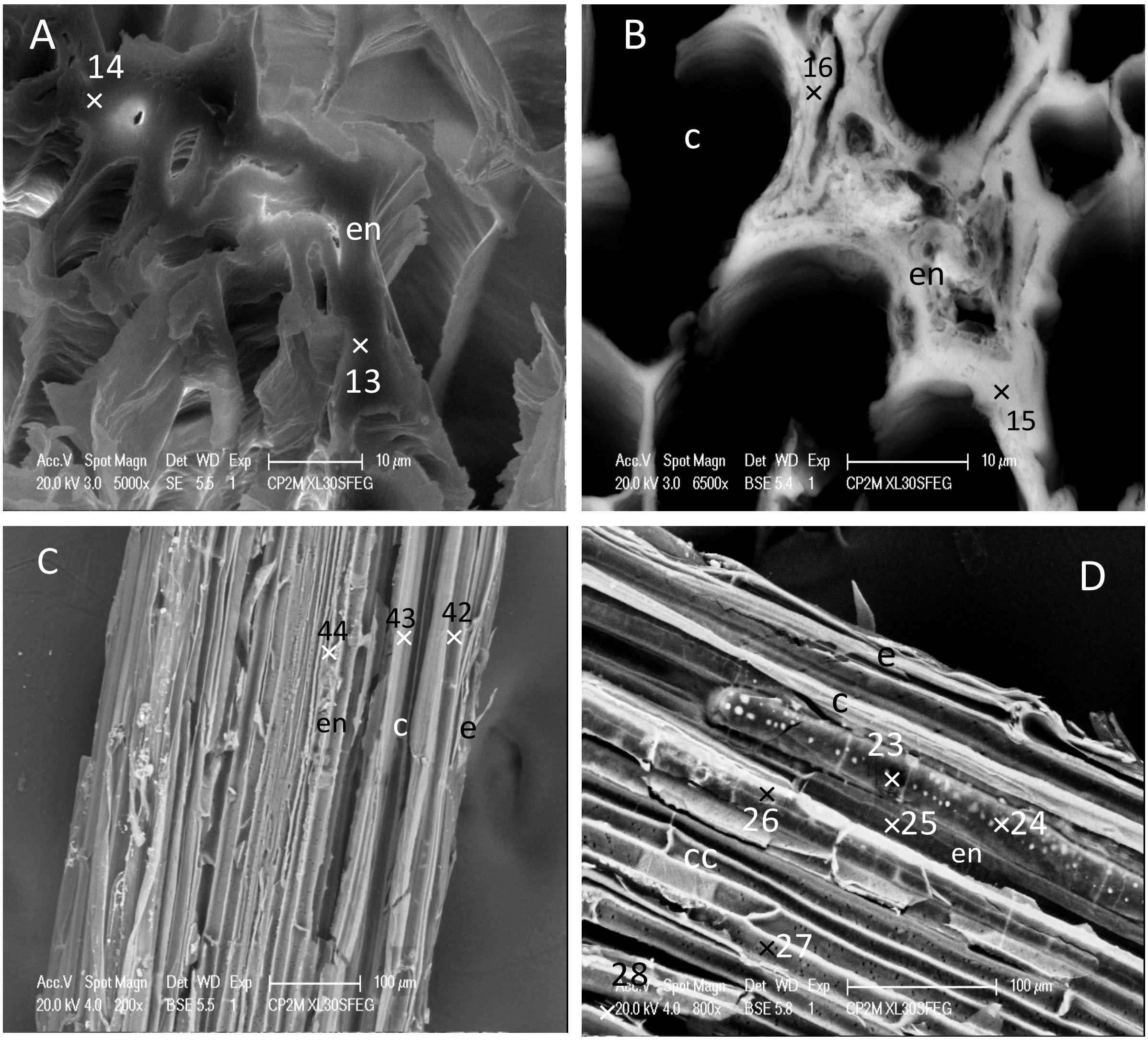

Figure 3. Scanning electron microscope (SEM) microphotographs of root cross-sections of wheat exposed to 50 µM Cd+1 mM Si. A, B, C, D- scanning electron microscopy micrographs. Different letters indicate different layers of root cross section i.e. e - epidermis, c - cortex, en - endodermis, pe - pericycle, cc - central cylinder and mx - metaxylem. Different numbers indicate sites at which EDX spectra were taken. A- 5000×, B- 6500×, C-200×, D-800×.

