EFFECT OF ZEOLITE APPLICATION ON CADMIUM ACCUMULATION IN RED AMARANTH *(Amaranthus spp.)* GROWN IN CONTAMINATED SOIL

MD. MASUM BELLAH

DEPARTMENT OF SOIL SCIENCE
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

JUNE, 2020

EFFECT OF ZEOLITE APPLICATION ON CADMIUM ACCUMULATION IN RED AMARANTH *(Amaranthus spp.)* **GROWN IN CONTAMINATED SOIL**

BY

MD. MASUM BELLAH

REGISTRATION NO.: 12-04869

A Thesis
Submitted to the Department of Soil Science
Sher-e-Bangla Agricultural University, Dhaka
In partial fulfilment of the requirements
For the degree of

MASTER OF SCIENCE (MS)
IN
SOIL SCIENCE
SEMESTER: JANUARY- JUNE, 2020

APPROVED BY:

Prof. Dr. Saikat Chowdhury
Supervisor
Department of Soil Science
Sher-e-Bangla Agricultural University

Dr. Md. Ashraf Hossain
Co-Supervisor
Director
Training and Communication Wing
Bangladesh Agricultural Research Institute

Prof. Dr. Alok Kumar Paul
Chairman
Department of Soil Science
Sher-e-Bangla Agricultural University

DEPARTMENT OF SOIL SCIENCE

Sher-e-Bangla Agricultural University

Sher-e-Bangla Nagar, Dhaka-1207

CERTIFICATE

This is to certify that the thesis entitled, **'Effect of Zeolite Application on Cadmium Accumulation in Red Amaranth** *(Amaranthus spp.)* **Grown in Contaminated Soil'** submitted to the Department of Soil Science, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **Master of Science in Soil Science,** embodies the result of a piece of bona fide research work carried out by **Md. Masum Bellah,** Registration number: 12-04869 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has duly been acknowledged.

Dated: June, 2020
Dhaka, Bangladesh

Prof. Dr. Saikat Chowdhury
Supervisor
Department of Soil Science
Sher-e-Bangla Agricultural
University

Dedicated

To

My Beloved Parents

ABBREVIATIONS

Elaborated Form		Abbreviated Form
And others (Co-workers)	=	*et al.*
Parts Per Million	=	ppm
Coefficient of Variation	=	CV
Degree centigrade	=	°C
Degree of freedom	=	df
Example	=	viz.
Million tons	=	mt
Non-significant	=	NS
Per Hectare	=	ha^{-1}
Percentage	=	%
Phosphorus	=	P
Potassium	=	K
Randomized Complete Block Design	=	RCBD
Sher-e-Bangla Agricultural University	=	SAU
Standard Error	=	SE
that is	=	i.e.
tons	=	t

ACKNOWLEDGEMENT

All praises are only for the Almighty Allah, the great, the gracious, merciful and supreme ruler of the universe to enable the author to complete the research work and thesis successfully for the degree of Master of Science (MS) in Soil Science.

The author expresses the deepest sense of gratitude, sincere appreciation and heartfelt indebtedness to his reverend research supervisor **Prof**. **Dr. Saikat Chowdhury**, Department of Soil Science, Sher-e-Bangla Agricultural University, Dhaka for his scholastic guidance, innovative suggestions, constant supervision and inspiration, valuable advice and helpful criticism in carrying out the research work and preparation of this manuscript.

Special thanks to his co-supervisor **Dr. Md. Ashraf Hossain,** Director Training and Communication Wing Bangladesh Agricultural Research Institute, Gazipur, for his assistance in the research and supreme help during works and continuous encouragement.

The author expresses the sincere respect to **Prof. Dr. Alok Kumar Paul,** Chairman, Department of soil science and all the teachers of the Sher-e-Bangla Agricultural University, Dhaka for providing the facilities to conduct the experiment and for their valuable advice and sympathetic consideration in connection with the study.

The author also felts to express his sincere thanks to all the staff of the Department of Soil Science, Sher-e-Bangla Agricultural University, Dhaka, for their sincere help and co-operation during the whole study period.

The Author

June, 2020

LIST OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Present work was carried out in the net house of Department of soil science, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh in order to study the effect of zeolite on Cd uptake in red amaranth plant as well as the effect of zeolite on soil health. The experiment was conducted in Completely randomized design (CRD) with four replications. Five levels of zeolite (Control, 1%, 2%, 3% and 4% of soil weight) in combination with two levels of Cd (Control and 15 ppm Cd) were used as treatment. In case of stem, leaf and root uptake, highest Cd concentrations (36.06, 65.46 and 46.99 ppm Cd respectively) were found in treatment 2 (Control zeolite + 15 ppm Cd), where no zeolite and maximum Cd was applied. On the other hand, maximum Cd remediation occurred in treatment 10 where maximum zeolite was applied (Zeolite @4% of soil weight + 15 ppm Cd). Higher concentrations of total Cd in bulk and rhizosphere soil were found in the treatments where higher rates of zeolite were applied. However, concentrations of bio-available Cd got lowered gradually in soils with higher rates of zeolite application. This indicates that more Cd was successfully immobilized by zeolite. Zeolite application also positively influenced the soil properties such as bulk density, particle density, soil pH and soil electrical conductivity. Therefore, it can be concluded that zeolite can be used as an effective tool for Cd remediation as well as a good soil amendment.

CHAPTER I

INTRODUCTION

Cadmium (Cd) is a highly carcinogenic metal that can cause toxic reactions even in low concentration (Khan *et al*., 2015). Cd is a non-essential trace element, and does not play any identified role in the growth and development of human, plants and animals. Generally, it occurs in lithosphere (0.2 mg kg^{-1}), sedimentary rocks (0.3 mg kg^{-1}) and soil (0.53 mg kg^{-1}) (Kabata-Pendias and Pendias, 2001). Cd enrichment in soil occurs from both natural and anthropogenic sources, and is considered to be of great environmental concern. Geological weathering is a major natural source of Cd in soil (Liu *et al.,* 2013), while mining, smelting, wastewater irrigation, industrial and vehicular emissions, manufacturing, and agrochemicals are primary anthropogenic sources of Cd (Nawab *et al.,* 2016). Uncontrolled and improper waste disposal practices have significantly increased the concentrations of Cd in soil.

Uptake of Cd from soil by plants depends on its concentration and bioavailability, while a small amount is directly taken up from atmosphere through dry deposition of contaminated dust (Clemens, 2006). The entry of heavy metals to plant cells occurs through the same transport systems used for carrying out the uptake of macro and micronutrients. The uptake of Cd occurs through trans-membrane carriers involved in the uptake of magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn) and copper (Cu) (Clemens, 2006). From contaminated soil, Cd can easily be taken up by plant roots and then transported to above ground parts where it interacts with biochemical and physiological processes, affecting the plant morphology and growth rate (Uraguchi *et al.,* 2009). The transfer of Cd to vegetables from Cd polluted soil has been reported in several studies.

Generally, many plant species are tolerant to a certain amount of Cd, but at certain concentrations can induce phytotoxicity. Concentrations of Cd causing phytotoxicity vary greatly with plant species. Hyper accumulators can accumulate above 0.01% of their shoot dry weight without showing toxicity symptoms (Verbruggen *et al*., 2009). Cd concentrations>5 to 10 μg g^{-1} Dry Matter are toxic to most plants. Street et al. (2010) reported a decrease in leaf length and fresh weight of leaves of wild garlic with the application of Cd. Cd decreases the chlorophyll content and concentration of adenosine triphosphate ATP. It inhibits the leaf photosynthesis by affecting the biosynthesis of chlorophyll and the function of the centers of photochemical reaction (Jing et al., 2005). Cd significantly reduced the dry weight of roots and shoots of wheat (Zhang et al., 2002).

Cd has significant negative impacts on plant nutritional values and growth rate and most of the plants grown in Cd contaminated soil are nutrient deficient (Khan et al., 2015). Yang et al. (1996) reported Cd effects on the uptake of various nutrients by plants. At the root region, Cd causes mineral deficiency by competing for absorption with minerals having similar chemical properties like Ca and Mg (Barcelo and Poschenrieder, 1990). High concentrations of Cd may cause the reduction of Ca, Mg, and K in the tissues of tomato (Khan et al., 2015) and cucumber plants (Burzynski, 1988). Bioaccumulation of Cd causes the alteration of various physiological functions by affecting N metabolism (Chaffei et al., 2004).

Natural zeolite as a potential vast resource was first found in 1756 and mined in various deposits throughout the world (Ulusoy and Simsek 2005). Currently, it can also be produced synthetically to tailor the properties for specific applications (Badora *et al*. 1998). So, generally speaking, zeolite is a class of alkaline porous alumio-silicates (Joshi *et al.*, 2002) with a negative charge (Mohamed 2001), having a three-dimensional framework, neutralized by introducing exchanged cations in the structure sites of it (Mondales et al., 1995). The exchanging efficiency depends on the micro-porosity and exchanging capacity of the particular zeolite (Sponer 2001).

These characteristics vary widely depending on the origin of the material (Mozgawa 2004).

The technique of remediation with zeolite has been used for a long time, but the theory has not been made an agreement (Castaldi *et al.*, 2005). Scientists summarized that zeolite can basically lead to the immobilization of metals in three ways (Querol *et al.*, 2006). Firstly, zeolites dissolve supplying alkalinity to the acid polluted soils, causing the precipitation of insoluble phases. These neoformed phases contain metals as major constituents (Chen *et al.*, 2000) or as minor components co-precipitated in hydroxides (Chlopecka and Adriano 1996). Secondly, the increase in alkalinity promotes the metal sorption via surface complexation processes. Mineral surfaces have a positive charge at low pH values due to the sorption of protons, and they acquire a negative charge as pH increases owing to the deprotonation of the surface unsaturated bonds (Nardin *et al.*, 1995). pH value makes cations increase through stable complexex with the negative radicals on the surfaces. Especially, natural zeolite plays a significant role in surface complexation because of their higher specific surface (Shanableh and Kharabsheh 1996). Thirdly, metal retention may also take place regardless of pH value due to the cation exchange in zeolite. Zeolite is crystalline aluminum-silicates, with group I or II elements as counter ions. Its structure is made up of a framework of $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ tetrahedra linked to each other at the corners by sharing their oxygen. The substitution of Si(IV) by Al(III) in the tetrahedra accounts for a negative charge of the structure, which may give rise to a high cation exchange capacity (CEC) (up to 5 mequiv/g) when the open spaces allow the access of cations (Mohamed 2001).

Objective(s):

The objectives of the study were-

1. To assess the effect of zeolite application on cadmium accumulation in leafy vegetables grown in contaminated soil.
2. To observe the influence of zeolite amendment on some selected soil properties.

CHAPTER II

REVIEW OF LITERATURE

A pool of literature pertaining to present study regarding phytotoxicity of Cd in plants, mechanism of Cd toxicity tolerance by plants and remediation of Cd by zeolite with a brief description of red amaranth is presented in this chapter.

2.1. Effect of Cadmium on Vegetables

Cd is one of the heaviest toxic metals that cause harmful effects in crops (Sethy and Ghosh, 2013). Plant uptake of Cd is determined by the bioavailability of Cd in contaminated soil (Clemens, 2006). Cd is a heavy metal readily absorbed and quickly translocated, which is symplastically transported through the root cortex to the shoots (Tudoreanu and Phillips, 2004). The toxic symptoms of Cd in plants are growth retardation, photosynthetic activity alternations, stomatal movement changes, disruption in enzymatic activity, protein metabolism and membrane functioning.

2.2. Cd Impacts on Plant Growth and Biomass

Cadmium negatively impacts plant growth. Slight but significant decreases in the yield of cucumber from shooting dry matter reflected a negative effect of concentrations of Cd in hydroponic culture as low as 0.05 mM. Cadmium uptake of cucumber plants as affected during growth by fluctuations in nutrient solution Cd concentration (Tack et al., 1998). In hydroponic conditions, the application of 10-mM Cd stress significantly reduced the root length, surface area and root tips of three pepper cultivars compared to the control (Huang et al., 2015). Dose- and cultivar-dependent application of Cd (2 and 10 mM) in hydroponic condition reduced the fresh weights of root, stem, and leaves of two pepper cultivars (Xin et al., 2014). Cadmium stress was shown to decrease the leaf area and leaf dry matter biomass of pepper (*Capsicum annuum L.*) as compared to the control, but the response varied among five cultivars studied (Leon et al., 2002). In a pot experiment, the highest Cd stress, 60 mg kg^{-1}, decreased the shoot and root lengths and the dry weight of potato seedlings (*Solanum tuberosum L.*) as compared to the control (Hassan et al., 2016). For 90 days the tomato (*Solanum lycopersicum L.*) showed no fruit production in a nutrient solution in the presence of 100 mM Cd (Hediji et al., 2015).

Due to Cd toxicity, a significant decrease in total leaf area and dry weights of cabbage leaf, stem, and roots (*Brassica oleracea L.*) was noted (Jinadasa et al., 2016). Variable production of biomass among sensitive and tolerant *Vicia faba* cultivars has been observed under the same stressed Cd environment. Similarly, in leafy, root and leguminous plant species such as lettuce (*Lactuca sativa L.)* (Monteiro et al., 2009), radish (*Raphanus sativus L.*) (Varalakshmi and Ganeshamurthy, 2013), and soybean (*Glycine max L.)* (Wang et al., 2016), Cd caused a reduction in growth and biomass. This review discussed the issue that depends on the vegetable species as well as the dose and duration of Cd exposure to decrease in plant growth and biomass under Cd stress.

2.3. Morphological and Systemic Changes brought on by Contamination of Cadmium

The major source of contamination in food is Cd taken up by plants (Chunhabundit, 2016). Even at low concentrations, Cd is toxic to most living organisms. Cd is one of the elements that can be accumulated at levels above 0.01% of shooting dry weight in plants without causing toxic symptoms (Verbruggen et al., 2009). Cd gathers in the topsoil, in close relationship with the organic fraction, which is highly accessible for plants growing in acidic soils (Kirkham, 2006), thus enhancing its root exudate solubility. This occurs mainly in soil as Cd^{2+} (Verbruggen et al., 2009). The cell wall is established as a major place of storage of heavy metal in plants, and its accumulation in the cell wall is considered a critical mechanism for heavy metal tolerance (Vazquez et al., 2006). Roots are the first to be affected by heavy metals, as they produce more metal ions in roots than shoots (Singh et al., 2015). When analyzed under an electron microscope, Cd position in plants revealed that the root cell walls contain most of the metal relative to cytoplasm because the heavy metal attaches to the cell wall because of its negative load (Dal Corso et al., 2010).

Cd can be easily absorbed and transported to shoots by plant roots, resulting in cellular, molecular, biochemical, and physiological changes affecting plant growth and morphology (Song et al., 2017). Cd toxicity apparently inhibits the growth of plant roots and affects root morphology (Daud et al., 2009). Prolonged plant exposure to Cd may cause the roots to become mucilaginous, brown, and decomposed, with reduced shoots and root elongation, leaf rolling and chlorosis. Cd accumulation prevents the development of lateral root, while the main root is brown, rigid and twisted (Rascio and Navari-Izzo 2011). It is observed in the apical region through the disordered division and abnormal enlargement of the epidermal and cortical cell layers. Cd stress also triggers an extraordinary number of nucleus populations in differentiated roots (Fusconi et al., 2006), inhibits the mitotic index, induces chromosomal aberrations and mitotic aberrations, and retards the development of micronucleus. This also destroys the DNA of cells with root caps (Seth et al., 2008). Cd causes nuclei damage in root tip cells, inducing chromosomal and mitotic aberrations in onions (Seth et al., 2008), and alters RNA synthesis by inhibiting ribonuclease activity in rice (Shah and Dubey, 1995).

