# Cadmium (Cd) Stress in Rice; Phyto-Availability, Toxic Effects, and Mitigation Measures-A Critical Review

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**Abstract:** Cadmium (Cd) is a phytotoxic heavy metal polluting paddy fields, whereas its accumulation in the soil poses harmful threats to agro-ecosystem and human health. Cd toxicity alters rice plants morpho-physiological and biochemical features and has become a global concern for crop productivity and food safety. This article takes a relevant review of cadmium stress and its effects, phyto-availability as well as mitigation strategies in rice. Studies have revealed that cadmium toxicity causes oxidative stress in rice plants, thereby decreasing seed germinating ability, growth potentials, mineral nutrients uptake, photosynthetic pigments, and rice grain yield. However, considering the increasing threats of toxic metals in the environment especially in agricultural soils and the aging much focus being placed on Cd uptake and effects on plants with little on phyto-availability and varying mitigating strategies, the use of various management strategies, for example bio-char amendments, exogenous application of signaling molecules and plant growth regulators, irrigation and several other means are amongst the numerous approaches reviewed and recommended for mitigating Cd-contaminated paddy soils. In conclusion, advances in understanding the varying processes involved in soils accumulated Cd and ameliorating mechanisms is of tremendous significant to aid rice production with less environmental hazards.

Key words: Cd-toxicity; rice; soil amendment; soil contamination

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#### I. Introduction

Due to the rapid increase in industrialization and persistence urbanization, cadmium (Cd) deposition in agricultural soils and translocation to plant organs have increased soil contamination of paddy fields and threats to food safety (Rizwan et al. 2012). Even at very low concentration, cadmium forms a highly eco-toxic trace element, hazardous to both plants and animals. Agricultural soils are considered the main source of nutrients for supporting rice growth and development, however, the increasing deposition of heavy metals like cadmium (Cd), lead (Pb), chromium (Cr) and copper (Cu) into the environment poses threats to future crop production (Adrees et al. 2015; Ali et al. 2015; Habiba et al. 2015; Ashraf et al. 2015; Anjum et al. 2016). Cd in agricultural soils do not only affect rice growth as well as posing health risk and hazards to humans but also reduces land available for crop production and thus increases food insecurity (Adrees et al. 2015). High Cd concentration causes several physio-biochemical disorders in growing plants (Rizwan et al. 2012). varying toxicity in paddy fields and translocation to different rice plant organs have become problematic for rice growth and is posing serious concerns to agro-ecosystems and human health (Song and Chen 2014). Research has shown that Cd is being deposited in agricultural soils through industrial emission of pollutants, irrigation with contaminated waste water and other waste from anthropogenic sources discharged in the soil (Ramadan and Al-Ashkar 2007; Kuo et al. 2006; Ghosh and Singh. 2005), the application of sewage sludge containing Cd content, phosphate fertilizers application and other waste disposal (Rizwan et al. 2012), as well as metal smelting (Douay et al. 2009). This is mostly true in places like China where population increase and rapid industrial development coupled with less pollution controls measures, have contributed to the increase in heavy metal contamination of agricultural soils (Ji 2000). Results from soil survey conducted in China revealed that a minimum of 13, 330 hectares of agricultural land in 11 provinces were contaminated by varying degrees of Cd (Zhang and Huang 2000), examples of these areas include; soils in 'Zhangtu' and 'Sheyang' provinces, that were found to be highly contaminated with heavy metals, in which Cd concentration in both rice grains and soils were above the maximum limits set for cereal food and agricultural soils (Huang et al. 2004). Therefore, understanding the varying aspect of Cd soil-plant interactions becomes eminent.