Annexe 3: Effects of various sources of silicon on alleviation of cadmium and copper toxicity in durum wheat (Triticum turgidum L. cv. Claudio W.) (See chapter 4 part III).

Table 1: pH values of initial minerals mixtures by different weights. Values are mean ± SD=3

		Mean value of pH		
Treatments	**Mixture type**	**75%-25%**	**85%-15%**	**95%-5%**
Control	Q+C	8.79±0.01	8.89±0.00	9.00±0.01
	Q+D	5.47±0.02	5.65±0.22	5.61±0.01
Cd	Q+C	8.80±0.00	8.92±0.02	8.95±0.01
	Q+D	5.66±0.01	5.63±0.21	5.92±0.01
Cu	Q+C	8.77±0.01	8.86±0.02	8.86±0.04
	Q+D	6.16±0.03	6.18±0.01	6.33±0.01

Table 2: Initial Na_2CO_3 extracted ASi (%age) from initial minerals mixtures by different weights. Values are mean ± SD=3

		ASi (%age)		
Treatments	**Mixture type**	**75%-25%**	**85%-15%**	**95%-5%**
Control	Q+C	0.29±0.09	0.23±0.02	0.28±0.02
	Q+D	2.74±0.14	2.07±0.23	0.89±0.02
Cd (2ppm)	Q+C	0.29±0.05	0.24±0.01	0.33±0.02
	Q+D	2.67±0.14	1.84±0.17	0.95±0.03
Cu (20ppm)	Q+C	0.24±0.05	0.21±0.01	0.27±0.00
	Q+D	1.33±0.30	1.30±0.17	0.83±0.07