2.4. Effect on Photosynthesis

The Cd toxicity decreased pepper seedlings' net photosynthetic output and water-use efficiency (WUE) relative to control (Leon et al., 2002). It has been documented that in 90-day old tomato leaves exposed to 100 mM Cd, chlorophyll and carotenoid content decreased while a lower concentration of 20 mM did not affect the photosynthetic pigments compared to the control (Hediji et al., 2015). Vegetable response to the photosynthetic rate and chlorophyll content under Cd stress differed among soybean genotypes (Shamsi et al., 2014). In younger soybean leaves, a greater decrease in total chlorophyll content, net photosynthetic rate, and stomatal conductivity was observed compared with mature leaves in hydroponics at concentrations of Cd less than 50 and 100 mM Cd for 10 days (Xue et al., 2014). This implies that Cd's effect on photosynthetic activity varies with age of the leaf, i.e. mature versus young. In peas (*Pisum sativum* L.) (Agrawal and Mishra, 2009), soybean (Xue et al., 2014), tomato (Hassan et al., 2016), lettuce (Monteiro et al., 2009), and potato (Xu et al., 2013), the Cd stress decreased chlorophyll material.

2.5. Role of Cd on Nutrient Access

Under Cd stress, the uptake and accumulation of various nutrients in vegetables can be significantly modified (Zhi et al., 2015). In pea seedlings, the Cd stress (5 mg kg^{-1} soil) decreased mineral nutrients such as potassium (K), phosphorus (P), calcium (Ca), manganese (Mn) , zinc (Zn), sulfur (S), and boron (B), and the response varied between the sensitive and tolerant pea genotypes (Metwally et al., 2005). Soil exposure containing 1.12 mg kg^{-1} Cd decreased the concentration of Mn while iron (Fe) and Zn concentrations increased in soybean cultivar seeds, which could have a beneficial effect of lower concentrations of Cd on Fe and Zn, considering that these elements are below optimum for a significant proportion of the population (Zhi et al., 2015). Cd application in the nutrient solution reduced concentrations of Zn, Mn, Ca and K in the aerial portions of tomato seedlings (Bertoli et al., 2012). Cadmium decreased the concentration of Mn in tomato seedlings, indicating the antagonistic effect of Cd on the absorption and translocation of Mn (Dong et al. 2006). The contents of Ca, Zn and Cu in tomato shoots decreased for 90 days due to CD stress due to exposure to 100 mM Cd and increased in roots, while the contents of K and Mg decreased compared to control in all parts of the plants (Hediji et al., 2015). Compared to control, excess Cd decreased mineral elements including Mn, Zn, Cu, Fe, and Ca in the leaves, stem, and cabbage roots (Jinadasa et al., 2016). More recently, Cd toxicity was reported to have significantly reduced the mineral elements in potatoes, lettuce, and tomatoes depending on the species (Khan et al., 2016). A reduction of mineral elements mediated by Cd has also been documented in lettuce (Monteiro et al., 2009). The excess Cd in tomato seedlings in an in vitro decreased the mineral nutrients including Ca, Mg K, Mn, Zn, Cu, and Fe, while when grown hydroponically they did not affect the concentration of those nutrients. In addition, tomato shooting and root biomass differentiated in both experiments regardless of the potato cultivars studied (Gonçalves et al., 2009). This means that the effect of Cd in tomato seedlings on mineral nutrients varies with the dose of Cd, the genotypes and the conditions of experimental culture. The reduction in plant uptake of mineral nutrients under Cd stress may be due to increased competition between Cd and mineral elements at the root surface during plant uptake (Rizwan et al., 2016).

In comparison, Cd treatments of 1, 2.5, and 5 mg kg^{-1} have been reported to synergistically increase concentrations of nutrient elements including P, K, Ca, Mg, Fe, and Zn in pseudostems and leaves of 25 welsh onions (*Allium fistulosum* L.) cultivars (Li et al., 2016). For these onion cultivars, however, opposite results could be obtained under higher Cd tension, and this needs to be investigated for depth.

2.6. Other Physiological Disorders in Plants as Caused by Cd toxicity

Photosystem II (PSII) is affected by Cd toxicity (Baker, 1991), and the changes in chlorophyll in fluorescent structures can easily identify its damage or failure under stress (Maxwell and Johnson, 2000). The two CO_2-fixing enzymes, Ribulose-1, 5-bisphosphate carboxylase (RuBisCO) and phosphoenol pyruvate carboxylase (PEPCase), are the main targets for damage to Cd. RuBisCO's activity is reduced by altering its structure, replacing the Mg ions, the vital cofactors of carboxylation reactions, and swinging towards oxygenation reactions (Siedlecka et al., 1998). PSII's water oxidizing complex (OEC) is also affected by the replacement of Ca^{2+} in Ca/Mg clusters that make up the oxygen evolving centers (Sigfridsson et al., 2004), as well as by the modification of the Ob binding site (Geiken et al., 1998).

Cd decreases the overall chlorophyll and carotenoid content of *B. napus* and improves non-photochemical quenching (Larsson et al., 1998). Cd greatly decreases normal H^+-K^+ exchange, plasma membrane ATPase activity, and a variety of other enzymes, including glucose-6-phosphate dehydrogenase, glutamate dehydrogenase, malic enzymes, isocitrate dehydrogenase (Mattioni et al., 1997), and rubisco and carbonic anhydrase (Siedlecka et al., 1998). The phosphoenolpyruvate carboxylase polypeptide was stated to have increased significantly, with no further synthesis of glutamate dehydrogenase and glutamate synthase polypeptides in *Z. mays* seedlings are exposed to 20 mM Cd (Ju et al., 1997); while chromatin alterations have been observed in pea plants (Hadwiger et al., 1973). Cd ions are chemically similar to Zn ions and may interfere with the activity of Zn-finger transcription factors, replace the Zn ions and interfere with transcription mechanisms (di Toppi and Gabbrielli, 1999). It also replaced Ca^{2+} ions in calmodulin proteins, causing intracellular calcium levels to be disrupted and calcium-dependent signaling pathways altered (Perfus-Barbeoch et al., 2002) by a mechanism similar to that observed with Zn transcription factors. The concentration of Ca^{2+} in cells during Cd stress (Dal Corso et al., 2008) activates calmodulin-like proteins to interact with Ca^{2+} ions by modifying their conformation to control a number of mechanisms, including ion transport, gene regulation, metabolism and stress tolerance (Yang and Poovaiah, 2003).

Cd decreases micro- and macronutrient absorption and thus affects transportation practices in plants (Hernandez et al., 1996). Cd exhibits detrimental effects by interfering with the absorption, transportation and subsequent distribution of nutrient elements such as Ca, Mg, P, K, S, Fe, Mo, Zn, Mn, B and Cu in plant species such as sugar beets (Chang et al., 2003), peas (Metwally et al., 2005), and barley (Guo et al., 2007). It affects the absorption of nitrate, and its transportation from roots to shoots, by inhibiting the nitrate reductase activity observed in *Silene cucubalus* plants (Mathys, 1975). Cd also causes changes in the composition of lipids and fatty acids, which alters membrane functionality (Popova et al., 2009). High Cd concentrations also contribute to an irregular metabolism of nutrients, such as plant imbalances between proteins and sugars (Costa and Spitz, 1997). This also has a detrimental effect on the sugars and amino acid content of some plant species (Wu et al., 2004) by raising their concentrations, suggesting a starch hydrolysis inhibition (Bishnoi et al., 1993).

2.7. Cd Mediated Mechanism for Toxicity in Vegetables

Specific mechanisms are responsible for reductions in plant production, biomass, and mineral nutrient absorption in soils and plants under Cd stress. Over production of ROS in vegetables caused higher levels of Cd, which increased malondialdehyde (MDA) in pea (Agrawal and Mishra, 2009) and soybean cultivars compared to untreated control (Shamsi et al., 2014). Similarly, MDA content in tomato leaves (Zhao et al., 2016), mung bean (Hassan and Mansoor, 2014), lettuce (Monteiro et al., 2009), and potato (Xu et al., 2013) increased with Cd stress. Compared to the regulation, the content of thiobarbituric acid reactive substances (TBARS) increased in the roots of tomato seedlings followed by leaves and fruits under Cd stress, but the response differed with the dose and length of Cd stress applied (Gratao et al., 2008). Similarly, Cd stress increased the content of TBARS in tomato leaves in a dose-dependent manner relative to control (Ben Ammar et al., 2007). The stress from Cd increased hydrogen peroxide (H_2O_2) production in mung bean (Hossain et al., 2010). The excess Cd content in mung bean seedlings increased by 0.3 mM compared to the control but the response varied between two studied genotypes (Hassan and Mansoor, 2014). This means that the response of plants to Cd stress may differ within the genotypes of the same species. By can lignification, p-hydroxyphenyl (H) and syringyl (S) units in soybean roots, Cd stress can decrease root growth (Finger-Teixeira et al., 2010). In vegetables the excess Cd caused genotoxicity. Deoxyribonucleic acid (DNA) damage in the root nuclei of potato seedlings has been reported to have increased by Cd (Gichner et al., 2008).

Plants might have attained a well-developed immune system to cope with metal stress that mainly involves enzymatic and non-enzymatic antioxidants and metal sequestration in metabolically inactive parts such as root cell walls and leaf vacuoles. Several studies have reported alterations in antioxidant enzyme activity in vegetables under Cd stress (Gratao et al., 2008; Chamseddine et al., 2009). Cadmium stress enhanced the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase, and guaiacol peroxidase (GPX) in pepper plant leaves as compared to control, but the response varies among the studied cultivars (Leon et al., 2002). Similarly, Cd stress decreased CAT and increased the development of SOD and peroxidase (POD) in pea seedlings compared to seedlings with no Cd stress (Agrawal and Mishra, 2009). The response to antioxidants varies between genotypes of the same species. For eg, ascorbate peroxidase (APX) activity decreased, while glutathione (GSH) activity increased in genotypes of Cd responsive peas (Metwally et al., 2005). Similarly, 5.0 mmol L^{-1} Cd exposure increased the activity of antioxidant enzymes, SOD and POD in genotypically dependent soybean seedlings (Shamsi et al., 2014). GPX and CAT activities rose, while APX declined under Cd stress in mung bean genotypes (Hassan and Mansoor, 2014). Overall, plants may thrive under certain levels of Cd stress by enhancing the activity of antioxidant enzymes, but under higher Cd stress these enzyme activities decreased, which could be due to higher oxidative stress in plants.

Cd detoxification in vegetables can require sequestration in root cell walls and leaf vacuoles (Wang et al., 2015). It has been documented that Cd immobilization in plants can include the stem cell wall, leaves, and pepper cultivar fruits (Xin et al., 2014; Xin and Huang, 2014). However, sequestration of Cd differed between species and genotypes within the same species in various metabolically inactive sections (Xin et al., 2014). By producing phytochelatins (PCs) in different plant parts, plants could tolerate Cd stress. The Cd stress (500 mg L^{-1}) increased the synthesis of PCs in the cabbage leaf, stem, and roots compared with the control subjected to 1 mg Cd L^{-1} (Jinadasa et al., 2016). By enhancing the endogenous production of PGRs under metal stress the plants may withstand such metal stress. The 100 mM Cd stress decreased proline and total ascorbate and increased the contents of a-tocopherol, asparagine, and tyrosine in tomato leaves compared to control (Hediji et al., 2010). In tomato wild type, the content of proline increased in a dose dependent manner under Cd stress (Zhao et al., 2016).

2.8. Cd Tolerance Mechanism in Plants

Cd toxicity is associated with oxidative stress in plant cells, promoting ROS generation (Olmos et al., 2003), and inhibiting or activating anti-oxidant enzymes (Iannelli et al., 2002), resulting in oxidative damage to the cells and lipid peroxidation (Chien et al., 2002). Cd, a non-redox metal, is unable to carry out single electron transfers and does not contain ROS such as superoxide anion (O^{2-}), single oxygen ($1O_2$), hydrogen peroxide (H_2O_2) or hydroxyl radicals (OH^-); however, it causes oxidative stress by interacting with antioxidant protection systems (Gratao et al., 2005). Cd slows antioxidant enzyme activity (Lin et al., 2007), resulting in foliar damage and an inhibition of plant growth (Dell'Amico et al., 2008). Heyno et al. (2008) stated that Cd stimulates ROS production in the chain for the transfer of mitochondrial electrons. In peroxisomes it also stimulates NADPH oxidase by accumulation of hydrogen peroxide, followed by accumulation of cellular oxygen and fatty acid hydroperoxide (Gill and Tuteja, 2010). Alterations in free radical scavengers such as superoxide dismutase (SOD) and catalase (CAT), ascorbate peroxidase (APOX), mono-dehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), peroxidases (POD), glutathione reduatse (GR) and some nonenzymatic scavengers glutathione (GSH), ascorbic acid (AsA) were observed in various species, including *P. sativum, Glycine max*.

In plants, higher activity of antioxidant enzymes and nonenzymatic constituents was observed in order to withstand stress during Cd intoxication. The induction of enzymatic protection systems, such as SOD and CAT, to minimize oxidative damage in Cd-stressed plants will quench ROS (Wu et al., 2003). The biochemical changes that occur in plants under Cd stress include ROS production such as O_2, H_2O_2 and OH^- (Cho and Park, 2000). When plants undergo stress due to Cd accumulation, oxidative damage may result from the imbalance between ROS production and their detoxification through the altered antioxidant system (Gomez et al., 1999). Tolerance to Cd stress is associated with increased scavenging or detoxification capacity of activated oxygen species (Hashem et al., 2016). SOD, CAT, APOX and nonenzymatic antioxidants such as GSH, AsA, and α-tocopherol are the enzymatic antioxidant defense systems in plant cells. SOD, a major scavenger, is a metalloprotein that catalyzes superoxide dismutation to H_2O_2 and molecular oxygen (Allen, 1995). H_2O_2 is also toxic to cells that need CAT and/or POD to detoxify additionally to water and oxygen (Zhu et al., 2004).

Plants also react to Cd metal toxicity by synthesizing phytochelatins (PCs), which act as chelators of heavy metals. Sulfur,

which is a component of phytochelatin, plays an important role in their synthesis and, eventually, in detoxifying Cd by forming Cd-binding peptides (CdBP) (Cobbett and Goldsbrough, 2002). Phytochelatins [(π-Glu-Cys)n-Gly] are the cys-rich peptides that are enzymatically synthesized using GSH as a phytochelatin synthase (PCS) substrate, which is found to be activated when exposed to heavy metal Cd (Thangavel et al., 2007). By binding to Cd ions, PCs are transported to the vacuole and play an important role in stress-tolerant plants (Mendoza Cozatl et al., 2005). Transgenic approaches have also revealed that PCs play an important role in Cd tolerance in many plants, as observed by the overexpression of the *Brassica juncea* π-glutamyl cysteine synthetase gene, resulting in increased biosynthesis of GSH and PCs, and increased tolerance to Cd (Zhu et al., 2004). In a study by Shanmugaraj et al. (2013), PCS gene transcripts increased in Brassica cultivars during treatment with Cd, indicating enhanced phytochelatine biosynthesis to protect the plant from heavy metal stress.

2.9. Zeolite: Its Potential Use in Agriculture

Zeolites are crystalline aluminosilicates. They are among the most common minerals in sedimentary rocks and are reported to be especially common in tuffaceous rocks. They have been found in rocks of diverse age, lithology, and geologic setting, and are valuable indicators of the depositional and post-depositional (diagenetic) environments of the host rocks. They are tectosilicates exhibiting an open three-dimensional structure containing cations needed to balance the electrostatic charge of the framework of silica and alumina tetrahedra and containing water.