#### Cadmium (Cd) stress in rice; phyto-availability, toxic effects, and mitigation measures-a critical review

Rice (Oryza sativa L.) forms the staple food for a greater proportion of the worlds' population (Kosolsaksakul et al. 2014). It has been widely reported that rice can readily take up Cd and translocate it to shoot and then to grains (Song et al. 2015). Thus, Cd enters the food chain through consumption of Cd contaminated rice. Even at minimal concentrations in food, the toxic effects to humans are severe (Aziz et al. 2015). In short, rice grown on Cd-contaminated soils faces production and quality challenges (Jallad 2015). Cd uptake and translocation in different plants parts is concentration dependent and genotype specific (Sebastian and Prasad 2014). During rice growth and development, varying morpho-physiological, biochemical and structural changes caused by Cd toxicity often results in leaf chlorosis, growth inhibition, reduction in rice growth and biomass accumulation that consequently lead to plant death (Srivastava et al. 2014). Moreover, increased oxidative stress in rice seedlings by over-production of reactive oxygen species (ROS) such as malondialdehyde (MDA) contents, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), electrolyte leakage (EL), as well as reducing plant growth and yield (Srivastava et al. 2014). Altering the leaf and root ultrastructure causes structural damage to rice photosynthetic apparatus during growth. In summary, the need for increasing awareness on toxic effects of Cd as well as phyto-availability and amendment strategies in rice is of tremendous significant to sustainable rice production and food safety. Several studies have adopted different amendment approaches at varying scope and locations. Wu et al. (2015); Cao et al. (2015) and Farooq et al. (2015) have all used plant growth regulators (PGRs) for mitigating Cd toxicity during rice growth and found significant improvements in rice growth when applied with PGRs under Cd-tainted soils. Studies have shown that Cd toxicity in rice largely depends on soil Cd bioavailability and elements contents which compete with Cd during plant uptake (Fahad et al. 2015), hence, the application of mineral elements such as nitrogen (N), phosphorus (P), selenium (Se), zinc (Zn) and iron (Fe), have proven to decrease Cd toxic effects in rice (Zhou et al. 2015; Hu et al. 2014; Fahad et al. 2015). The application of crop residues in the form of manure or compost, and biochar are among the organic amendments strategies used under Cd stress conditions (Wang et al. 2015; Suksabye et al. 2016; Zhang et al. 2015). Breeding schemes involving low Cd-accumulating rice cultivars selection, agronomic practices such as; crop rotation, planting patterns, irrigation management have also proven effective means for reducing Cd toxic effects in rice (Aziz et al. 2015; Yao et al. 2015; Hu et al. 2015). This review, therefore, took a broad assessment of cadmium toxicity in rice with focus on phyto-availability, effects and amendment strategies for ensuring higher rice performance and reducing consumption of Cd contaminated rice.

# II. Cadmium accumulation and phyto-availability in rice organs

Cd is one of the non-essential elements that can easily be absorbed by plants. Hence, it becomes more toxic to plants than other metals such as Ni, Cu, Zn and Pb (Balsberg 1989). Cd is easily taken up in ionic form  $(Cd^{2+})$  by the roots of different rice varieties at different rates and translocated to the various plants parts through xylem flow. In plants Cd toxicity is often 2–20 times higher than other heavy metals (Jagodin 1995). The physiological translocation and accumulation of Cd in rice plant often comprises four major transport processes. They include; root Cd uptake from the soil, from root to shoot translocation through xylem flow, the redirection of Cd to rice plant nodes, and the remobilization of Cd from leaves to the rice grains (Fig. 1). Several researchers have carried out studies and conducted quantitative trait locus (QTL) analyses to identify the gene responsible for aiding this translocation processes (Ishikawa et al. 2010; Ishikawa et al. 2005; Tezuka et al. 2010; Ueno et al. 2009). They all concluded that QTL analysis forms a very useful approach which provides a clear genotypic difference among cultivars during Cd accumulation in rice plant organs especially in shoots and grains.

In most cases, Cd accumulation in rice shoots and grains are often less in japonica cultivars as compared to indica cultivars (He et al. 2006). Furthermore, some specific cultivars among indica rice do often accumulate much higher Cd in the vegetative tissues and grains (Uraguchi et al. 2009). Several transporters have recently been identified through forward and reverse genetics approaches as Cd transporter in rice plant. Cadmium often passes through the xylem into the leaves after it has been rapidly taken up by the roots, some rice varieties seemed very sensitive to low Cd concentrations, uptake and distribution of micro and macro nutrients slow down roots and shoots growth, due to the alterations in the photosynthesis rate (Sandalio 2001). Even though Cd is not necessary for plant growth, its ions however, are often readily taken up by the plant roots and translocated to the above ground vegetative parts (Shamsi 2008). Grain yield expresses the union of several growth components in plant, and beyond certain Cd level in soil, the quality of field products decreases as well as yield of crops (Hassan 2005). Cd phyto-toxicity is another important problem for plant growth, this is mostly in some areas highly polluted with heavy metals, where decline in agricultural crop production has been observed. In rice, Cd toxicity has been reported to inhibit seedling vigor, stunted growth, proline induction and certain novel proteins synthesis, and the decrease in activities of many key hydrolytic enzymes as a result of translocation (Shah et al. 2001). However, there are several mechanisms behind Cd uptake and translocation in plants which needs further investigation. In summary, Cd is taken up by the rice roots and then translocated to shoot mainly through the xylem and finally remobilized and translocated to rice grains.