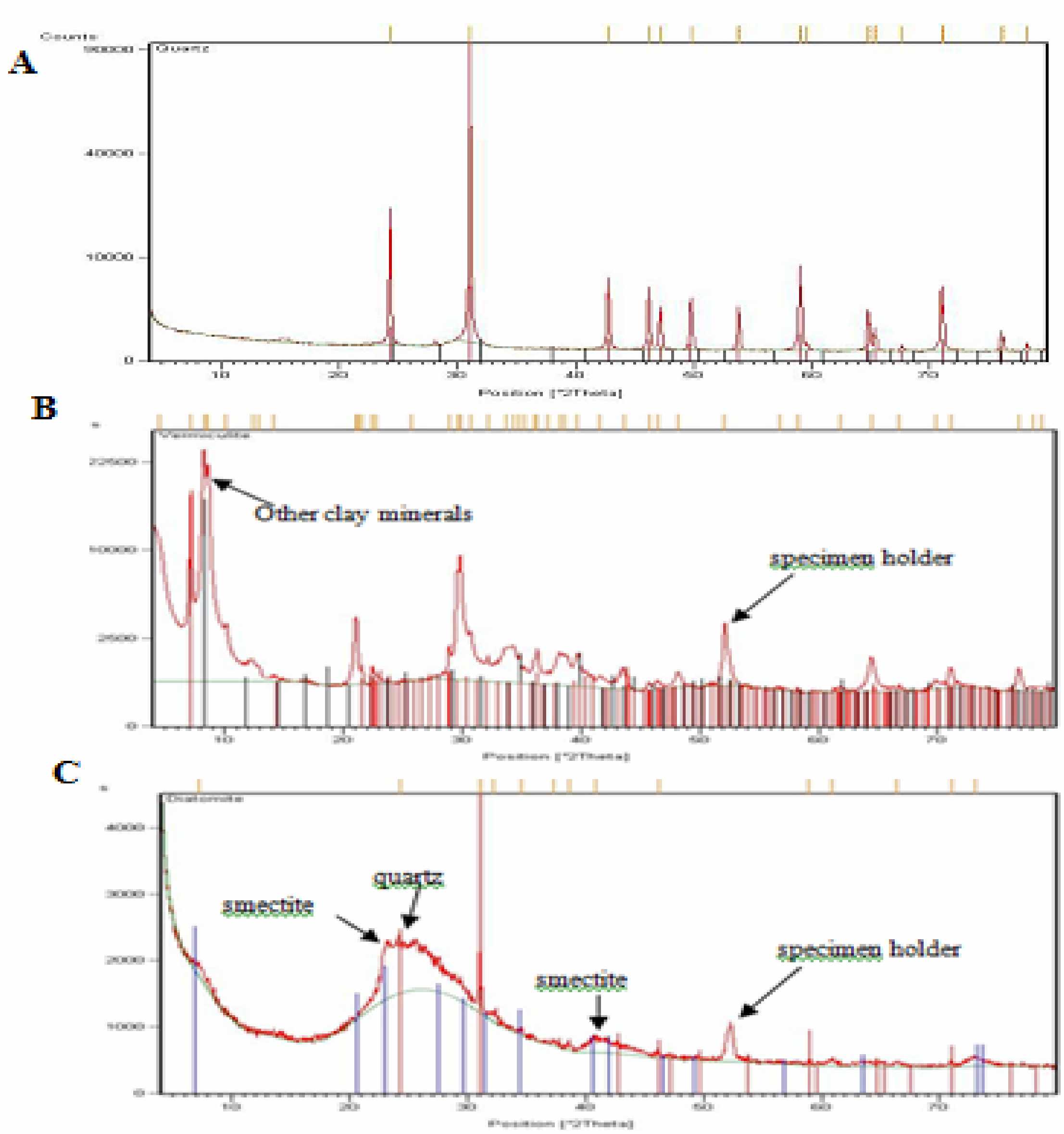

Figure 1. shows the X-ray diffraction pattern for quartz (A), vermiculite (B) and diatomite (C).

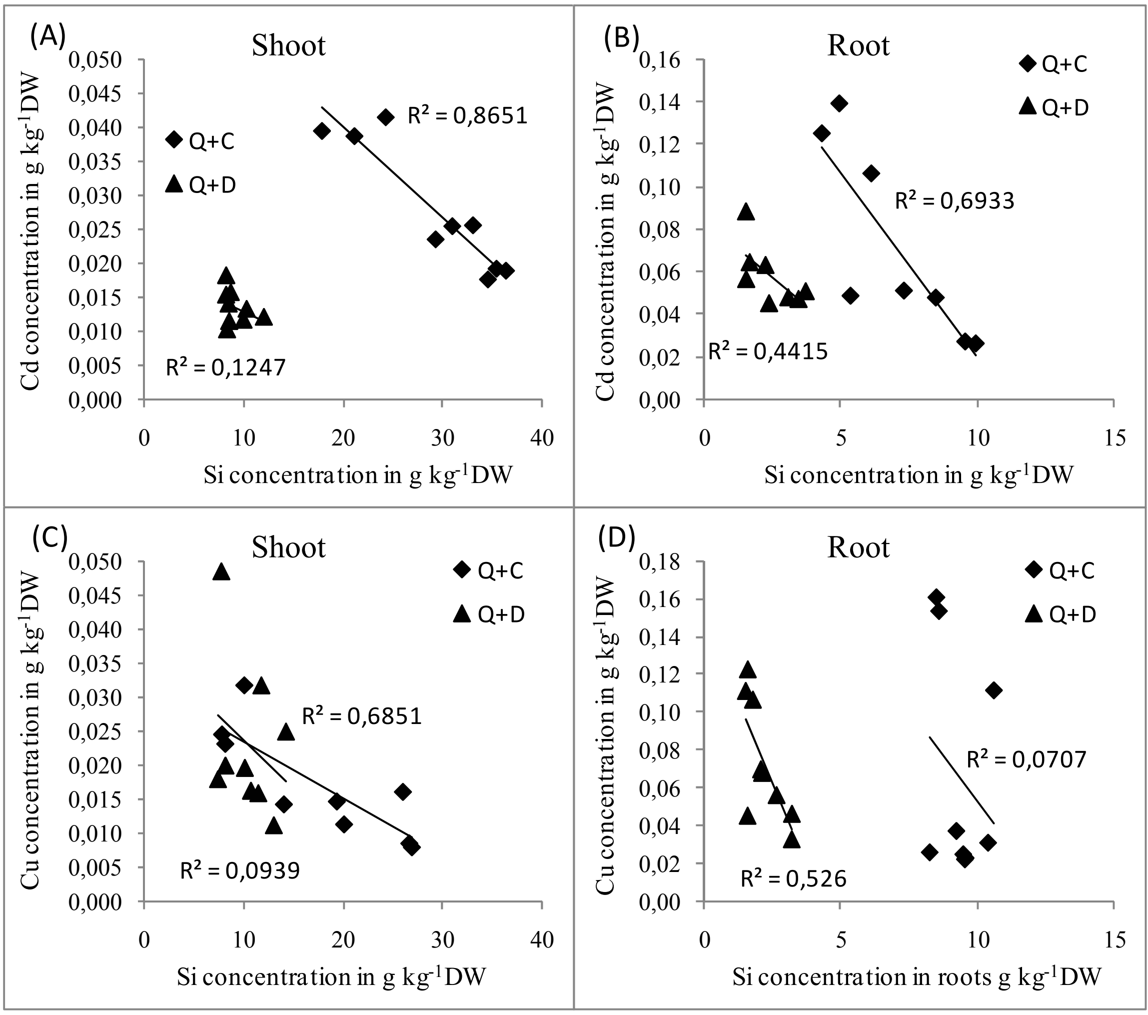

Figure 2. Cd and Cu concentration in shoots and roots correlated with relative Si concentrations both in shoots and roots.