2.10. Origin and history of Zeolites

Identification of zeolite as a mineral goes back to 1756, when a Swedish mineralogist, Alex Fredrik Cronstedt, collected some crystals from a copper mine in Sweden. He found that upon rapidly heating the material stilbite, it produced large amounts of steam from water that had been adsorbed by the material. Based on this, he called the material zeolite, from the Greek words meaning “boiling stones,” because of ability to froth when heated to about 200 C. Following Cronstedt’s findings, zeolites were considered as minerals found in volcanic rocks for a period of 200 years. Natural zeolites form where volcanic rocks and ash layers react with alkaline groundwater. Zeolites also crystallize in postdepositional environments over periods ranging from thousands to millions of years in shallow marine basins. Naturally occurring zeolites are rarely pure and are contaminated to varying degrees by other minerals, metals, quartz, etc. For this reason, naturally occurring zeolites are excluded from many important commercial applications where uniformity and purity are essential.

Most of the initial research on the use of zeolites in agriculture took place in the 1960s in Japan. A brief review of the literature has pointed out that Japanese farmers have used zeolite rock over years to control the moisture content and to increase the pH of acidic volcanic soils. Ion-exchange properties of zeolites can be utilized in agriculture because of their large porosity and high cation-exchange capacity. They can be used as both carriers of nutrients and a medium to free nutrients.

2.11. Classification of Zeolites

The Si/Al ratio is an important characteristic of zeolites. The charge imbalance due to the presence of aluminum in the zeolite framework determines the ion-exchange characters of zeolites and is expected to induce potential acidic sites. The Si/Al ratio is inversely proportional to the cation content, however directly proportional to the thermal stability. The surface selectivity changes from hydrophilic to hydrophobic when the ratio increases. Silica molecular sieves (silicalite-1) have a neutral framework; are hydrophobic in nature, and have no ion-exchange or catalytic properties.

Zeolites are classified on the basis of silica:alumina ratio as follows:

1. Zeolites with low Si:Al ratio (1.0 to 1.5)
2. Zeolites with intermediate Si:Al ratio (2 to 5)
3. Zeolites with high Si:Al ratio (10 to several thousands).

As the Si to Al ratio continues to increase, the catalytic activity often tends to pass through a maximum because of two opposing effects: increasing effectiveness of each acid center on the one hand, and decreasing number of acid centers on the other. The aluminous zeolites are excellent desiccants whereas the most siliceous zeolites tend to be organophilic nonpolar sorbents (Barrer, 1986). Flanigen (1980) considered that “low silica” zeolites or aluminum-rich zeolites contain the maximum number of cation-exchange sites balancing the framework aluminum, and thus the highest cation contents; “intermediate silica” zeolites exhibit a common characteristic in terms of improved stability over the “low silica” zeolites and “high silica" zeolites representing heterogeneous hydrophilic surfaces within a porous crystal. The surface of the high silica zeolites approaches a more homogeneous characteristic with an organophilic hydrophobic selectivity and exchange capacities Flanigen (2001) has classified zeolites based on pore diameter, namely, small-pore zeolites, medium-pore zeolites, large-pore zeolites, and extra-large-pore zeolites:

a. Small-pore zeolites (8-rings) with free pore diameter of 0.3-0.45 nm
b. Medium-pore zeolites (10-rings) with free pore diameter of 0.45-0.6 nm
c. Large-pore zeolites (12-rings) with free pore diameter of 0.6-0.8 nm
d. Extra-large-pore zeolites (14-rings) with free pore diameter of 0.8-1.0 nm.

2.12. Physical and Chemical Properties of Zeolites

Two major processes have been identified as kinetics of ion-exchange process in zeolites, namely, particle diffusion and film diffusion. Zeolites are one of the greatest cationic interchangers and their cationic inter-change capacity is two to three times greater than other types of minerals found in soils. Zeolites are potential adsorbents due to the ability of their microporous structures to adsorb molecules at relatively low pressure (Kamarudin et al., 2003). There is a wide variation in the cation-exchange capacity of zeolites because of the differing nature of various zeolite cage structures, natural structural defects, adsorbed ions and their associated minerals. Thus, in short, zeolites are natural materials with the ability to exchange ions, absorb gases and vapors, act as molecular-scale sieves, and catalyze reactions owing to fixed pore sizes and active sites in the crystal lattice. The size of clinoptilolite channels controls the size of the molecules or ions that can pass through them and therefore a zeolite like clinoptilolite can act as a chemical sieve allowing some ions to pass through while blocking others (Mumpton, 1999). Their internal areas mostly fall in the range of 400-850 $m^2\ g^{-1}$ for zeolites (Barrer, 1986). Zeolites vary widely in their chemical composition, particularly with respect to contents of SiO_2, CaO, K_2O, Al_2O_3, Na_2O, and Fe_2O_3. Dixon and Ming (1987) outlined the techniques for separation of clinoptilolites from soil by combining the low specific gravity and fine particle-size characteristics of clinoptilolite in soils. Clinoptilolite was separated from the silt fractions of the chemically treated and untreated samples using a heavy liquid.

2.13. Major Natural Zeolites of Agricultural Importance

Of more than 48 natural zeolites species known, clinoptilolite is the most abundant in soils and sediments. Among the natural zeolites, clinoptilolite (Abadzic and Ryan 2001) is most commonly used in agricultural practices as a soil amendment and for promoting nitrogen retention in soils (Polat et al., 2004). Clinoptilolite is a member of the heulandite group of natural zeolites, a temperature-stable heulandite seems to be the most abundant zeolite in soils over a wide variety of pH conditions, from slightly acidic to strongly alkaline (Dixon and Ming, 1986). They are the most well-known and one of the most useful zeolites. Extensive deposits of clinoptilolite are found in Western United States, Bulgaria, Hungary, Japan, Australia, and Iran (Mumpton, 1999). Clinoptilolite has a high cationic interchange capacity and a great affinity for NH_4^+ ions (Inglezakis, 2004).

It was reported by Polat et al. (2004) that, of the 40 naturally occurring zeolites studied by research groups, the most well-known ones are clinoptilolite, erionite, chabazite, heulandite, mordenite, stilbite and phillipsite.

2.14. Zeolite Nutrient Interactions

Some of the characteristics of zeolites that potentially make them desirable for improving the properties of soils are a large internal porosity that results in water retention, a uniform particle-size distribution that allows them to be easily incorporated, and high cation-exchange capacity that retains nutrients (Ok et al., 2003). The addition of zeolite has improved the nutrient status of sand-based root zones, especially selective retention of NH_4^+ and K^+ ions (Petrovic, 1993).

2.15. Soil Urease Adsorption

Urease adsorption on zeolite and the various properties of adsorbed urease were investigated to find out the influence of zeolite on activity and properties of urease by Choi and Park (1988). Free urease in solution was adsorbed on zeolite until maximum adsorption, and the amount of maximum adsorption was 11.3 mg urease/100 mg zeolite at pH 7.0. It is apparent that free urease was adsorbed on the outer surface of zeolite by cation-exchange reaction, and more than 70% of urease was adsorbed within 30 min. The activity of adsorbed urease decreased by 89.6%, whereas Km value increased to 34.4 mM, which is higher than that of free urease. The optimum pH of adsorbed urease widened 6.5-7.0, compared to that of free urease 7.0. Results of Bernardi et al. (2010) have shown the potential for urea and zeolite to improve the efficiency of nitrogen use, since the use of the mineral provided similar effects to the treatments with lower volatility losses and were inferior only to those sources that do not exhibit volatility losses and to that with urease inhibitor. Addition of zeolite to soil reduced soil urease activity through urease adsorption on the zeolite (Ramesh et al., 2010).

2.16. Nitrate Leaching

Huang and Petrovic (1994) advocated the application of zeolites in order to reduce the leaching of nitrates in golf courses located on sandy soils. Amendment of clinoptilolite zeolite to sandy soils has been reported to lower nitrogen concentration in the leachate and to increase moisture and nutrients in the soil due to increased soil surface area and cation-exchange capacity (He et al., 2002). MacKown and Tucker (1985) found that zeolite applications decreased nitrification and leaching losses, NH_4^+-clinoptilolite decreased nitrification by about 11%. The decrease resulted from retention of NH_4^+ by clinoptilolite in places where nitrifying bacteria could not oxidize NH_4^+.

2.17. Ammonium Trapping

The small internal tunnels of clinoptilolite zeolite as an example have been found to physically protect ammonium ions from too much nitrification by microorganisms (Ferguson and Pepper, 1987). Composting experiments have shown that by incorporating clinoptilolitic tuff with animal waste, ammonia can be retained by the zeolite (Witter and Lopez-Real, 1988). The slow retention and liberation capacity of NH_4^+ ions that have been incorporated in the channels forming crystalline structure is generally attributed to zeolites, and particularly clinoptilolites (Allen et al., 1996). The availability of internal space volume is another interesting characteristic of zeolites for separation/purification applications (Kamarudin et al., 2003). Zeolite may initially immobilize NH_4^+ N in the soil when it is applied, reducing N availability to the crop and resulting in the negative effects on growth (Wiedenfeld, 2003). Besides retaining large quantities of ammonium ion, these minerals also interfere with the process of nitrification. Zeolites have reduced ammonia emissions from animal manures. Zeolite reduced total ammonia loss by 16% (Witter and Kirchmann, 1989). Ahmed et al. (2002) have found that zeolite mixtures significantly reduced NH_3 loss by between 32 and 61% compared with straight urea (46% N) and zeolite (0.75 and 1 g kg^{-1} of soil).

2.18. Improving Soil Physio-Chemical and Microbial Properties

Natural zeolites are extensively used to improve soil physical environment, particularly in sandy and clay poor soils. Application of zeolite to the tune of one-fifth of the soil weight was found to be the best medium for tomato plants (Unlu et al., 2004). Bansiwal et al. (2006) emphasized that zeolites were commonly used as soil conditioners. Khan et al. (2008) demonstrated that zeolite application at soybean planting time encouraged the initiation of vegetative phenology on allophanic soil. Zeolite has an effect to mitigate the salt damage to plants and that the leaching of $CaCl_2$ substitutes adsorbed Na in zeolite for Ca. Substituted zeolite gives high productivity to sand. Zeolite amendment is an effective way to improve soil condition in an arid and semiarid environment (Yasuda et al., 1998). Application of natural zeolite increased the available nitrogen, phosphorus, calcium, and magnesium of the medium. A study by Wiedenfeld (2003) concluded that the slight effect of zeolite application observed in a study suggests that its potential benefit might be realized only under poorer conditions where the needs for improvement in nutrient retention and moisture holding capacity are greater. Farmers add the zeolites to the soil to control soil pH and to improve ammonium retention. The CEC of soil may be increased by using zeolites as soil amendments (DeSutter and Pierzynski, 2005). Chander and Joergensen (2002) found an increase in soil microbial biomass and incorporation of added ^{14}C into microbial biomass after zeolite amendment. The microbial populations could respond to zeolite amendment in different ways but not usually toxic to organisms.

2.19. Enhancing Nitrogen Use Efficiency

There are several reports in the literature showing that the addition of zeolite to the source of N can improve the nitrogen use efficiency (Gruener et al., 2003; McGilloway et al., 2003; Rehakova et al., 2004). Surface-modified zeolites offer a great promise as anion carriers for slow release of nutrients (Bansiwal et al., 2006) The high potential of zeolites as nitrogen fertilizers has been demonstrated. Their use would diminish environmental problems and increase fertilizer efficiency. It has been verified that when mixed with nitrogen, phosphorus, and potassium compounds, zeolite enhances the action of such compounds as slow-release fertilizers, both in horticultural and extensive crops. Natural zeolites have high tendency of ammonium selective properties (Kithome et al., 1998). The main use of zeolites in agriculture is for nitrogen capture, storage, and slow release. It has been shown that zeolites, with their specific selectivity for ammonium (NH_4^+), can take up this specific cation from either farmyard manure, composts, or ammonium-bearing fertilizers, thereby reducing losses of nitrogen to the environment. There is a new possibility, which is the addition of zeolite to the organic substrate.

Natural zeolites, due to their structure and properties, inert and nontoxic material can be used as a slowly releasing carrier of fertilizer (Rehakova et al., 2004). It is possible to obtain an increase in the efficiency of nitrogen fertilizer in forage crops when nitrogenated clinoptilolites are used in comparison with the use of urea. Ferguson and Pepper (1987) suggested that the effects of zeolite on N uptake and plant growth would vary with soil type, and that maximum benefit would be expected on coarse-textured low cation-exchange capacity soils.

2.20. Remediation of Heavy Metal Contaminated Soils

Reducing the plant availability of heavy metals (Cd, Pb, Cr, Zn, Cu, etc.) in soils is critical for optimizing agricultural production in areas with heavy metal contaminated soils. Phytoavailability of heavy metals correlates best with their concentrations in soil solution rather with their total content in soil (Kabata-Pendias and Brummer, 1992). The removal of heavy metals in polluted areas is very difficult because they persist in soils for very long periods. However, the fixation of heavy metals in a non-available form could be a useful method for soils that are already contaminated by heavy metals. Heavy metal phyto-availability may be reduced if the metals are sorbed or precipitated from the soil solution. One of the ways to heavy metal immobilization may be the application of zeolites. Zeolites in general have large cation-exchange capacity and expectedly attract positive-charged ions and, therefore, are widely used for sequestration of cationic pollutants like heavy metals (Kumar et al., 2007). Natural and artificial zeolites increase ion-exchange sites in soils in addition to offering absorption sites for small molecules, due to their porous structure. Consequently, zeolites are able to retain heavy metals in soil. Some zeolites, for example, clinoptilolite, are stable in acid conditions up to pH. High affinity of zeolites to heavy metals has been demonstrated (Tsadilas, 2000). Alexander and Christos (2003) studied Pb adsorption in soil and zeolite, and reported that clinoptilolite zeolite sorbed 20-30 times more Pb than the soil. Application of zeolite leads to a decrease of Pb concentrations in soil solution retaining the metal in the solid phase, where it should be less available for plants. Calculations based on the value of maximal sorption capacity of zeolite reveal that 1% added zeolite can retain 3.6 mmol Pb kg^{-1} or 750 mg kg^{-1} soil. Advantage of zeolite for soil remediation is its high efficiency independent of soil pH in the pH range 3-5 (Alexander and Christos, 2003). Leggo et al. (2006) showed that organo-zeolitic soil systems offer an opportunity to revegetate land made barren by metal pollution and as a consequence reduction of erosion and dissemination of contaminants.

2.21. Red Amaranth: An Overview

Recent decades have witnessed a resurgence of interest in *Amaranthus* sp. as nutraceutical and natural protector against chronic ailments. A native of tropical America, *Amaranthus* (meaning immortal in Greek) was a staple crop in the Aztec, Mayan, Incan civilizations. Currently it is widely cultivated and consumed throughout India, Nepal, China, Indonesia, Malaysia, Phillipines; whole of Central America, Mexico; Southern and Eastern Africa.

Genus *Amaranthus* belongs to order Caryophyllales, family Amaranthaceae, sub-family Amaranthoideae. It includes branched annual herbs with about 70 different species, 17 of which are edible. National Botanical Research Institute of India (NBRI) has built up perhaps, one of the best qualitative collections of Amaranth 'germplasm' in the world, comprising nearly 400 accessions, referable to 20 species, of which nearly half belong to the grain type. *Amaranthus* extracts have been used in ancient Indian, Nepalese, Chinese and Thai medicine to treat several conditions including urinary infections, gynecological conditions, diarrhea, pain, respiratory disorders, diabetes and also as diuretic. In India, root extract of *A. spinosus* is given as a vermicide among the Santhali and Paharia tribes of eastern Bihar, while an aqueous decoction of the plant is used for chronic diarrhea in southern Orissa. Some tribes apply *A. spinosus* to induce abortion. The juice of *A. spinosus* is used by tribals of Kerala to prevent swelling around stomach while leaves are boiled without salt and consumed for 2–3 days to cure jaundice. However, anti-cancerous, anti-viral, hepatoprotective, neuroprotecive, cardioprotective and antidiabetic properties, of *Amaranthus* with relevance to current global health scenario are currently in the limelight.

Natural crude extracts from plants have been used in traditional medicine to treat various ailments, *Amaranthus* spp. is one of them; though its complete therapeutic uses are still unexplored. Scientific interest in *Amaranthus* and its health promoting benefits has increased significantly in the recent past with various reviews presenting nutraceutical properties of Amaranth; its composition, antioxidant properties, applications, and processing.