# 2.1 Root Cd uptake from the soil

Soil Cd is taken up by rice roots in ionic form, it is mainly absorbed in the form of Fe phytosiderophore, however,  $Fe^{2+}$  transporters have been identified to play a very significant role in  $Fe^{2+}$  uptake by roots (Ishimaru et al. 2006). OsIRT 1 and 2 both contain  $Cd^{2+}$  as well as  $Fe^{2+}$  influx activities in yeasts, which suggest that OsIRTs play eminent role in root Cd uptake, this is mostly after release of pounded water during water management (Ishimaru et al. 2006). It is suggested that during rice cultivation in flooded paddy fields, OsIRTs possibly induce lower levels of available iron, after water release, induced OsIRTs contribute to available Cd uptake in aerobic conditions. Over expressing OsIRT1 leads to Cd accumulation in rice roots and shoots under soil Cd, but this was not observed in field condition as reported by Lee and An (2009). This shows that, the potential involvement of OsIRT1 in root Cd uptake is largely affected by the soil environmental conditions. This requires further in-depth research to further investigate OsIRT1 role in root Cd uptake.

#### 2.2 Root to shoot Cd translocation through xylem flow

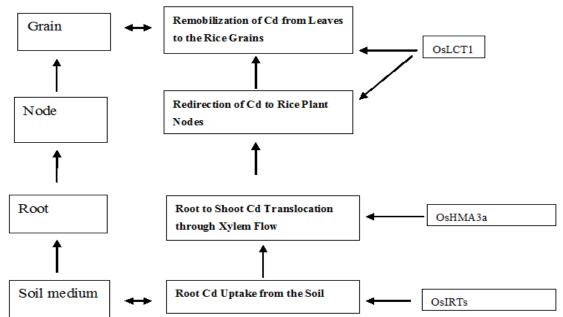
Xylem ability to mediate Cd translocation into shoots is a major determinant for Cd accumulation in many plants including rice (Uraguchi et al. 2009). In other plants such as A. thaliana and A. halleri which are Cd/Zn hyper accumulators, the key xylem Cd transporters have been identified. In A. thaliana, the P1B-type ATPaseAtHMA2 and AtHMA4 were identified to regulate the translocation of Cd and Zn from root to shoot (Hussain et al. 2004). While in A. halleri, AhHMA4 (a homolog of AtHMA4) plays a vital role in translocation of Cd and Zn into shoots (Hanikenne et al. 2008). Following xylem Cd transport gene in A. thaliana and A. halleri identification, OsHMA3 has also been identified as a key regulator for xylem transporting of Cd in rice through vacuolar sequestration mediation of Cd in the root cells compartment (Miyadate et al. 2011). There are some unique features in OsHMA3 when compared to AtHMA4 and AhHMA4. All of them mediate Cd efflux transportation, but OsHMA3 has been reported not to transport other metals such as Zn, whereas AtHMA4 and AhHMA4 transport both Zn and Cd. Another eminent difference includes subcellular localization differing between OsHMA3 and others. OsHMA3 is believed to be localized in the vacuolar membrane, but AtHMA4 and AhHMA4 are localized in the plasma membrane. OsHMA3 and Arabidopsis HMA4s major differences can also be found in their physiological function during rice growth. In the Nipponbare background, RNAi mediates knocking down of OsHMA3 and increased root to shoot Cd translocation while the over expression reduces shoot Cd accumulation, this suggests that, in root cells, OsHMA3 plays a functional role in vacuolar compartmentation of Cd and hence, reduces xylem Cd loading and accumulation in the shoots (Ueno et al. 2010), whereas, AtHMA4 and AhHMA4 catalyzes Cd loading into the xylem. This finding reveals the processes involved for reducing Cd translocation in the shoots of some japonica and also substituting single amino acid in OsHMA3 from some indica rice cultivars though does not partition Cd from the edible rice grains.

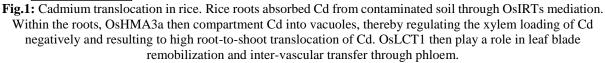
#### 2.3 Redirection of Cd to rice plant nodes

During rice growth, nearly 100 percent of the Cd deposited to the grains was mediated by phloem (Tanaka et. al. 2007). Xylem to phloem Cd transfer at nodes was observed even though report has clearly not justified the presence of transporters for phloem Cd transport in rice plants so far (Fujimaki et al. 2010), even though phloem transportation of Cd through panicle neck showed variation based on rice genotype (Kato et al. 2010). This shows that transporters at nodes for phloem exist and involve in Cd transportation to grains.

#### 2.4 Remobilization of Cd from Leaves to the Rice Grains

According to Rodda et al. (2011), phloem transport was observed to be mediated by Cd remobilization from the leaf blades to rice grains. In another research conducted by Uraguchi et al. (2011), a transporter gene involving in phloem Cd transport has been identified, this transporter gene (OsLCT1) is also known to be the homolog of wheat Low affinity cation Transporter (Clemens et al. 1998), it encodes a Cd efflux transporter on the plasma membrane and OsLCT1 expression was observed to be higher in the leaf blades and nodes at the reproductive stages. In the uppermost node, OsLCT1 was found mainly expressed in diffused vascular bundles connected to the panicles. The Cd levels in grains and phloem released from leaf blades were substantially reduced in RNAi plants compared to control plants, even though Cd concentration in xylem sap did not differ. These findings indicate that OsLCT1 plays a part in inter-vascular Cd transfer from larger vascular bundles to diffuse ones connected to the panicles. This shows the first identification of a phloem Cd transporter in rice which suggests the need for further research on verifying the significance of phloem as a transporter. An overview of Cd effects in rice has been shown in Fig. 1.