Annexe 4: Silicon-mediated enhancement of heavy metals tolerance in durum wheat plant grown in soil (See chapter 5 part II).

Table 1. Total Si uptake in mg per pot of wheat shoots and roots grown in a soil without and with Cd addition and treated with increasing doses of amorphous silica

Si application (ton ha^{-1})	Without Cd		With Cd	
	shoot	root	shoot	root
0	32.6±2.9	8.2±2.8	8.8±1.3	3±0.1
1	33.4±2.0	7.2±.6	8.5±0.6	2.6±0.7
10	42±3.9	8.2±2.2	11.1±2.2	3.1±0.5
15	33.4±5.2	14.8±3.2	8.5±1.7	1.4±0.3

Table 2: Soil pH measured after crop harvest. Values are mean ± SD (n =3 without plant and n= 4 with plant)

Si application (ton ha^{-1})	Without Cd		With Cd	
	without plant	With plant	without plant	With plant
0	7.32 ± 0.19	7.34 ± 0.10	7.45 ± 0.04	7.56 ± 0.14
1	7.3 ± 0.12	7.36 ± 0.11	7.57 ± 0.05	7.47 ± 0.02
10	7.39 ± 0.08	7.46 ± 0.16	7.42 ± 0.08	7.61 ± 0.14
15	7.42 ± 0.08	7.37 ± 0.01	7.41 ± 0.05	7.63 ± 0.16

Table 3: Effect of ASi on DTPA-TEA extractable Zn and Cu (mg/kg^{-1} DW) concentrations in a soil without and with Cd addition and planted or not with wheat. Bars represent SD of four replicates (with plant) or three replicates (without plant) and * means significance relative to control of Student's t-test at $p<0.05$ and (**) at $p<0.01$ and (***) at $p<0.001$.

ASi application (ton ha^{-1})		Zn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)	
		without plant	with plant	without plant	with plant
Without Cd	0	18.3 ± 0.6	4.9 ± 0.3	2.6 ± 0.1	2.8 ± 0.1
	1	9.8***± 0.0	4.7 ± 0.1	2.5 ± 0.1	2.7* ± 0.1
	10	17.6 ± 0.5	4.9 ± 0.5	2.6 ± 0.1	2.8 ± 0.1
	15	12.1* ± 3.6	5.1 ± 0.5	2.5 ± 0.0	2.8 ± 0.4
With Cd	0	3.4 ± 0.1	3.5 ± 0.2	2.7 ± 0.1	2.8 ± 0.1
	1	3.3 ± 0.1	3.1* ± 0.1	2.7 ± 0.0	2.8 ± 0.1
	10	3.4 ± 0.1	3.2* ± 0.1	2.7 ± 0.06	2.8 ± 0.1
	15	3.3 ± 0.05	3.5 ± 0.1	2.5* ± 0.05	2.7± 0.08