CHAPTER III

MATERIALS AND METHODS

This experiment was done to find out the impact of Zeolite on cadmium absorption by red amaranth plant. This section provides a brief overview about experimental time, spatial reference, soil and weather conditions of the experimental area, experimental information, treatments, experimental design and layout, intercultural activities, data collection and statistical analysis.

3.1. Experimental period

The study was undertaken during the period from October, 2018 to April, 2019 in rabi season.

3.2. Description of the experimental site

The experiment was conducted in the research field of Sher-e-Bangla Agricultural University. Sher-e-Bangla Nagar, Dhaka. The location of the site was 23°74' N latitude and 90°35' E longitude with an elevation of 8.2 meter from sea level. The soil belonged to The Madhupur Tract, AEZ - 28 (FAO, 1988). Top soil was silty clay in texture with distinct dark yellowish-brown mottles.

3.3. Climate condition

The geographical location of the experimental site was under the subtropical climate, characterized by three distinct seasons, winter season from November to February and the pre-monsoon period or hot season from March to April and monsoon period from May to October.

3.4. Planting Material

The test crop was Red Amaranth (cv. BARI Lalshak-01). The seeds were collected from Horticultural Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur.

3.5. Treatments of the experiment

Table 1. Treatments of the experiment

Treatments	Description
Zl_0Cd_0	No Zeolite + No Cadmium (Control)
Zl_0Cd_{15}	No Zeolite + 15 ppm Cadmium
Zl_1Cd_0	1% Zeolite of soil wt. + No cadmium
Zl_1Cd_{15}	1% Zeolite of soil wt. + 15 ppm cadmium
Zl_2Cd_0	2% Zeolite of soil wt. + No cadmium
Zl_2Cd_{15}	2% Zeolite of soil wt. + 15 ppm cadmium
Zl_3Cd_0	3% Zeolite of soil wt. + No cadmium
Zl_3Cd_{15}	3% Zeolite of soil wt. + 15 ppm cadmium
Zl_4Cd_0	4% Zeolite of soil wt. + No cadmium
Zl_4Cd_{15}	4% Zeolite of soil wt. + 15 ppm cadmium

3.6. Experimental design and layout

The experiment was laid out following Completely Randomized Design (CRD) with five replications.

3.7. Zeolite collection

Natural zeolite clinoptilolite was collected from Bangladesh Agricultural Research Institute (BARI).

3.8. Preparation of the pots

Collected soil was dried, pulverized for two weeks in first week of October, then dried soil was mixed with Cd concentration at different rates and remained for six weeks as barren for proper integration. After that, different percentage of pulverized zeolite was thoroughly mixed with soil of each pot, that contained 7 kg of total mixture of soil.

3.9. Application of fertilizers and manure

The fertilizers N, P, K and S in the form of Urea, TSP, MP and Gypsum, respectively were applied. The entire amount of TSP, MP and Gypsum, two-third of urea were applied during the final preparation of pot. Rest of urea was top dressed after first irrigation.

Table 2. Recommended doses of fertilizer and manure for Lalshak cultivation

Name of fertilizer/ manure	**Amount/Ha**
Urea	200-250kg
TSP	100-150kg
MOP	150-200kg

Source: Mondal 2011

3.10. Intercultural Operations

After the seedlings emergence, different types of intercultural operations such as irrigation, weeding, top dressing of fertilizer and crop protection measures were accomplished for ensuring better growth and development of the Lalsak seedlings. Weeding was done manually at two times; one is at 15 DAS and another at 35 DAS. Fungicide Autostin was applied to get rid of fungal attack in the pots. Irrigation was done as per necessity.

3.11. Harvesting, Drying and Cleaning

The crop was harvested depending upon the maturity of plant manually from each pot at the first week of April, 2020. The harvested crop of each pot was bundled separately, properly tagged and allowed to sun drying. After enough sun drying, whole plant was put on desiccator at 27°C. Before that fresh weight, height, no of leaves of each plant was taken. After three days of drying, dry weight was taken, and separated into root, leaves and stem portion. All of dry portion then pulverized in a grinding machine for Cd analysis.

3.12. Data Collection

3.12.1. Cadmium analysis

Dried plant materials were separated into three parts as root, stem and leaves. Segregated materials then pulverized and prepared for Cd analysis in laboratory, and measured in ppm unit.

3.12.2. Rhizosphere and bulk soil

Randomly collected rhizosphere and bulks soil from plant root zone, tagged, dried, pulverized and prepared for Cd analysis for the estimation of available Cd and total Cd.

3.13. Cadmium concentration analysis

Soil and plant samples were analyzed for total Cd using an atomic absorption spectrophotometer (AAS) after digestion of the samples. EDTA extractable Cd was treated as bio-available Cd in this study. 0.5 M DTPA solution was used to extract Cd from the soil samples. In brief, 10 g of soil was taken in a 250 ml conical flask containing 50 ml of DTPA solution. The soil suspension was then shaken on a horizontal shaker for 15h at 170 cycles per min. The suspension was then centrifuged at 2,000g for 5 minutes and the supernatant was filtered through a suitable filter paper. Cd in the extract was then determined by an AAS.

3.14. Statistical analysis

Data recorded for Cd uptake against application of zeolite percentage was compiled and tabulated in proper form for statistical analysis. Analysis of variance was done with the help of MSTAT-C computer package program. The mean differences among the treatments were evaluated with DMRT test (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Effect of Zeolite on the cadmium concentration in Stem

Table 3. Effect of Zeolite on the cadmium concentration in Stem

Treatments	Concentration of Cadmium (ppm)
Zl_0Cd_0	1.18 f
Zl_0Cd_{15}	36.06 a
Zl_1Cd_0	1.15 f
Zl_1Cd_{15}	33.22 b
Zl_2Cd_0	1.07 f
Zl_2Cd_{15}	31.85 c
Zl_3Cd_0	0.98 f
Zl_3Cd_{15}	30.38 d
Zl_4Cd_0	0.91 f
Zl_4Cd_{15}	28.32 e
$LSD_{0.05}$	**0.37**
CV (%)	**1.32**

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability by LSD

It is evident that different zeolite concentration in soil influenced the Cd uptake by stem. Importantly, from Table 3, it is clear that zeolite application had positive influence on Cd concentration in stem of red amaranth. It is found that highest concentration of Cd in stem was present in treatment Zl_0Cd_{15}, where the highest dose of Cd (15 mg/kg soil) was applied in soil without zeolite application. From this treatment, the highest stem concentration (36.06 ppm) was found which was statistically different from any other treatment of the experiment. It was followed by treatment Zl_1Cd_{15}, where highest cadmium concentration was subject to be treated by zeolite in low concentration and the cadmium stem concentration was 33.22 ppm. The cadmium concentration in treatment Zl_1Cd_{15} was reduced by 8.54% and showed statistical difference from any other treatment. The cadmium concentration (31.85 ppm) was then followed by treatment Zl_2Cd_{15}, reduction of concentration was 13.21% compared to highest uptake. Further, treatment Zl_3Cd_{15} observed 30.38 ppm Cd uptake by stem and Cd concentration was 18.69% less than that of highest concentration. In case of treatment Zl_4Cd_{15} where both zeolite and cadmium were applied in their highest concentration and it was observed that stem concentration of cadmium was 28.32 ppm which is 27.33% less than highest concentration. It is proved that increasing zeolite concentration decreased the Cadmium uptake by plant. On the other hand, treatment 1,3,5,7 and 9 was experienced with no cadmium application with gradual increase in zeolite concentration. The Cd concentration in stem was 1.18 ppm (Zl_0Cd_0), 1.15 ppm (Zl_1Cd_0), 1.07 ppm (Zl_2Cd_0), 0.98 ppm (Zl_3Cd_0) and 0.91 ppm (Zl_4Cd_0) respectively. No statistical variation found among these treatments. Overall, Cd presence in soil caused a significant increase in Cd concentration accumulated within plants, also Jalil *et al.* (1994) reported that, adding 0, 0.5 and 0.1 µM $CdCl_2$ to fertigation solution of three wheat cultivars, increased shoot and root Cd concentration in all cultivars and treatments significantly. Rehakova *et al.* (2004) observed the lowest content of heavy metals in plants treated by zeolite in contaminated soil. Their results imply that natural zeolite is effective in improving soil properties, also the intake of Cd from soil decrease significantly. The findings indicated a general negative influence of Cd contamination on shoot uptake but in contrast zeolite could ameliorate adverse effects by reducing its absorption by plants.

4.2. Effect of Zeolite on the cadmium concentration in Leaf

Table 4. Effect of Zeolite on the Cd concentration in Leaf

Treatments	**Concentration of Cadmium (ppm)**
Zl_0Cd_0	1.43 f
Zl_0Cd_{15}	65.46 a
Zl_1Cd_0	1.32 f
Zl_1Cd_{15}	58.83 b
Zl_2Cd_0	1.13 fg
Zl_2Cd_{15}	55.41 c
Zl_3Cd_0	0.86 g
Zl_3Cd_{15}	51.70 d
Zl_4Cd_0	0.71 g
Zl_4Cd_{15}	48.41 e
$LSD_{0.05}$	**0.45**
CV (%)	**0.92**

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability by LSD

It is evident that different zeolite concentration in soil influenced the Cd accumulation in red amaranth leaves. Importantly, from Table 4, it is clear that zeolite application had positive influence on Cd concentration in leaves of red amaranth. It is found that highest concentration of Cd in leaf was present in treatment Zl_0Cd_{15}, where no zeolite was applied and highest amount of Cd (15 mg/kg soil) was applied in soil. From this treatment the highest leaf concentration (65.46 ppm) was found which was statistically different from any other treatment of the experiment. It was followed by treatment Zl_1Cd_{15} where highest Cd concentration was subject to be treated by zeolite in low concentration and the Cd concentration in leaf was 58.83 ppm. The Cd concentration of treatment Zl_1Cd_{15} was reduced by 11.26% and showed statistical difference from any other treatment. The Cd concentration (55.41 ppm) was then followed by treatment Zl_2Cd_{15}, reduction of concentration was

18.13% compared to highest concentration. Further, treatment Zl_3Cd_{15} observed 51.703 ppm Cd accumulation in leaf and Cd concentration was 26.61% less than that of highest concentration. In case of treatment Zl_4Cd_{15} where both zeolite and Cd were applied in their highest levels and it was observed that leaf concentration of Cd was 48.41 ppm which is 35.21% less than highest concentration. It is proved that increasing zeolite concentration decreased the Cd concentration in leaf of red amaranth. On the other hand, treatments 1,3,5,7 and 9 were experienced with no Cd application with gradual increase in zeolite concentration. The Cd concentration in leaf was 1.43 ppm (Zl_0Cd_0), 1.32 ppm (Zl_1Cd_0), 1.13 ppm (Zl_2Cd_0), 0.863 ppm (Zl_3Cd_0) and 0.71 ppm (Zl_4Cd_0), respectively. No statistical variation was found among treatments 1, 3 and 5. Similarly, treatment 7 and 9 had no variation statistically. Overall, Cd presence in soil caused a significant increase in Cd concentration accumulated within leaves. Subsequently, zeolite application leads to decrease in Cd concentration.

Cadmium accumulation in leaf can severely affect the overall physiology of plant. Ouzounidou *et al.* (1997) reported that the growth reduction and the inhibition of chlorophyll content and photosynthesis observed in the upper plant parts seemed principally due to indirect Cd effects on the content of essential nutrients. Cadmium treatment was shown to damage the structure of chloroplasts, as manifested by the disturbed shape and the dilation of the thylakoid membranes. Phytoavailability of heavy metals correlates best with their concentrations in soil solution rather with their total content in soil. Zeolites in general have large cation-exchange capacity and expectedly attract positive-charged ions and, therefore, is widely used for sequestration of cationic pollutants like heavy metals (Kumar *et al.*, 2007).

4.3. Effect of Zeolite on the Cadmium concentration in Root

Table 5. Effect of Zeolite on the cadmium concentration in Root

Treatments	Concentration of Cadmium (ppm)
Zl_0Cd_0	1.17 f
Zl_0Cd_{15}	46.99 a
Zl_1Cd_0	1.09 f
Zl_1Cd_{15}	41.4 b
Zl_2Cd_0	1.03 f
Zl_2Cd_{15}	38.60 c
Zl_3Cd_0	0.97 f
Zl_3Cd_{15}	36.51 d
Zl_4Cd_0	0.94 f
Zl_4Cd_{15}	33.24 e
$LSD_{0.05}$	**0.74**
CV (%)	**2.14**

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability by LSD

It is observed that different zeolite concentration in soil influenced the Cd accumulation in red amaranth roots. Importantly, from Table 5, it is clear that zeolite application had positive influence on Cd concentration in roots of red amaranth. It is found that highest concentration of Cd in root was present in treatment Zl_0Cd_{15}, where no zeolite was applied and highest amount of Cd (15 mg/kg soil) was applied in soil. From this treatment the highest root concentration (46.99 ppm) was found which was statistically different from any other treatment of the experiment. It was followed by treatment Zl_1Cd_{15} where highest Cd concentration was subject to be treated by zeolite in low concentration and the Cd concentration in root was 41.4 ppm. The Cd concentration in treatment Zl_1Cd_{15} was reduced by 13.51% and showed statistical difference from any other treatment. The Cd concentration (38.60 ppm) was then followed by treatment Zl_2Cd_{15}, reduction of concentration was

21.67% compared to highest concentration. Further, treatment Zl_3Cd_{15} observed 36.51 ppm Cd accumulation in root and Cd concentration was 28.67% less than that of highest concentration. In case of treatment Zl_4Cd_{15} where both zeolite and Cd were applied in their highest concentration and it was observed that root accumulation of Cd was 33.24 ppm which is 41.37% less than highest concentration. It is proved that increasing zeolite concentration decreased the Cd concentration in roots of red amaranth. On the other hand, treatments 1,3,5,7 and 9 were experienced with no Cd application with gradual increase in zeolite concentration. The Cd concentration in root was 1.17 ppm (Zl_0Cd_0), 1.08 ppm (Zl_1Cd_0), 1.03 ppm (Zl_2Cd_0), 0.97 ppm (Zl_3Cd_0) and 0.94 ppm (Zl_4Cd_0) respectively. No statistical variation found among these treatments. Overall, Cd presence in soil caused a significant increase in Cd concentration within roots.

Cadmium affects cyto-histology and morphology of the roots. In particular, they alter the lateral root primordia organization and development with negative consequences on root system architecture. This is due to a disturbance of IAA biosynthesis and transport, as indicated by the altered expression of both ASA2 and YUCCA2 biosynthetic genes, and AUX1 and PIN5b transporter genes (Ronzan *et al.* 2018). Our findings show conformity with the findings of Eshghi *et al.* (2015). They reported that amid Cd stress, plants root structure can be developed by application of zeolite. Further, according to the study of Abdi *et al.* (2006), Zeolite also increased net photosynthetic rate, stomatal conductance, water use efficiency, mesophyll efficiency, petiole length, leaf area, specific leaf weight, fresh and dry weights of shoots and roots, fruit weight and number of achenes of strawberry.