# **III.** Factors affecting cadmium phyto-availability in soils and translocation in rice 3.1 Soil cadmium concentration

The concentration of Cd in agricultural soils forms a major influencing factor to rice plants Cd uptake during cultivation, such influence is not only limited to rice plant uptake but also translocation to edible grains. Even though several researches have proven that there is no linear correlation between Cd plants uptake to the concentration of Cd in the soil (Fujimoto and Uchida 1979). Solution culture experiment of about four hours on roots exposed to a concentration of 0.02 and 10.24 mg/L cadmium has shown that rice plants do actively uptake Cd from solution concentrations below 0.15 mg/L Cd and depress uptake at concentrations above 0.15 mg/L. In flooded pots experiments, it was observed that, even though Cd uptake increased as the solution Cd increased from 0 to 6.25 mg/L, the percentage of shoot translocated Cd reduced greatly (Ito and Limura 1976). Yu et al. (2006) conducted two seasons submerged field trials by adding CdNO<sub>3</sub> with Cd concentration varying from 1.85 mg/kg to 77.55 mg/kg, the results showed that maximum grain Cd concentration was 0.31 mg/kg at low soil Cd concentration. This shows that soil Cd concentration do influence translocation to grains. However, the key mechanism ameliorating maximum Cd transocation from straw to grains remain uninvestigated on a broader perspective.

#### 3.2 Sources of Cadmium

The sources of Cd are a critical aspect in directing research focus on how rice plant behaves under Cd toxic conditions. Interpretation of laboratory trials using metal salts as Cd source must be cautiously done compared to field situation where Cd source is often from the addition of phosphate fertilizers, wastes and bio-solids. Kikuchi et al. (2007) conducted a two and half years field trials in both flooded and non-flooded cultivation, the results showed that, Cd in the form of cow manure added to the soil was more readily bioavailable to rice plants than one in added phosphate fertilizers. Research has also shown that, pH plays a vital role in Cd liability when Cd is added to soils in soluble form (Degryse et al. 2004). When Cd was induced to the soil as smelting waste and sludge amendment which are in less soluble form, the liability of Cd was not predicted well by soil organic matter content or pH, this calls for further studies on Cd source variation to Cd phyto-availability to rice plant.

# 3.3 Cadmium long term aging in soils and Soil pH

Long term aging effects also affect Cd bioavailability in soils and translocation to rice plants. Several researches have reported the long term or aging effect of Cd in soils to decrease the bioavailability and solubility of soil Cd (Kirkman 2006). In similar crop to rice such as wheat, Hamon et al. (1998) observed aging effects of Cd to have decreased the bioavailability of Cd in wheat over a period of field trials.

Soil pH has a greater influence on availability and liability of soil Cd (McLaughlin et al., 2006). Several factors including; the influence of surface charge on Cd affinity for absorption sites (McBride 1989), CdCO<sub>3</sub> and Cd (OH)<sub>2</sub> precipitation at high pH, and solution speciation changes, may result to the pH effects on bioavailability and liability in soils. Researches have reported that there is higher Cd bioavailability in soils with lower pH or neutral state than those of higher pH state (McLaughlin et al. 2006). As with most heavy metals, the concentration and accumulation of cadmium in the soil is to a larger extent influence by the soil pH. Reports have shown that under acidic conditions, the solubility of Cd increases and little cadmium adsorption by hydrous oxides, soil colloids, and organic matter takes place (U.S. EPA 1999). At pH > 6, cadmium is thus adsorbed by the soil in solid phase or precipitated, and the concentrations of dissolved cadmium reduced greatly. Cadmium then forms soluble complexes alongside organic and inorganic ligands, most especially with chloride ions. These complexes formed then increase cadmium mobility in soils (U.S. EPA 1999),. In general, chloride forms a soluble complex with Cd<sup>2+</sup> as CdCl<sup>+</sup>, which decreases Cd<sup>2+</sup> adsorption to soil particles (Adriano et al. 2005). As opposed to inorganic ligand ions, Cd<sup>2+</sup> adsorption by kaolinite, a variable charged mineral, is enhanced by organic matter presence via an adsorbed organic layer formation on the clay surface (Adriano et al. 2005).