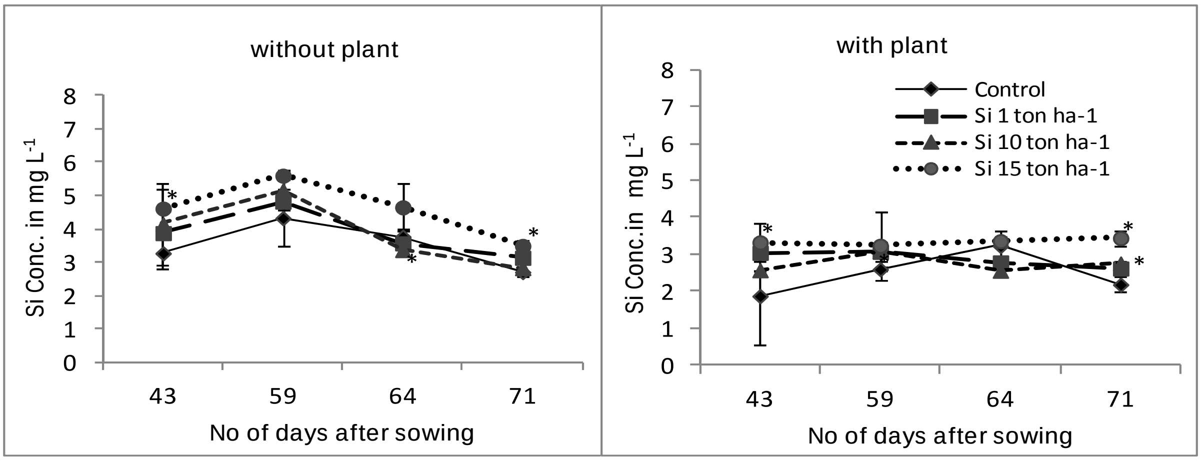

Figure 1. Effect of amorphous silica (ASi) application on the Si concentration in the soil solution without Cd addition. Bars represent SD of two replicates and * means significance relative to control of Student's t-test at $p<0.05$.

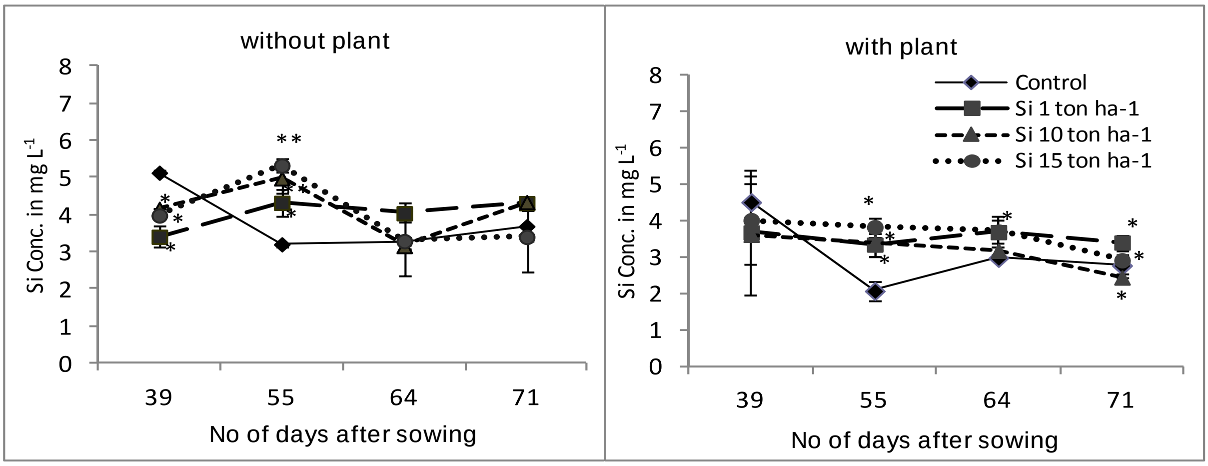

Figure 2. Effect of amorphous silica (ASi) application on the Si concentration in the soil solution with Cd addition. Bars represent SD of two replicates and * means significance relative to control of Student's t-test at $p<0.05$ and (**) at $p<0.01$.

Table 4: Zinc and Cu concentration in μg L^{-1} measured in the soil solution collected 59 days after sowing in pots of a soil without and with Cd addition and planted or not with wheat. Values are mean ± SD (n = 2). * means significance relative to control of Student's t-test at $p<0.05$. nd means no data available.

Si applications (ton ha^{-1})		Zn (μg L^{-1})		Cu (μg L^{-1})	
		without plant	with plant	without plant	with plant
Without Cd	0	10.9 ± 1.1	9.9 ± 3.8	10.1 ± 7.5	9.3 ± 3.5
	1	12.8 ± 5.4	11.6 ± 6.9	13.6 ± 8.1	15.9 ±11.9
	10	9.4 ± 2.0	10.4 ± 1.0	12.5 ± 5.1	17.9 ± 6.6
	15	8.0 ± 3.4	7.8 ± 2.6	6.5 ± 2.8	14. ± 11.7
With Cd	0	3.9 ± 2 .14	7.8 ± 2.7	5.2 ± 3.6	6.4 ± 2.3
	1	nd	7.6 ± 0.7	12.0* ± 0.6	7.3 ± 1.6
	10	5.1 ± 0.9	6.4 ± 1.1	5.0 ± 2.2	8.8 ± 0.9
	15	5.7 ± 2.8	8.1 ± 3.9	5.8 ± 3.3	9.7* ± 0.8