4.4. Effect of Zeolite on total cadmium concentration in Bulk soil

Table 6. Effect of Zeolite on total cadmium concentration in Bulk soil

Treatments	Concentration of Cadmium (ppm)
Zl_0Cd_0	0.77 g
Zl_0Cd_{15}	7.99 e
Zl_1Cd_0	0.82 fg
Zl_1Cd_{15}	8.62 d
Zl_2Cd_0	0.86 fg
Zl_2Cd_{15}	9.17 c
Zl_3Cd_0	0.91 f
Zl_3Cd_{15}	9.41 b
Zl_4Cd_0	0.95 f
Zl_4Cd_{15}	10.36 a
$LSD_{0.05}$	**0.13**
CV (%)	**1.61**

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability by LSD

It is observed that different zeolite concentration in soil influenced the total Cd concentration in bulk soil. Importantly, from Table 6, it is clear that zeolite application had positive influence on Cd concentration in bulk total of soil. The more zeolite had applied in soil, the more accumulation happened. It is found that highest concentration of Cd in bulk total was present in treatment Zl_4Cd_{15}, where highest amount of zeolite and highest amount of Cd (15 mg/kg soil) was applied in soil. From this treatment the highest concentration (10.36 ppm) was found which was statistically different from any other treatment of the experiment. It was followed by treatment Zl_3Cd_{15} where highest Cd concentration was subject to be treated by zeolite in lower concentration and the Cd concentration in bulk soil was 9.41 ppm. The Cd accumulation in treatment Zl_3Cd_{15} was reduced by 10.09% and showed statistical difference from any other treatment. The Cd concentration (9.17 ppm) was then followed by treatment Zl_2Cd_{15}, reduction of accumulation was 13.01% compared to treatment Zl_4Cd_{15}. Further, treatment Zl_1Cd_{15} observed 8.62 ppm Cd concentration was 20.18% less than that of highest concentration. In case of treatment Zl_0Cd_{15} where no zeolite was applied and Cd were applied in the highest concentration, it was observed that concentration of Cd was 7.99 ppm which is 29.71% less than highest concentration found. It is proved that increasing zeolite concentration retained more Cd concentration in bulk soil. On the other hand, treatment 1,3,5,7 and 9 was experienced with no Cd application with gradual increase in zeolite concentration. The Cd concentration in bulk was 0.95 ppm (Zl_4Cd_0), 0.91 ppm (Zl_3Cd_0), 0.86 ppm (Zl_2Cd_0), 0.823 ppm (Zl_1Cd_0) and 0.77 ppm (Zl_0Cd_0) respectively. Result obtained from treatment 1 was statistically different from any other treatments but no statistical variation found among the treatments 3, 5 and 7. Subsequently, the higher the zeolite applied in soil, the higher the accumulation of Cd occurred.

Mahabadi *et al.* (2007) performed column and batch experiments with the aim to investigate the effects of zeolite (clinoptilolite) addition on Cd leaching according to the texture of the polluted soil. The results of the batch experiments indicated that the use of 15% zeolite reduced Cd leaching by 98%, 97%, 91%, and 93% in loam, loamy sand, clay, and sand textures, respectively. The column experiment showed that 9% zeolite is the optimal quantity for reducing Cd leaching in clay and sand, whereas 15% is optimal for loamy soil.

4.5. Effect of Zeolite on available cadmium concentration in Bulk soil

Table 7. Effect of Zeolite on available cadmium concentration in Bulk soil

Treatments	Concentration of Cadmium (ppm)
Zl_0Cd_0	0.37 f
Zl_0Cd_{15}	4.16 a
Zl_1Cd_0	0.36 f
Zl_1Cd_{15}	3.84 b
Zl_2Cd_0	0.32 fg
Zl_2Cd_{15}	3.42 c
Zl_3Cd_0	0.32 fg
Zl_3Cd_{15}	2.94 d
Zl_4Cd_0	0.28 g
Zl_4Cd_{15}	2.73 e
$LSD_{0.05}$	**0.07**
CV (%)	**2.28**

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability by LSD

It is observed that different zeolite concentration in soil influenced the available Cd concentration in bulk soil. Importantly, from Table 7, it is clear that zeolite application had positive influence on available Cd concentration in bulk soil. The more zeolite had applied in soil, the less available Cd concentration was found in the experiment. It is found that highest concentration of Cd in bulk available was present in treatment Zl_0Cd_{15}, where lowest amount of zeolite and highest amount of Cd (15 mg/kg soil) was applied in soil. From this treatment the highest concentration (4.153 ppm) was found which was statistically different from any other treatment of the experiment. It was followed by treatment Zl_1Cd_{15} where highest Cd concentration was subject to be treated by zeolite in higher concentration and the Cd concentration in available form soil was 3.843 ppm. The Cd availability in treatment Zl_1Cd_{15}

was reduced by 8.06% and showed statistical difference from any other treatment. The Cd concentration (3.423 ppm) was then followed by treatment Zl_2Cd_{15}, reduction of concentration was 21.32% compared to treatment Zl_4Cd_{15}. Further, treatment Zl_3Cd_{15} observed 2.945 ppm Cd availability in soil and Cd concentration was 41.01% less than that of highest concentration. In case of treatment Zl_4Cd_{15} where highest amount of zeolite and Cd were applied in the highest concentration, it was observed that concentration of Cd was 2.73 ppm which is 51.95% less than highest concentration found. It is proven that increasing zeolite concentration retained more Cd concentration in bulk soil. Consequently, less amount of Cd was found in available form. On the other hand, treatments 1,3,5,7 and 9 were experienced with no Cd application with gradual increase in zeolite concentration. The Cd concentration in bulk available was 0.373 ppm (Zl_0Cd_0), 0.353 ppm (Zl_1Cd_0), 0.32 ppm (Zl_2Cd_0), 0.316 ppm (Zl_3Cd_0) and 0.277 ppm (Zl_4Cd_0) respectively. No statistical variation found among the treatments 1, 3, 5 and 5, 7, 9. Overall, Cd presence in soil caused a significant increase in Cd concentration accumulated within roots. Subsequently, the higher the zeolite applied in soil, the least the availability of Cd occurred.

According to the findings of Gworek (1992), the addition of synthetic zeolite pellets to soils contaminated with Cd significantly reduced the concentrations of Cd in the roots and shoots of a range of crop plants. Use of synthetic zeolites types 4A and 13X, at application rates of 1% by soil weight, caused reductions in Cd concentrations of up to 86% in leaves of lettuce grown in pots, compared to controls with no added zeolites.

4.6. Effect of Zeolite on total cadmium concentration in Rhizosphere soil

Table 8. Effect of Zeolite on total cadmium concentration in Rhizosphere soil

Treatments	Concentration of Cadmium (ppm)
Zl_0Cd_0	0.55 i
Zl_0Cd_{15}	7.67 e
Zl_1Cd_0	0.63 i
Zl_1Cd_{15}	8.13 d
Zl_2Cd_0	0.85 h
Zl_2Cd_{15}	8.74 c
Zl_3Cd_0	1.09 g
Zl_3Cd_{15}	9.08 b
Zl_4Cd_0	1.28 f
Zl_4Cd_{15}	9.28 a
$LSD_{0.05}$	**0.13**
CV (%)	**1.72**

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability by LSD

It is observed that different zeolite concentration in soil influenced the total Cd concentration in rhizosphere soil. Importantly, from Table 8, it is clear that zeolite application had positive influence on Cd concentration of rhizosphere total of soil. The more zeolite had applied in soil, the more accumulation happened. It is found that highest total concentration of Cd in rhizosphere soil was present in treatment Zl_4Cd_{15}, where highest amount of zeolite and highest amount of Cd (15 mg/kg soil) was applied in soil. From this treatment the highest concentration (9.276 ppm) was found which was statistically different from any other treatment of the experiment. It was followed by treatment Zl_3Cd_{15} where highest Cd concentration was subject to be treated by zeolite in lower concentration and the Cd concentration in rhizosphere soil was 9.08 ppm. The Cd accumulation in treatment Zl_3Cd_{15} was reduced by 2.15% and showed statistical difference from any other treatment. The Cd concentration (8.743 ppm) was then followed by treatment Zl_2Cd_{15}, reduction of accumulation was 6.09% compared to treatment Zl_4Cd_{15}. Further, treatment Zl_1Cd_{15} observed 8.126 ppm Cd adsorption and Cd concentration was 14.11% less than that of highest concentration. In case of treatment Zl_0Cd_{15} where no zeolite was applied and Cd were applied in the highest concentration, it was observed that accumulation of Cd was 7.67 ppm which is 20.89% less than highest concentration found. It is proved that increasing zeolite concentration retained more Cd concentration in rhizosphere soil. On the other hand, treatment 1,3,5,7 and 9 was experienced with no Cd application with gradual increase in zeolite concentration. The total Cd concentration in bulk soil was 1.28 ppm (Zl_4Cd_0), 1.083 ppm (Zl_3Cd_0), 0.846 ppm (Zl_2Cd_0), 0.633 ppm (Zl_1Cd_0) and 0.546 ppm (Zl_0Cd_0) respectively. Result obtained from treatment 1 and 3 was statistically similar but significant variation found among the treatments 5, 7 and 9. Subsequently, the higher the zeolite applied in soil, the higher the accumulation of Cd occurred in rhizosphere soil.

4.7. Effect of Zeolite on available cadmium concentration in Rhizosphere soil

Table 9. Effect of Zeolite on available cadmium concentration in Rhizosphere soil

Treatments	Concentration of Cadmium (ppm)
Zl_0Cd_0	1.12 f
Zl_0Cd_{15}	7.30 a
Zl_1Cd_0	0.84 g
Zl_1Cd_{15}	7.14 b
Zl_2Cd_0	0.64 h
Zl_2Cd_{15}	6.85 c
Zl_3Cd_0	0.53 i
Zl_3Cd_{15}	6.73 d
Zl_4Cd_0	0.41 j
Zl_4Cd_{15}	6.48 e
$LSD_{0.05}$	**0.08**
CV (%)	**1.23**

In a column means having similar letter(s) are statistically identical and those having dissimilar letter(s) differ significantly as per 0.05 level of probability by LSD

It is observed that different zeolite concentration in soil influenced the available Cd concentration in rhizosphere soil. Importantly, from Table 9, it is clear that zeolite application had positive influence on available Cd concentration in rhizosphere soil. The more zeolite had applied in soil, the less available Cd concentration was found in the experiment. It is found that highest concentration of available Cd in rhizosphere soil was present in treatment Zl_0Cd_{15}, where the lowest amount of zeolite and highest amount of Cd (15 mg/kg soil) was applied in soil. From this treatment, the highest concentration of available Cd (7.30 ppm) was found which was statistically different from any other treatment of the experiment. It was followed by treatment Zl_1Cd_{15} where the highest Cd concentration was subject to be treated by zeolite in higher concentration and the Cd concentration in available form in rhizosphere soil was 7.14 ppm. The Cd availability in treatment Zl_1Cd_{15} was reduced by 2.24% and showed statistical difference from any other treatment. The available Cd concentration (6.85 ppm) was then followed by treatment Zl_2Cd_{15}, reduction of concentration was 6.56% compared to treatment Zl_4Cd_{15}. Further, treatment Zl_3Cd_{15} observed 6.72 ppm Cd availability in rhizosphere and Cd concentration was 8.63% less than that of highest concentration. In case of treatment Zl_4Cd_{15} where the highest amount of zeolite and Cd were applied in the highest concentration, it was observed that concentration of Cd was 6.47 ppm which is 12.82% less than highest concentration found. It is proved that increasing zeolite concentration retained more Cd concentration in rhizospheric soil. Consequently, less amount of Cd was found in available form. On the other hand, treatments 1,3,5,7 and 9 were experienced with no Cd application with gradual increase in zeolite concentration. The available Cd concentration in bulk soil was 1.12 ppm (Zl_0Cd_0), 0.84 ppm (Zl_1Cd_0), 0.64 ppm (Zl_2Cd_0), 0.53 ppm (Zl_3Cd_0) and 0.42 ppm (Zl_4Cd_0) respectively. All the treatments were statistically different from each other. Overall, Cd presence in soil caused a significant increase in Cd concentration accumulated within rhizosphere. Subsequently, the higher the zeolite applied in soil, the least the availability of Cd occurred.

Overall, it was observed that Cd accumulation by plant system may be reduced by application of zeolite. Zeolite successfully adsorb more Cd and make it unavailable, thus escape metal stress for roots. But due to high root: shoot ratio, the accumulation of Cd in leaf was higher than that of stem and root.

4.8. Effect of Zeolite on Soil pH

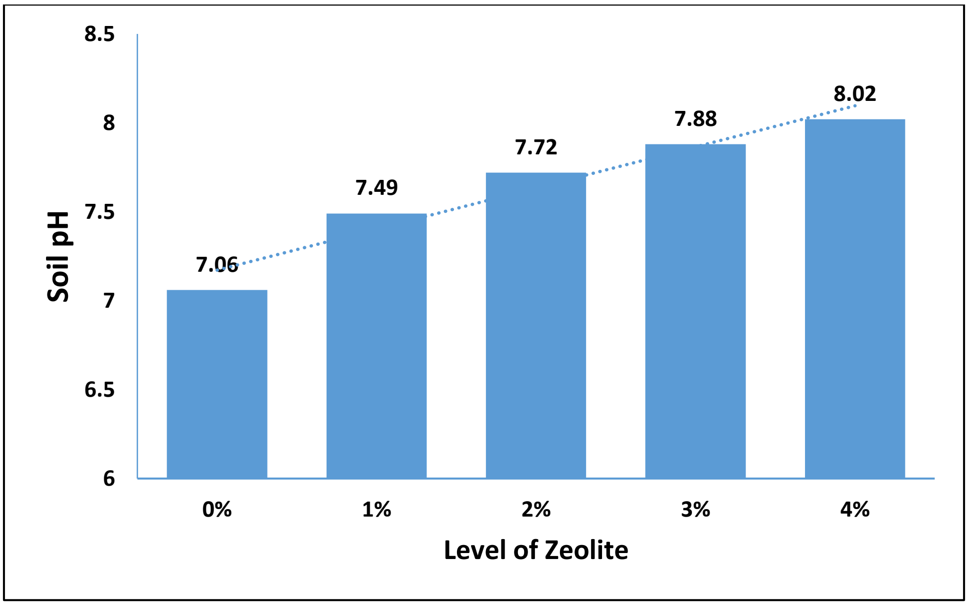

Figure 1. Effect of Zeolite on Soil pH.

It is evident that level of zeolite influenced the soil pH, increase of zeolite increased the soil pH. From Figure 1, it is found that soil pH was 7.06 where no zeolite (0%) was applied. In treatment zeolite 1%, soil pH was 7.49. Soil pH was increased to 7.72 for zeolite 2%. It was followed by zeolite 3% where soil pH was 7.88 and lastly the highest level of pH (8.02) was found from zeolite 4%.

4.9. Effect of zeolite on electrical conductivity

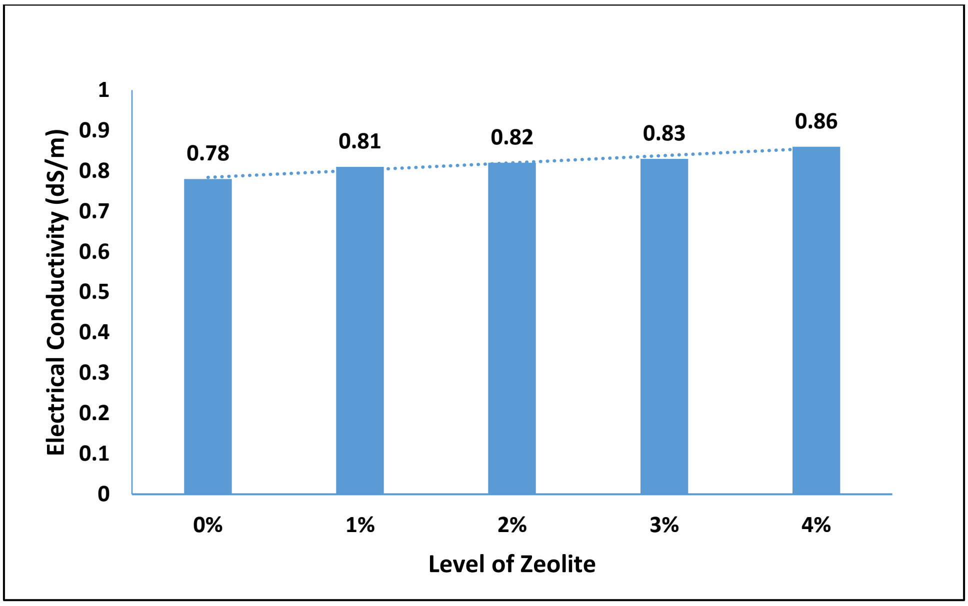

Figure 2. Effect of Zeolite on electrical conductivity.