# 3.4 Soil redox potential

Soil redox potential is universally known for its influence on Cd liability, solubility and rice plants uptake (Contin et al. 2007). The major processes influencing Cd and other heavy metals solubility include; biogeochemical cycling of sulfur, Fe and Mn oxyhydroxides. Metals become less soluble as redox potential decreases from positive state to a negative one. The extent of Cd solubility effects may be different in redox conditions of different soils. Ge et al. (2007) proved that Cd solubility is lowered in some soils but increased in other soils when three different paddy soils were subjected to incubation experimentation.

# 3. 5 Sulphur biochemical cycling

Sulfur cycling in the soil may be an influencing factor to the accumulation of paddy rice grain Cd content. Studies have shown that, Cd present in flooded soils is released in sulfide phases (Inahara et al. 2007). Though not verified in field conditions, Pure Cd sulfide (CdS) has the tendency of oxidizing any pure Zn sulfide (ZnS) that results in a greater relative bioavailability in the soil and catalyses Cd rice plants uptake and translocation during grain filling stage (Chaney et al. 1996). Studies have also shown that, metal sulfide formation in submerged soils is greatly affected by microbial reduction of SO<sub>4</sub><sup>2-</sup> to dissolve sulfide (S<sup>2-</sup>), this process requires and its often been influenced by the availability of organic carbon (Du Liang et al. 2009).

#### 3.6 Mn and Fe Oxyhydroxides

Adsorbent minerals presence in the soil affect Cd rice uptake in different parts. Oxyhydroxide affinity of metals in the soil is being affected by metal properties like ionic state, hydrolyze tendency, oxidation state, electronegativity and properties of oxyhydroxides minerals such, surface morphology, point of zero charge, soil pH and crystalinity (McBride 1989). Li et al. (2005) stated that Cd bioavailability to rice plant cultivated on soils with higher Fe contents was lower, which is similar to studies undertaken by McLaughlin et al. (2006) on wheat. Since Mn and Fe are known as adsorbent minerals and as primary sink for Fe and Zn in oxidized soil environment, it is therefore, expected that, soils high in these mineral will provide less Cd for rice plants uptake (Du Liang et al. 2009).

# 3.7 Chlorides

Chloride presence in agricultural soils has proven to increase rice up take of Cd (Ohtani et al. 2007). After soil was sparked with ammonium chloride salt, the Cd concentration in the soil and shoots increased. This is the opposite when soil is sparked with ammonium nitrate, ammonium sulfate or dyhydrogen phosphate. The increase in Cd up take in rice

#### 3.8 Organic matter content

Organic matter presence in soils can bind heavy metals through the processes of chelation, adsorption and complexation, however, it also mobilizes metals by forming soluble metal complexes alongside organic ligands with low molecular weight (Du Laing et al. 2009). The release of reductive organic matter has proven to be eminent in controlling heavy metals released in wetland soils (Grybos et al 2007). Organic matter addition has been shown to reduce Cd bioavailability/solubility in agricultural soils. Kashem and Singh (2004) in pot experiments under flooded and non-flooded conditions found that organic matter addition decreased solubility of Cd. In another experiment, using three different soils, Kashem and Singh (2001) reported that total uptake of Cd in rice (shoot, straw and polished rice) was greatly reduced by approximately 30% after applying organic matter, less effects was observed in soils that were naturally rich in organic matter.

#### **3.9** Bio-solids incorporation in the soil

Though bio-solids themselves may contain Cd, they are still well known to limit Cd uptake by rice plants in Cd contaminated soils. It is thus important to discover whether the organic or inorganic component of the bio-solids that is responsible for this. If the organic component binds Cd in the soil, then there is the potential for metals release from the organic matter since the decomposition occurs with aging (Stacey et al. 2001). Li et al. (2001) in their study found that the bio-solids that are associated with reduction in Cd availability is not only dependent on the soil organic fraction. This was determined by the production of adsorption isotherms for different soil samples at pH 4.5, 5.5 and 6.5 amended with bio-solids and control soils, each tested with the organic carbon removed. Inorganic fraction in the soil was found to have a significant contribution to the total soil Cd sorptive capacity, and after bio-solids amendment, was able to sorb more Cd. Hettiarachchi et al. (2003) also agreed that increased Cd sorption by bio-solids amended soil at pH 5.5 is due to a combination of organic and inorganic components of the bio-solids. They further reported that retention of added Cd also increased after bio-solids amendment, and the biosolids fraction responsible for such effect was found to be Fe and Mn rather than organic matter. Synchrotron based techniques; µ-XANES and µ-XRF use, showed that Cd in bio-solids was strongly associated with Fe and Mn phases (Hettiarachchi et al. 2006). However, when 70-75% of the organic carbon was removed from the bio-solids, there was little change to the association with Fe phases. This lends indirect evidence to the hypothesis that metals in bio-solids are associated mainly with the inorganic fraction. Other studies have found that organic manure application depresses Cd uptake by rice plants but only for added Cd rather than Cd already found in the soil (Zhang et al. 2002). It was also found that pig manure was a more effective depressant of Cd uptake.