It is evident (Figure 2) that level of zeolite influenced the electrical conductivity, increase of zeolite had increased electrical conductivity. In this Figure it is seen that, electrical conductivity was 0.78 dSm^{-1} where no zeolite (0%) was applied. In case of zeolite 1%, electrical conductivity was 0.81 dSm^{-1}. Electrical conductivity was increased to 0.82 dSm^{-1} for zeolite 2% and 0.83 dSm^{-1} for zeolite 3%. In last treatment, zeolite 4% represents the highest level of electrical conductivity 0.86 dSm^{-1}.

4.10. Effect of Zeolite on soil bulk density

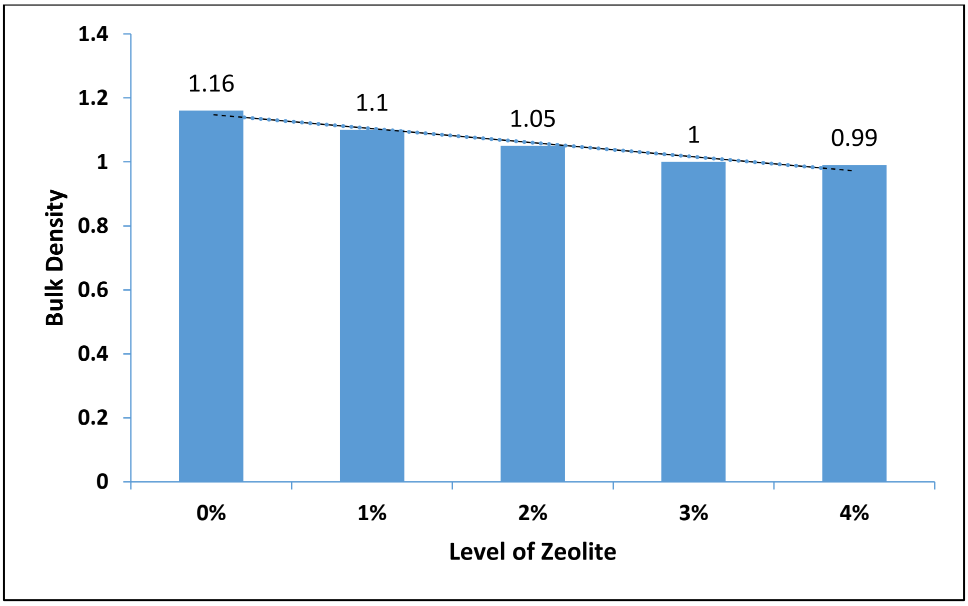

Figure 3. Effect of Zeolite on soil bulk density.

It is explicit that soil bulk density had decreased when the increased the level of zeolite. From this Figure 3, it clearly shows that 1.16 soil bulk density where no zeolite (0%) was applied. In treatment zeolite 1%, soil bulk density was 1.1. Soil bulk density was 1.05 for level of zeolite 2% and it also decreased 1 for level of zeolite 3%. Highest level of zeolite 4% showed (0.99), the lowest level of soil bulk density.

4.11. Effect of Zeolite on soil particle density

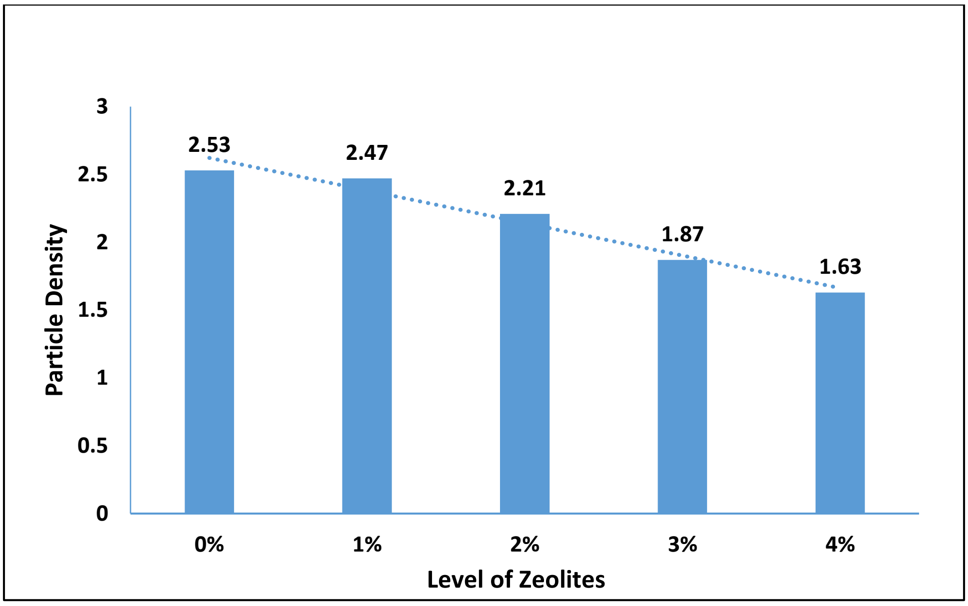

Figure 4. Effect of zeolite on particle density.

It is also explicit that soil bulk density had decreased when the increased the level of zeolite. From this Figure 4, it clearly shows that the highest particle density (2.53) from where no zeolite (0%) was applied. In treatment zeolite 1%, soil particle density was 2.47. Soil bulk density was 2.21 for level of zeolite 2% and it also decreased to 1.87 for level of zeolite 3%. Highest level of zeolite 4% showed (1.63), the lowest level of soil particle density.

4.13. Effect of zeolite water holding capacity (%)

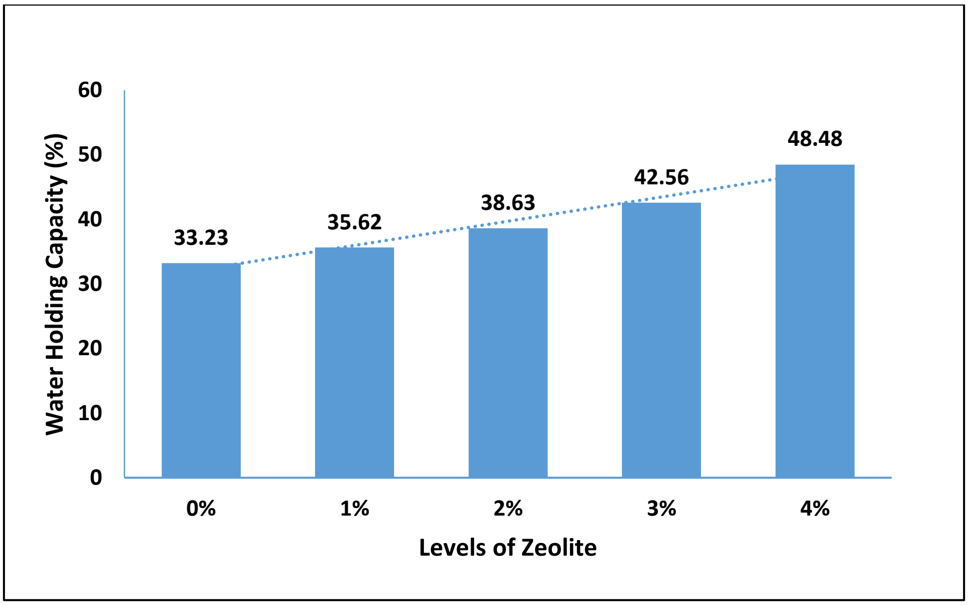

Figure 6. Effect of zeolite water holding capacity (%).

From the Figure 6, it is seen that zeolite had a positive influence on water holding capacity. The lowest water holding capacity was 33.23% where no zeolite (0%) was applied. In case of zeolite 1%, water holding capacity was 35.62 % which is 7.19 % higher than zeolite 0%. It was followed by zeolite 2% where water holding capacity was 38.63% and it was 16.25% higher than that of zeolite 0%. From zeolite 3% water holding capacity was obtained 42.56% which is 28.07% higher than that of zeolite 0%. However, the highest amount (48.48%) of water holding capacity obtained from zeolite 4% which is 45.89% higher than that of zeolite 0%.

CHAPTER V

SUMMARY, CONCLUSION AND RECOMMENDATION

The study was conducted at the research field of Sher-e-Bangla Agricultural University, Dhaka during the period from October 2018 to April 2019 in order to assess the effect of zeolite on Cd accumulation in leafy vegetables and to observe the influence of zeolite on some soil properties. The test crop was Red Amaranth (BARI Lalsak-1).

The treatment consisted of four doses of zeolite viz. Zl_0= no zeolite, Zl_1= 1% zeolite of soil wt, Zl_2= 2% zeolite of soil wt, Zl_4= 4% zeolite of soil wt. and two levels of Cd viz. Cd_0= Control (no Cd), Cd_{15} = 15 ppm Cd. There were 10 treatments and 4 replications altogether. The experiment was laid out in Completely Randomized Design (CRD) with five replications. The collected data were analyzed statistically for the treatment effect evaluation.

Evidently different zeolite concentrations in soil influenced the Cd accumulation in stem. It is found that highest concentration (36.06 ppm) of Cd in stem was present in treatment Zl_0Cd_{15}, where no zeolite was applied and highest amount of Cd (15 mg/kg soil) was applied in soil. On the other hand, in case of treatment Zl_4Cd_{15} where both zeolite and Cd were applied in their highest concentration and it was observed that stem absorption of Cd was 28.32 ppm which is 27.33 % less than highest accumulation.

Similarly, highest concentration (65.46 ppm) of Cd in leaf was found in treatment Zl_0Cd_{15}, where no zeolite was applied but the highest amount of Cd (15 mg/kg soil) was applied in soil. In case of treatment Zl_4Cd_{15} where both zeolite and Cd were applied in their highest concentration and it was observed that leaf accumulation of Cd was 48.41 ppm which is 35.21% less than highest absorption.

The highest concentration (46.997 ppm) of Cd in root was observed in treatment Zl_0Cd_{15}, where no zeolite was applied but highest amount of Cd (15 mg/kg soil) was applied in soil. The lowest concentration was found in treatment Zl_4Cd_{15}, where both zeolite and Cd were applied in their highest rates and it was observed that root accumulation of Cd was 33.237 ppm which is 41.37% less than highest absorption.

It was observed that the highest concentration of total Cd in bulk soil was present in treatment Zl_4Cd_{15}, where highest amount of zeolite and highest amount of Cd (15 mg/kg soil) was applied in soil. Whereas, in case of treatment Zl_0Cd_{15}, where no zeolite was applied but Cd were applied in the highest concentration, it was observed that accumulation of Cd was 7.987 ppm which is 29.71% less than highest concentration found. It is due to the fact that increasing zeolite rate might have retained more Cd concentration in bulk soil.

The more zeolite had applied in soil, the less available Cd concentration was found in post-harvest soil. It is found that highest concentration (4.153 ppm) of available Cd in bulk soil was present in treatment Zl_0Cd_{15}, where the lowest amount of zeolite and highest amount of Cd (15 mg/kg soil) was applied in soil. Further, treatment Zl_4Cd_{15}, where the highest amount of zeolite and Cd were applied, the concentration of available Cd in post-harvest soil was found as 2.733 ppm, which is 51.95% less than highest concentration found.

The highest concentration (9.276 ppm) of total Cd in rhizosphere soil was found in treatment Zl_4Cd_{15}, where the highest amount of zeolite and highest amount of Cd (15 mg/kg soil) was applied in soil. In treatment Zl_0Cd_{15}, where no zeolite was applied but Cd were applied at the highest concentration, it was observed that accumulation of total Cd was 7.67 ppm which is 20.89% less than the highest concentration found.

Zeolite application positively influenced available Cd concentration in rhizosphere soil. The more zeolite applied in soil; the less available Cd concentration was found. Increasing zeolite concentration retained more Cd concentration in rhizosphere soil. Consequently, less amount of Cd was found in available form.

Zeolite had also important effect on soil properties such as pH, electrical conductivity, bulk density and water holding capacity. Highest soil pH (8.02) was found from Zl_4 whereas, the lowest (7.06) obtained from Zl_0. The highest electrical conductivity (0.86 dS/m) found from Zl_4 and the lowest from (0.78 dS/m) $Zl_{0,}$ where no zeolite was applied. Soil bulk density decreased with the increase of zeolite application. The lowest level of bulk density (0.99 g/ml) obtained from Zl_4 whereas highest (1.16 g/ml) bulk density found from Zl_0. Soil water holding capacity followed similar trend as influenced by zeolite application. The value increased when zeolite level in soil increases and vice-versa.

From these findings, it can be concluded that application of zeolite at 4% of soil wt. appeared to be a promising practice to reduce absorption of Cd in leafy vegetables grown in contaminated soil and a good way of improving soil physio-chemical properties (pH, bulk density, electrical conductivity) which might result in good soil aggregation and water holding capacity and promising to increase plant growth and yield.

Considering the findings of the present experiment, further studies in the following areas may be suggested:

1. The effect of zeolite on bioavailability of different heavy metals (Pb, Hg, Al) in industrially polluted soils of Bangladesh.
2. Similar experiments may be carried out by using another porous material like biochar to examine its effect on accumulation of heavy metals in different crops.

CHAPTER VI

REFERENCES

Abadzic, S.D. and Ryan, J.N. (2001). Particle release and permeability reduction in a natural zeolite (clinoptilolite) and sand porous medium. *Environ. Sci. Tech.* 35: 4501-4508.

Abdi, Gh., Khosh-Khui, M. and Eshghi, S. (2006). Effects of natural zeolite on growth and flowering of Strawberry (*Fragaria xananassa* Duch.). *Intl. J. Agric. Res.***1**: 384-389.

Agrawal, S.B. and Mishra, S. (2009). Effects of supplemental ultraviolet-B and cadmium on growth, antioxidants and yield of *Pisumsativum* L. *Ecotoxicol. Environ. Saf.* 72: 610-618.

Ahmed, O.H., Aminuddin, H. and Husni, M.H.A. (2002). Reducing ammonia loss from urea and improving soil-exchangeable ammonium retention through mixing triple superphosphate, humic acid and zeolite. *Soil Use Manag.***22**(3): 315-319.

Alexander, A.P. and Christos, D.T. (2003). Lead (II) retention by Alfisol and clinoptilolite: Cation balance and pH effect. *Geoderma.* 115: 303-312.

Allen, E.R., Hossner, L., Ming, D. and Henninger, D. (1996). Release rates of phosphorus, ammonium, and potassium in clinoptilolite phosphate rock systems. *Soil Sci. Soc. America J.* 60: 1467-1472.

Allen, R.D. (1995). Dissection of oxidative stress tolerance using transgenic plants. *Plant Physiol.* 107: 1049-1054.

Badora, A., Furrer, G., Grunwald, A. and Schulin, A. (1998). Immobilizationof zinc and cadmium in polluted soils by polynuclear Al-13 and almontmorillonite. *J. Soil Contam.* **7:**573–588.

Baker, N.R. (1991). A possible role for photosystem II in environmental perturbations of photosynthesis. *Physiol. Plant.* 81: 563-570.

Bansiwal, A.K., Rayalu, S.S., Labhasetwar, N.K., Juwarkar, A.A. and Devotta, S. (2006). Surfactant-modified zeolite as a slow release fertilizer for phosphorus. *J. Agric. Food Chem.* 54: 4773-4779.

Barcelo, J. and Poschenrieder, C. (1990). Plant water relations as affected by heavy metal stress: *a review. J. Plant Nutr.***13**: 1–37.

Barrer, R.M. (1986). Porous crystals: A perspective. *Pure Appl. Chem.* **58**(10): 1317-1322.

Ben Ammar, W., Nouairi, I., Zarrouk, M. and Jemal, F. (2007). Cadmium stress induces changes in the lipid composition and biosynthesis in tomato (*Lycopersicon esculentum* Mill.) leaves. *Plant Growth Regul.* 53: 75-85.

Bernardi, A.C.C., Mota, E.P., Souza, S.C.H., Cardoso, R.D. and Oliviera, P.P.A. (2010). Ammonia volatilization, dry matter yield and nitrogen levels of Italian ryegrass fertilized with urea and zeolite. 19th world congress of soil science, soil solutions for a changing world 1-6 August 2010 (pp. 22-26). Brisbane, Australia.

Bertoli, A.C., Gabriel Cannata, M., Carvalho, R., Ribeiro Bastos, A.R., Puggina Freitas, M. and dos Santos Augusto, A. (2012). *Lycopersiconesculentum* submitted to Cd-stressful conditions in nutrition solution: nutrient contents and translocation. *Ecotoxicol. Environ. Saf.* 86: 176-181.

Bishnoi, N., Sheoran, I. and Singh, R. (1993). Effect of cadmium and nickel on mobilization of food reserves and activities of hydrolytic enzymes in germinating pigeon pea seeds. *Biol. Plant.* 35: 583-589.

Burzynski, M. (1988). The uptake and accumulation of phosphorus and nitrates and the activity of nitrate reductase in cucumber seedlings treated with Pb and Cd. *Acta Soc. Bot. Pol.***57**: 349–359.

Castaldi,P., Santona,L. and Melis, P. (2005). Heavy metal immobilization by chemical amendments in a polluted soil and influence on white lupin growth. *Chemosphere.* 60: 365–371.

Chaffei, C., Pageau, K., Suzuki, A., Gouia, H., Ghorbel, M.H. and Masclaux-Daubresse, C. (2004). Cadmium toxicity induced changes in nitrogen management in *Lycopersicon esculentum* leading to a metabolic safeguard through an amino acid storage strategy. *Plant Cell Physiol*. **45**(11): 1681–1693.

Chamseddine, M., Wided, B.A., Guy, H., Marie-Edith, C. and Fatma, J. (2009). Cadmium and copper induction of oxidative stress and antioxidative response in tomato (*Solanum lycopersicon*) leaves. *Plant Growth Regul.* 57: 89-99.

Chander, K. and Joergensen, R.G. (2002). Decomposition of ^{14}C labelled glucose in a Pb-contaminated soil remediated with synthetic zeolite and other amendments. *Soil Biol. Biochem.* 34: 643-649.

Chang, Y., Zouari, M., Gogorcena, Y., Lucena, J.J. and Abadia, J. (2003). Effects of cadmium and lead on ferric chelate reductase activities in sugar beet roots. *Plant Physiol. Biochem.* 41: 999-1005.

Chen, Z.S., Lee, G.J. and Liu, J.C. (2000). The effects of chemical remediation treatments on the extractability and speciation of cadmium and lead in contaminated soils. *Chemosphere*.**41:**235–242.

Chien, H.F., Lin, C.C., Wang, J.W., Chen, C.T. and Kao, C.H. (2002). Changes in ammonium ion content and glutamine synthetase activity in rice leaves caused by excess cadmium are a consequence of oxidative damage. *Plant Growth Regul*. 36: 41-47.

Chlopecka, A. and Adriano, D.C. (1996). Mimicked in-situ stabilization of metals in a cropped soil: bioavailability and chemical form of zinc. *Environ. Sci. Technol.***30:**3294–3303.

Cho, U.H. and Park, J.O. (2000). Mercury-induced oxidative stress in tomato seedlings. *Plant Sci.* 156: 1-9.

Choi, J. and Park, M. (1988). Adsorption of urease on zeolite. *J Korean Soc Soil Sci Fert.* 21: 380-385.

Chunhabundit, R. (2016). Cadmium exposure and potential health risk from foods in contaminated area, Thailand. *Toxicol. Res.* 32: 65–72.

Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochimie.* 88: 1707–1719.

Clemente, R., Escolar, A. and Bernal, P. (2006). Heavy metals fractionation and organic matter mineralisation in contaminated calcareous soil amended with organic materials. *Bioresour. Technol*. **97**: 1894–1901.

Cobbett, C. and Goldsbrough, P. (2002). Phytochelatins and Metallothioneins: roles in heavy metal detoxification and homeostasis. *Annu. Rev. Plant Biol.* 53: 159-182.

Costa, G. and Spitz, E. (1997). Influence of cadmium on soluble carbohydrates, free amino acids, protein content of in vitro cultured *Lupinusalbus*. *Plant Sci.* 128, 131–140.

Dal Corso, G., Farinati, S. and Furini, A. (2010). Regulatory networks of cadmium stress in plants. *Plant Signal. Behav.*5: 663–667.

Dal Corso, G., Farinati, S., Maistri, S. and Furini, A. (2008). How plants cope with cadmium: staking all on metabolism and gene expression. *J. Integr. Plant Biol.* 50: 1268-1280.

Daud, M.K., Sun, Y., Dawood, M., Hayat, Y., Variath, M.T., Wu, Y.X., Raziuddin, Mishkat, U., Salahuddin, Najeeb, U. and Zhu, S. (2009). Cadmium-induced functional and ultrastructural alterations in roots of two transgenic cotton cultivars. *J. Hazard. Mater.* 161: 463–473.

Dell'Amico, E., Cavalca, L. and Andreoni, V. (2008). Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant *rhizobacteria*. *Soil Biol. Biochem.* 40: 74-84.

DeSutter, T.M. and Pierzynski, G.M. (2005). Evaluation of soils for use as liner materials: A soil chemistry approach. *J. Environ. Qual.* 34: 951-962.

di Toppi, L.S. and Gabbrielli, R. (1999). Response to cadmium in higher plants. *Environ. Exp. Bot.* 41: 105-130.

Dixon, J.B., and Ming, D.W. (1987). Technique for the separation of clinoptilolite from soils. *Clays Clay Min.* **35**(6): 469-472.

Dong, J., Wu, F. and Zhang, G. (2006). Influence of cadmium on antioxidant capacity and four microelement concentrations in tomato seedlings (*Lycopersiconesculentum*). *Chemosphere*. 64: 1659-1666.

Eshghi, S., Mahmoodabadi, M.R., Abdi, G.R. and Jamali, B. (2015). Zeolite ameliorates the adverse effect of Cadmium contamination on growth and nodulation of soybean plant (*Glycine max* L.). *J. Biol. Environ. Sci.***4**: 43-50.

Ferguson, G.A. and Pepper, I.L. (1987). Ammonium retention in sand amended with clinoptilolite. *Soil Sci. Soc. America J.* 51: 231-234.

Finger-Teixeira, A., Lucio Ferrarese, M. de L., RicardoSoares, A., da Silva, D. and Ferrarese-Filho Osvaldo, O. (2010). Cadmium-induced lignification restricts soybean root growth. *Ecotoxicol. Environ. Saf.* 73: 1959-1964.

Flanigen, E.M. (1980). Molecular sieve zeolite technology- The first twenty-five years. *Pure Appl. Chem*. 52: 2191-2211.

Flanigen, E.M. (2001). In H. van Bekkum, E. M. Flanigen, P. A. Jacobs, & J. C. Jansen (Eds.), Introduction to zeolite science and practice (2nd ed.). Amsterdam: Elsevier (Studies in Surface Science and Catalysis 137, 11).

Fusconi, A., Repetto, O., Bona, E., Massa, N., Gallo, C., Dumas-Gaudot, E. and Berta, G. (2006). Effects of cadmium on meristem activity and nucleus ploidy in roots of *Pisumsativum*L. cv. Frisson seedlings. *Environ. Exp. Bot.* 58: 253–260.

Geiken, B., Masojidek, J., Rizzuto, M., Pompili, M.L. and Giardi, M.T. (1998). Incorporation of [^{35}S] methionine in higher plants reveals that stimulation of the D1 reaction Centre II protein turnover accompanies tolerance to heavy metal stress. *Plant Cell Environ.* 21: 1265-1273.

Gichner, T., Patkova, Z., Szakova, J., Znidar, I. and Mukherjee, A. (2008). DNA damage in potato plants induced by cadmium, ethyl methanesulphonate and g-rays. *Environ. Exp. Bot.* 62: 113-119.

Gill, S.S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 48: 909-930.

Gomez, J.M., Herna´ndez, J.A., Jimenez, A., del Rio, L.A. and Sevilla, F. (1999). Differential response of antioxidative enzymes of chloroplasts and mitochondria to long-term NaCl stress of pea plants. *Free Radic. Res*. 31: 511-518.

Gonçalves, J.F., Antes, F.G., Maldaner, J., Pereira, L.B., Tabaldi, L.A., Rauber, R., Rossato, L.V., Bisognin, D.A., Dressler, V.L., de Moraes Flores, E´.M. and Nicoloso, F.T.(2009). Cadmium and mineral nutrient accumulation in potato plantlets grown under cadmium stress in two different experimental culture conditions. *Plant Physiol. Biochem.* 47: 814-821.

Gratao, P.L., Monteiro, C.C., Antunes, A.M., Peres, L.E.P. and Azevedo, R.A. (2008). Acquired tolerance of tomato (*Lycopersiconesculentum* cv. Micro-Tom) plants to cadmium-induced stress. *Ann. Appl. Biol.* 153: 321-333.

Gruener, J.E., Ming, D.W., Henderson, K.E. and Galindo, C., Jr. (2003). Common ion effects in zeoponic substrates: Wheat plant growth experiment. *Micropor. Mesopor. Mat.* 61: 223-230.

Hadwiger, L.A., Broembsen, S. and Eddy, R. (1973). Increased template activity in chromatin from cadmium treated pea tissues. *Biochem. Biophys. Res. Commun*. 50: 1120-1128.

Hashem, A., Abd Allah, E.F., Alqarawi, A.A., AlHuqail, A.A., Egamberdieva, D. and Wirth, S. (2016). Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance. *Saudi J. Biol. Sci.* 23: 272-281.

Hassan, M. and Mansoor, S. (2014). Oxidative stress and antioxidant defense mechanism in mung bean seedlings after lead and cadmium treatments. *Turk. J. Agric. For*. 38: 55-61.

Hassan, W., Bano, R., Bashir, S. and Aslam, Z. (2016). Cadmium toxicity and soil biological index under potato (S*olanum tuberosum* L.) cultivation. *Soil Res.* 54: 460-468.

He, Z.L., Calvert, D.V., Li, A.K. and Alva-Banks, D.J. (2002). Clinoptilolite zeolite and cellulose amendments to reduce ammonia volatilization in a calcareous sandy soil. *Plant Soil*: 247: 253-260.

Hediji, H., Djebali, W., Belkadhi, A., Cabasson, C., Moing, A., Rolin, D., Brouquisse, R., Gallusci, P. and Chaibi, W. (2015). Impact of long-term cadmium exposure on mineral content of *Solanum lycopersicum* plants: consequences on fruit production. *South Afr. J. Bot*. 97: 176-181.

Hernandez, L.E., Carpena-Ruiz, R. and Garate, A. (1996). Alterations in the mineral nutrition of pea seedlings exposed to cadmium. *J. Plant Nutr*. 19: 1581-1598.

Heyno, E., Klose, C. and Krieger-Liszkay, A. (2008). Origin of cadmium-induced reactive oxygen species production: mitochondrial electron transfer versus plasma membrane NADPH oxidase. *New Phytol.* 179: 687-699.

Hossain, M.A., Hasanuzzaman, M. and Fujita, M. (2010). Up-regulation of antioxidant and glyoxalase systems by exogenous glycinebetaine and proline in mung bean confer tolerance to cadmium stress. *Physiol. Mol. Biol. Plants.* 16: 259-272.

Huang, B., Xin, J., Dai, H., Liu, A., Zhou, W., Yi, Y. and Liao, K. (2015). Root morphological responses of three hot pepper cultivars to Cd exposure and their correlations with Cd accumulation. *Environ. Sci. Pollut. Res.* 22: 1151-1159.

Huang, Z.T. and Petrovic, A.M. (1994). Clinoptilolite zeolite influence on nitrate leaching and nitrogen use efficiency in simulated sand based golf greens. *J. Environ. Qual.***23**(6): 1190-1194.

Iannelli, M.A., Pietrini, F., Fiore, L., Petrilli, L. and Massacci, A. (2002). Antioxidant response to cadmium in *Phragmitesaustralis* plants. *Plant Physiol. Biochem.* 40: 977-982.

Inglezakis, V., Loizidou, M., and Grigoropoulou, H. (2004). Ion exchange studies on natural and modified zeolites and the concept of exchange site accessibility. *J. Colloid Interf. Sci.* 275: 570-576.

Jalil, A., Selles, F. and Clarke, J.M. (1994). Effect of cadmium on growth and the uptake of cadmium and other elements by durum wheat. *J. Plant Nutr.* **17**(11): 1839-1858.

Jinadasa, N., Collins, D., Holford, P., Milham, P.J. and Conroy, J.P. (2016). Reactions to cadmium stress in a cadmium-tolerant variety of cabbage (*Brassica oleracea* L.): is cadmium tolerance necessarily desirable in food crops? *Environ. Sci. Pollut. Res.* 23: 5296-5306.

Jing, D.O.N.G., Fei-bo, W.U. and Guo-ping, Z. (2005). Effect of cadmium on growth and photosynthesis of tomato seedlings. *J. Zhejiang Univ. Sci. B.* **6**(10): 974–998.

Joshi, U.D., Joshi, P.N., Tamhankar, S.S., Joshi, V.P., Idage, B.B., Joshi, V.V. and Shiraljar, V.P. (2002). Influence of the size of extra framework monovalent cations in X-types zeolite on their thermal behavior. *Thermochim. Acta*. **387**: 121–130.

Ju, G.C., Li, X.Z., Rauser, W.E. and Oaks, A. (1997). Influence of cadmium on the production of g-glutamylcysteine peptides and enzymes of nitrogen assimilation in *Zea mays* seedlings. *Physiol. Plant.* 101: 793-799.

Kabata-Pendias, A. and Pendias, H. (2001). Trace Element in Soil and Plants. 3rd ed. CRC Press, Boca Raton, USA.

Kabata-Pendias, A. and Pendias, H. (1992). Trace Elements in Soils and Plants, 2nd ed. (365 p) CRC Press, Boca Raton.

Kamarudin, K.S., Hamdan, H., and Mat, H. (2003). Methane adsorption characteristic dependency on zeolite structures and properties. Paper presented at the 17th Symposium of Malaysian Chemical Engineers, Copthorne Orchid Hotel, Penang, 29-30 December.

Khan, A., Khan, S., Alam, M., Khan, M.A., Aamir, M., Qamar, Z., Rehman, Z.U. and Perveen, S. (2016). Toxic metal interactions affect the bioaccumulation and dietary intake of macro- and micro-nutrients. *Chemosphere.* 146: 121-128.

Khan, A., Khan, S., Khan, M.A., Qamar, Z. and Waqas, M. (2015). The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review. *Environ. Sci. Pollut. Res.***22**(18): 13772–13790.

Khan, H., Khan, A.Z., Khan, R., Matsue, N. and Henmi, T. (2008). Zeolite application affects vegetative phenology of determinate and indeterminate soybean grown on Allophanic soil. *Intl. J. Agric. Res.***3**(2): 148-154.

Kirkham, M.B. (2006). Cadmium in plants on polluted soils: effects of soil factors, hyper accumulation and amendments. *Geoderma*. 137: 19-32.

Kithome, M., Paul, J.W., Lavkulich, L.M. and Bomke, A.A. (1998). Kinetics of ammonium adsorption and desorption by the natural zeolite clinoptilolite. *Soil Sci. Soc. American J.* 62: 622-629.

Kumar, P., Jadhav, P.D., Rayalu, S.S. and Devotta, S. (2007). Surface-modified zeolite- A for sequestration of arsenic and chromium anions. *Curr. Sci.* 92: 512.