#### **3.10 EDTA addition to soils**

Several evidences have shown that the addition of EDTA could affect Cd uptake in rice grains. Zn uptake in rice grain was enhanced when Zn was applied to the soil in the form of Zn-EDTA rather than ZnSO4 (Karak et al. 2006). This may also be true for Cd though not considered in the study. No details on the water management or Fe content of the soil were given however, which makes it very difficult to draw conclusions about the possible effect under variable redox conditions. Cd accumulation in rice roots, shoots and grains was significantly reduced (> 67%) by the addition of Fe-EDTA at 0.09 g/kg in pot experiments with soil Cd contamination of 5.7 mg/kg (Shao et al., 2008). This would prevent the uptake of Cd and Zn by the plant, and with increased free Fe(II), Fe would be more readily available for plant uptake.

#### 3.11 Silicon

Silicon (Si) alleviates Cd toxicity and translocation in rice plants, it is an important element to rice plants and is known for stimulating growth and yield, which is normally found in the shoot tissue in the form of  $SiO_2$  and at a concentration of 10 to 15% dry weight (Marschner 1995). Marschner (1995) found that Si inhibits the translocation of Cd to rice shoots; concentration of Cd in the root was increased by 21% but in the shoot decreased by 24%. Si limits Cd uptake in rice shoots due to its incorporating ability into the structural cell walls (Shi et al. 2005).

#### **3.12 Temperature effects**

A rise in temperature can enhance Cd translocation to rice shoots tissues. In an experiment conducted by Chino and Baba (1981), the found that relatively low temperature (20° C compared to 28° C) depressed Cd translocation though not the overall uptake of Cd in the rice plants. In other studies of related crops, higher temperatures have been shown to increase Cd uptake in. In pot experiments run for 1 year by using ryegrass grown at 9 and 20 ° C, heavy metal content of aboveground plant parts was increased by at least 1.5 times at the higher temperature (Almas and Singh 2001).

#### 3.13 Nutrient effects

Higher soil nutrients availability can decrease Cd bioavailability to rice plants (Hassan et al. 2005a). Adding of  $PO_4^{3-}$  into the soil has proven to have no effect on Cd uptake in rice plants, rather, pyrophosphate suppresses Cd uptake via an insoluble formation of Cd pyrophosphate complex (Koshino 1973). In contrast, Saito and Takahashi (1978) found that P excess or deficiency increases Cd uptake by rice plants grown in solution culture. Nitrogen content in the soils has proven to affect Cd translocation in rice, N deficiency causes the greatest Cd translocation during rice growth (Saito and Takahashi 1978).

#### **3.14Cultivar types**

Cadmium uptake and accumulation in rice grains differ for different rice cultivars. He et al. (2006) proved that Cd concentration in paddy rice grain had a variation from 0.06 to 0.99 mg/kg between 38 genotypes in a field condition experiment. Arao and Ae (2003) also observed differences in Cd uptake from two soil types

while comparing forty nine rice cultivars in pot experiments carried out under drained conditions over a two years period. Soil A which was grey lowland soil, was slightly polluted with a Cd concentration of 0.9 mg/kg while soil B was Andosols and heavily polluted with a Cd concentration of 7.4 mg/kg. Within the two years experiment, grain Cd concentration varied between the cultivars from 0.19 to 4.41 mg/kg in Soil A and 0.59 to 7.71 mg/kg in soil B (Arao and Ae 2003). similar variability among cultivars was also observed in a submerged rice field trial in China across two seasons with two soil Cd concentrations added as CdNO<sub>3</sub>, the differences in grain Cd concentrations between the rice cultivars was in an order of magnitude (Yu et al. 2006).

#### IV. Morpho-physiological and biochemical effects of Cd stress in rice

Agricultural practices involving inorganic fertilizers application and atmospheric deposition are ways through which cadmium is added to agricultural soils. These sources lead to increase in accumulation of Cd in edible rice grains. Over the last decade, one of the key concerns to farming communities had been the effects of cadmium deposition and concentration to farmlands. Research has shown that, rice cultivar grown in a rain protected greenhouse using containers, proved to take up more cadmium than the same rice cultivar grown in open field condition subjected under the same cadmium levels. This is largely due to the greater root development in confined containers and the roots tendencies to readily be in contact with cadmium contaminated soil, whereas, in field condition, roots may grow down below the cadmium contaminated soil level (De Vries and Tiller 1978; Page and Chang 1978). Therefore, considering factors aiding phyto-availability and Cd's toxic tendencies, uptake and toxicity effects on growing rice plants vary greatly on a wider scope.