Larsson, E.H., Bornman, J.F. and Asp, H. (1998). Influence of UV-B radiation and Cd^{2+} on chlorophyll fluorescence, growth and nutrient content in *Brassica napus*. *J. Exp. Bot.* 49: 1031-1039.

Leggo, P.J. Lede´sert, B. and Christie, G. (2006). The role of clinoptilolite in organo-zeolitic soil systems used for phytoremediation. *Sci. Tot. Environ.* 363: 1-10.

Leon, A.M., Palma, J.M., Corpas, F.J., Gomez, M., Romero-Puertas, M.C., Chatterjee, D., Mateos, R.M., Del Rio, L.A. and Sandalio, L.M. (2002). Antioxidative enzymes in cultivars of pepper plants with different sensitivity to cadmium. *Plant Physiol. Biochem.* 40: 813-820.

Lin, R., Wang, X., Luo, Y., Du, W., Guo, H. and Yin, D. (2007). Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.). *Chemosphere.*69: 89-98.

Liu, Y., Xiao, T., Ning, Z., Li, H., Tang, J. and Zhou, G. (2013). High cadmium concentration in soil in the Three Gorges region: geogenic source and potential bioavailability. *Appl. Geochem.***37**:149–156.

MacKown, C. and Tucker, T. (1985). Ammonium nitrogen movement in a coarse-textured soil amended with zeolite. *Soil Sci. Soc. America J.* 49: 235-238.

Mahabadi, A.A., Hajabbasi, M.A., Khademi, H. and Kazemian, H. (2007). Soil cadmium stabilization using an Iranian natural zeolite. *Geoderma.* **137**: 388-393.

Mathys, W. (1975). Enzymes of heavy-metal–resistant and nonresistant populations of *Silene cucubalus* and their interaction with some heavy metals in vitro and in vivo. *Physiol. Plant.* 33: 161-165.

Mattioni, C., Gabbrielli, R., Vangronsveld, J. and Clijsters, H. (1997). Nickel and cadmium toxicity and enzymatic activity in Ni-tolerant and non-tolerant populations of *Sileneitalic.Pers. J. Plant Physiol.* 150: 173-177.

Maxwell, K. and Johnson, G.N. (2000). Chlorophyll fluorescence-a practical guide. *J. Exp. Bot.* 51: 659-668.

Mcgilloway, R., Weaver, R., Ming, D. and Gruener, J.E. (2003). Nitrification in azeoponic substrate. *Plant Soil.* 256: 371-378.

Mendoza Cozatl, D., LozaTavera, H., Hernandez Navarro, A. and Moreno-Sanchez, R. (2005). Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants. *FEMS Microbiol. Rev*. 29: 653-671.

Metwally, A., Safronova, V.I., Belimov, A.A. and Dietz, K.J. (2005). Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. *J. Exp. Bot.* 56: 167-178.

Mohamed, M.M. (2001). Heat capacities, phase transitions and structural properties of cation-exchanged H-mordenite zeolites. *Thermochim. Acta*. **372:** 75–83.

Mondales, K.D., Carland, R.M. and Aplan, F.F. (1995). The comparative ion exchange capacities of natural sedimentary and synthetic zeolites. *Miner. Eng.* **8:** 535–548.

Monteiro, M.S., Santos, C., Soares, A.M.V.M. and Mann, R.M. (2009). Assessment of bio- markers of cadmium stress in lettuce. *Ecotoxicol. Environ. Saf*. 72: 811-818.

Mozgawa, W. (2004). The influence of some heavy metals cations on the FTIR spectra of zeolites, *J. Mol. Struct.***555:** 299–304.

Mumpton, F. A. (1999). La rocamajica: Uses of natural zeolites in agriculture and industry. Proceedings of the National Academy of Sciences of the United States, 96, 346-3470.

Nardin, G., Randaccio, L. and Zangrando, E. (1995). Lead clustering in a zeolite-X.*Zeolites*.**15:** 684–688.

Nawab, J., Khan, S., Aamir, M., Shamshad, I., Qamar, Z., Din, I. and Huang, Q (2016). Organic amendments impact the availability of heavy metal (loid) s in mine-impacted soil and their phytoremediation by *Penisitum americanum* and Sorghum bicolor. *Environ. Sci. Pollut. Res*. **23**(3): 2381–2390

Ok, C.H., Anderson, S.H. and Ervin, E.H. (2003). Amendments and construction systems for improving the performance of sand-based putting greens. *Agron J*. 95: 1583-1590.

Olmos, E., Mart´ınez-Solano, J.R., Piqueras, A. and Hellin, E. (2003). Early steps in the oxidative burst induced by cadmium in cultured tobacco cell (BY-2 line). *J. Exp. Bot.* 54: 291-301.

Ouzounidou, G., Moustakas, M. and Eleftheriou, E. (1997). Physiological and ultrastructural Effects of Cadmium on Wheat (*Triticum aestivum* L.) Leaves. *Arch. Environ. Contam. Toxicol*. **32**: 154-160.

Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A. and Forestier, C. (2002). Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *Plant J.* 32: 539-548.

Petrovic, A.M. (1993). Potential for natural zeolite uses on golf courses. *USGA Green Section*. 31:11-14.

Polat, E., Karaca, M., Demir, H., and Onus, A.N. (2004). Use of natural zeolite (clinoptilolite) in agriculture. *J. Fruit Ornam. Plant Res.* 12(Spl. ed.): 183-189.

Popova, L., Maslenkova, L., Yordanova, R., Krantev, A., Szalai, G. and Janda, T. (2008). Salicylic acid protects photosynthesis against cadmium toxicity in pea plants. *Gen. Appl. Plant Physiol.* 34: 133-144 (Special issue).

Querol, X., Alastuey, A., Moreno, N., Alvarez-Ayuso, E., Garcia-Sanchez, A. and Cama, J. (2006). Immobilization of heavy metals in polluted soils by the addition of zeolitic material synthesized from coal fly ash. *Chemosphere*. **171:**171–218.

Ramesh, K., Biswas, A.K., Somasundaram, J., and Subba Rao, A. (2010). Nanoporous zeolites in farming: Current status and issues ahead. *Curr. Sci.* **99**(6): 760-765.

Rascio, N. and Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? *Plant Sci.* 180: 169-181.

Rehakova, M., Cuvanova, S., Dzivak, M., Rimar, J. and Gavalova, Z. (2004) Agricultural and agrochemical uses of natural zeolite of the clinoptilolite type. *Curr. Opin. Solid State Mater. Sci.* **8:** 397-404.

Rizwan, M., Ali, S., Abbas, T., Zia-ur-Rehman, M., Hannan, F., Keller, C., Al-Wabel, M.I. and Ok, Y.S. (2016). Cadmium minimization in wheat: a critical review. *Ecotoxicol. Environ. Saf.* 130: 43-53.

Ronzan, M., Piacentini, D., Fattorini, L., Della Rovere, F., Eiche, E., Riemann, M., Altamura, M.M. and Falasca, G. (2018). Cadmium and arsenic affect root development in *Oryza sativa* L. negatively interacting with auxin. *Environ. Exp. Bot.***151:** 64–75.

Seth, C.S., Misra, V., Chauhan, L.K. and Singh, R.R. (2008). Genotoxicity of cadmium on root meristem cells of *Allium cepa*: cytogenetic and Comet assay approach. *Ecotoxicol. Environ. Saf*. 71: 711–716.

Sethy, S.K. and Ghosh, S. (2013). Effect of heavy metals on germination of seeds. *J. Nat. Sci. Biol. Med*. 4: 272–275.

Shah, K. and Dubey, R.S. (1995). Effect of cadmium on RNA level as well as activity and molecular forms of ribonuclease in growing rice seedlings. *Plant Physiol. Biochem.* 33: 577–584.

Shamsi, I.H., Zhang, G., Hu, H., Xue, Q., Hussain, N., Ali, E., Shen, Q., Zheng, W., Zhang, Q., Liu, X., Jabeen, Z. and Lin, X. (2014). Assessment of the hazardous effects of Cd on physiological and biochemical characteristics of soybean genotypes. *Int. J. Agric. Biol.* 16: 41-48.

Shanableh, A. and Kharabsheh, A. (1996). Stabilization of Cd, Ni and Pb in soil using natural zeolite. *J. Hazard. Mater*.**45:** 207–217.

Shanmugaraj, B.M., Harish, M.C., Balamurugan, S. and Sathishkumar, R. (2013). Cadmium induced physio-biochemical and molecular response in *Brassica juncea*. *Int. J. Phytoremediation.* 15: 206-218.

Siedlecka, A., Samuelsson, G., Gardenstrom, P., Kleczkowski, L.A. and Krupa, Z. (1998). The "activatory model" of plant response to moderate cadmium stress-relationship between carbonic anhydrase and Rubisco. In: Garab, G. (Ed.), Photosynthesis: Mechanisms and Effects. Kluwer Academic, Dordrecht, Boston, London, pp. 2677-2680.

Sigfridsson, K.G.V., Berna´t, G., Mamedov, F. and Styring, S. (2004). Molecular interference of Cd^{2+} with Photosystem II. *Biochim. Biophys. Acta.* 1659: 19-31.

Singh, S., Parihar, P., Singh, R., Singh, V.P. and Prasad, S.M. (2015). Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. *Front. Plant Sci*. 6: 1143.

Song, Y., Jin, L. and Wang, X. (2017). Cadmium absorption and transportation pathways in plants. *Int. J. Phytoremediation.* 19: 133–141.

Sponer, J.E., Sobalik, Z., Leszczynski, J. and Wicthterlova, B. (2001). Effect of metal coordination on the charge distribution over the cation binding sites of zeolites. A combined experimental and theoretical study.*J. Phys. Chem. B.* **105**: 8285–8290.

Street, R.A., Kulkarni, M.G., Stirk, W.A., Southway, C. and Van Staden, J. (2010). Effect of cadmium on growth and micronutrient distribution in wild garlic (*Tulbaghia violacea). S. Afr. J. Bot.* **76**(2): 332–336.

Tack, F.M.G., Mozo, J.E. and Verloo, M.G. (1998). Cadmium uptake by cucumber plants as affected by fluctuations in nutrient solution cadmium concentration during growth. *Commun. Soil Sci. Plant Anal.* 29: 3015-3021.

Thangavel, P., Long, S. and Minocha, R. (2007). Changes in phytochelatins and their biosynthetic intermediates in red spruce (*Picea rubens* Sarg.) cell suspension culture under cadmium and zinc stress. *Plant Cell Tissue Organ Cult.* 88: 201-216.

Tsadilas, C.D. (2000). Effect of soil pH on the distribution of heavy metals among soil fractions. In I. Iskandar (Ed.), Environment restoration of metals contaminated soils (pp. 107-119). Boca Raton, FL: Lewis Publishers.

Tudoreanu, L. and Phillips, C.J.C. (2004). Modelling cadmium uptake and accumulation in plants. *Adv. Agron.* 84: 121–157.

Ulusoy, U. and Simsek,S. (2005). Lead removal by polyacrylamide–bentonite and zeolite composites: effect of phytic acid immobilization. *J. Hazard. Mater*. **127**: 163–171.

Unlu, H., Ertok, R. and Padem, H. (2004). The usage of zeolite in tomato seedling production medium. V. Vegetable production symposium (pp. 318-320). Canakkale/Turkey, 21-24 September 2004.

Uraguchi, S., Mori, S., Kuramata, M., Kawasaki, A., Arao, T. and Ishikawa, S (2009). Root toshoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. *J. Exp. Bot.* **60**(9): 2677–2688.

Varalakshmi, L.R. and Ganeshamurthy, A.N. (2013). Phytotoxicity of cadmium in radish and its effects on growth, yield, and cadmium uptake. *Commun. Soil Sci. Plant Anal.* 44: 1444-1456.

Vazquez, S., Goldsbrough, P. and Carpena, R.O. (2006). Assessing the relative contributions of phytochelatins and the cell wall to cadmium resistance in white lupin. *Physiol. Plant.* 128: 487-495.

Verbruggen, N., Hermans, C. and Schat, H (2009). Mechanisms to cope with arsenic or cadmium excess in plants. *Curr. Opin. Plant Biol.***12**(3): 364–370.

Wang, Y., Dong, Y., Wang, J. and Cui, X. (2016). Alleviating effects of exogenous NO on tomato seedlings under combined Cu and Cd stress Environ. *Sci. Pollut. Res.* 23: 4826-4836.

Wiedenfeld, B. (2003). Zeolite as a soil amendment for vegetable production in the Lower Rio Grande Valley. *Subtrop. Plant Sci.* 55: 7-10.

Witter, E. and Kirchmann, H. (1989). Peat, zeolite and basalt as adsorbents of ammoniacal nitrogen during manure decomposition. *Plant and Soil.* 115: 43-52.

Witter, E. and Lopez-Real, J. (1988). Nitrogen losses during the composting of sewage sludge and the effectiveness of clay soil, zeolites and compost in absorbing the volatilized ammonia. *Biological Wastes.* 23: 279-294.

Wu, F.B., Chen, F., Wei, K. and Zhang, G.P. (2004). Effect of cadmium on free amino acid, glutathione and ascorbic acid concentrations in two barley genotypes (*Hordeum vulgare* L.) differing in cadmium tolerance. *Chemosphere.*57: 447-454.

Wu, F.B., Zhang, G.P. and Dominy, P. (2003). Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. *Environ. Exp. Bot.* 50: 67-78.

Xin, J. and Huang, B. (2014). Subcellular distribution and chemical forms of cadmium in two hot pepper cultivars differing in cadmium accumulation. *J. Agric. Food Chem.* 62: 508-515.

Xin, J., Huang, B., Dai, H., Liu, A., Zhou, W. and Liao, K. (2014). Characterization of cadmium uptake, translocation, and distribution in young seedlings of two hot pepper cultivars that differ in fruit cadmium concentration. *Environ. Sci. Pollut. Res.* 21: 7449-7456.

Xu, D., Chen, Z., Sun, K., Yan, D., Kang, M. and Zhao, Y. (2013). Effect of cadmium on the physiological parameters and the subcellular cadmium localization in the potato (*Solanum tuberosum* L.). *Ecotoxicol. Environ. Saf.* 97: 147-153.

Xue, Z., Gao, H. and Zhao, S. (2014). Effects of cadmium on the photosynthetic activity in mature and young leaves of soybean plants. *Environ. Sci. Pollut. Res.* 21: 4656-4664.

Yang, T. and Poovaiah, B.W. (2003). Calcium/calmodulin-mediated signal network in plants. *Trends Plant Sci.* 8: 505-512.

Yang, X., Baliger, V.C., Martens, D.C. and Clark, R.B. (1996). Cadmium effects on influx and transport of mineral nutrients in plant species. *J. Plant Nutr*. **19**: 643–665.

Yasuda, H., Takama, K., Fukuda, T., Araki, Y., Suzuka, J. and Fukushima, Y. (1998). Effects of zeolite on water and salt control in soil. Bulletin of the Faculty of Agriculture, Tottori University. 51: 35-42.

Zhang, G., Fukami, M. and Sekimoto, H. (2002). Influence of cadmium on mineral concentrations and yield components in wheat genotypes differing in Cd tolerance at seedling stage. *Field Crop Res.* **77**(2): 93–98.

Zhao, S., Ma, Q., Xu, X., Li, G. and Hao, L. (2016). Tomato jasmonic acid-deficient mutant spr2 seedling response to cadmium stress. *J. Plant Growth Regul.* 35: 603-610.

Zhi, Y., He, K., Sun, T., Zhu, Y. and Zhou, Q. (2015). Assessment of potential soybean cadmium excluder cultivars at different concentrations of Cd in soils. *J. Environ. Sci.* (China) 35: 108-114.

Zhu, Y.G., Chen, S.B. and Yang, J.C. (2004). Effects of soil amendments on lead uptake by two vegetable crops from a lead-contaminated soil from Anhui, China. *Environ. Int.* 30: 351-356.