#### 4.1 Morphological Effects

Cadmium stress during rice growth causes a wide variety of responses, from cellular metabolism to gene expression as well as growth and productivity. Cd presence in agricultural soils severely affects the growth and development of cultivated rice and more than any other environmental stress. Many studies have reported Cd's inhibiting effect on rice plant height, as well as fresh and dry matter accumulation, leaf area index, root length, and other parameters. Its presence reduce rice growth rate by affecting various parts of root metabolism such as mineral and water uptake (Barcelo 1990), inhibition of enzyme activities (Tamas 2006), membrane function (Herńandez 1997), inhibition of cell division (Fusconi 2006), cell death (Ortega-Villasante, 2005), it primarily disturb cellular redox environment, causing oxidative stress in both roots and leaves (Ortega-Villasante 2005; Romero-Puertas 2004). Cd toxicity causes detrimental effect on rice plant growth (height) and chlorophyll content (SPAD values) (Larson 1998). Phytotoxic symptoms that subsequently induce some of these above mentioned toxic symptoms were observed in the form of root browning (Arduini 1994), leaf epinasty (Vazquez et al. 1989), and leaf chlorosis (Foy et al. 1978), leaf red-brownish discoloration (Malone et al.1997). In summary, exploring the variation in soil Cd phyto-availability will further provide useful bases in understanding the dynamics of soil Cd interaction.

Cd-levels	Experiment	Experimental	Relevant findings	Refrences
	type	location	8	
0, 30 mg kg <sup>-1</sup> of soil	hydroponic culture	Faisalabad (Pakistan)	The use of Auxine improved rice growth and yield under Cd toxicity (plant height, panicle and tiller number, 1000 grain weight etc). Increased straw Cd uptake but limit translocation to grains was also observed.	Farooq et al. (2015)
0, 50 µM Cd	Hydroponic	Nanjing (China)	$H_2O_2$ pre-treatment decreased Cd concentration in shoots as well as mitigated Cd stress.	Hu et al. (2009)
50 and 100 mg Cd /kg soil	Pot experiment	Rajarata (Sri Lanka)	Plant height, flag leaf area, root dry weight and Cd accumulation were significantly affected by Cd toxicity. Cd contents within the rice plant were observed as: grains< shoots< roots.	Herath et al.(2014)
100μM Cd(NO <sub>3</sub> ) <sub>2</sub>	Hydroponic	Shangdong(China)	H <sub>2</sub> O <sub>2</sub> accumulation and subsequent modification of the auxin signaling pathway and/or cell cycle gene expression	(Zhao et al. 2012)
82 mg Cd kg <sup>-1</sup> . P fertilizer (0-52-34, containing 52% $P_2O_5$ and 34% $K_2O$ ) (the control), 50, 200, and 1000 mg phosphorus(P) $kg^{-1}$	Pot experiment	Bangkok (Thailand)	Application of P increased soil pH and reduced the redistribution of Cd. Plant growth was also enhanced by P addition. Plant Cd uptake was only significantly reduced in mature plants receiving a P-application rate of 1000 mg P kg <sup>-1</sup> .	Siebers et al.(2013)
0, 0.3 mg kg-1 and 0.6 mg kg <sup>-1</sup>	Pot experiment in sunlight greenhouse	Beijing (China)	Cd concentrations in rice grains showed a significantly difference (P<0.05) in the twenty rice cultivars under the same Cd level in soil, and rice morphological characters significantly affected.	(Song et al. 2014)
5mM CdCl <sub>2</sub>	Hydroponic	Taipei (Taiwan)	H <sub>2</sub> O <sub>2</sub> accumulation dependent on	(Hsu and

Table 1. Consequences of Cd toxicity in rice and varying amendment means

			NADPH-oxidase and phosphatidylinositol 3-phosphate	Kao, 2007)
100,200, 400mM CdCl <sub>2</sub>	Hydroponic	Tainan, (Taiwan)	MAP kinase activity regulated by non-enzymatic (OH) and enzymatic ROS production ( $O_2^-$ or $H_2O_2$ ) involving NADPH oxidase, CDPKs, PI3 kinase, and closing of the mitochondrial pore Regulation of NADPH oxidase and CDPKs activity by $Ca^{2+}$	(Yeh et al. 2007)
15–60 mg <sup>1–1</sup> for 8 days	quartz sand culture	Shenyang (China)	Root growth Inhibited and total soluble protein content increased in rice seedlings with the increase of Cd concentration.	Wan, L et.al.,(2006)
$650 \text{ mg kg}^{-1}$	Pot experiment, 90 days	Bangkok (Thailand)	Microbes increased rice dry weight and decreased the concentration of Cd in rice grains.	Suksabye et al. (2016)
0, 20, and 50 mg kg <sup><math>-1</math></sup>	Pot experiment, 20 days	Silchar (India)	Bacterial inoculation increased rice growth under Cd stress and decreased Cd uptake in plants parts especially at higher Cd levels	Nath et. al. (2014)
200 μΜ	Rice grown on soaked filter paper with treatments for 8 days	Khon Kaen (Thailand)	Bacterial isoloates promoted the growth of rice and decreased Cd uptake by rice.	Siripornadul sil and Siripornadul sil (2013)

#### 4.2 Physiological Effects

Rice growth and sustainability under different environmental stress conditions depend on plant reproductive and vegetative growth patterns. Plant growth forms a function of complex interplay between sources and sinks limited by the roots and shoots system which establishes a functional equilibrium (Anjum et al. 2016). Studies have shown that several genes of the Cd responsive miRNAs encode proteins that are functioning in diverse biological processes, example, EF-hand proteins and Leucine Rich Repeat (LRR) receptor protein kinases were reported to be involving in signal transduction and in external or endogenous stimulus responses (Tichtinsky et al., 2003; Osakabe., et al, 2005; Lu et al., 2008). Reports for new mechanisms of Cd tolerance on rice genes showed a novel rice gene that low cadmium (LCD) was found involved in Cd accumulation and tolerance (Shimo, et al. 2011). Therefore, Knocking out LCD results in reduced Cd accumulation and increased growth under excess Cd concentration. However, LCD- GFP localized to the cytoplasm and nucleus, do suggest that LCD is not a membrane transporter of Cd, since LCD is not homologous to other genes, and many authors have already concluded that, LCD is a novel protein that is related to Cd homeostasis. A novel cysteine-rich peptides encoded by OsCDT1 are being observed to be involved in rice Cd tolerance and therefore, over expression of OsCDT1 in A-thaliana under Cd exposure increased the growth of plants (Kuramata et al. 2009).

Previously, it has also been shown that Cd is solidly phytotoxic and can limit the plant growth and leads consequently to its death (Lamoreaux and Chaney 1977). The negative effect of Cd on rice growth and its development increased the ratio of dry to fresh masses (DM/FM) in all rice organs (Greger and Lindberg 1986; Moya et al. 1993). During ontogenesis DM/FM ratio changes and it increase in young plants is a criterion for stress response that is indicative on the whole plant level (Baker 1993).

#### 4.2.1 Photosynthesis

Mechanisms of photosynthesis influenced by Cd accumulation in the soil directly affect rice performance, it affects and disrupts all major components of photosynthesis including carbon reduction cycle as well as control of stomatal  $CO_2$  supply, it also increases accumulation of carbohydrates, disruption of water balance and peroxidative destruction of lipids (Allen and Ort 2001). Effects of Cd on photosynthetic rate inhibition are due to less access of  $CO_2$  (Bazzaz et al. 1974). As a result of reduced chlorophyll content and enzymatic activities involved in  $CO_2$  fixation lead to decreased photosynthetic rate as well as limiting uptake and distribution of mineral nutrients in plants (Greger and Gren 1991). Detrimental photosynthetic effects of Cd toxicity on rice are exhibited on growth (height) and chlorophyll content (SPAD values) and increased metal concentration in the culture medium (Larson et al. 1998). Photosynthesis inhibition by Cd stress decreased the chlorophyll content and stomatal conductivity, this indirectly affect photosynthesis (Ouzounidou et al. 1997). It is presumed that toxic Cd strongly affects the leaves normal functioning during the earlier stages of vegetation (Lang et al. 1995).

#### 4.2.2 Rice roots signaling under Cd stress

Soil Cd contamination is gaining significant interest among researchers, since  $Cd^{2+}$  is readily taken up by the rice roots and translocated to other parts, its toxicity has been estimated to amount 2–20 times higher from other heavy metals (Jagodin et al. 1995). Cd is often loaded into the rice plants xylem and subsequently transported to the leaves after being taken up rapidly by the roots. Many rice cultivars are sensitive to low Cd concentrations, which influence uptake and distribution, macro and micronutrients inhibition and subsequently affects root and shoot growth, due to the alterations in the photosynthesis rate (Sandalio et al. 2001). In some rice cultivars, Cd toxicity has been reported to cause inhibition of seedling vigor, stunted growth, induction in synthesis of proline and certain novel proteins as well as decreasing many key hydrolytic enzymes activities (Shah et al. 2001) oxidation and cross-linking of proteins (Ortega-Villasante 2005), induction of DNA damage (Fojtov´a 2002; Gichner 2004). Further studies are still needed to examine the variation in long and short term effects

#### 4.3 Biochemical responses

#### 4.3.1 Reactive oxygen species (ROS) production

ROS themselves are signal molecules causing damage to plant cells as well as signaling participating molecules in recognizing and responding to stress factors (Wrzaczek et al. 2013). Among the different existing ROS, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) performs the role of primary messenger, due to its relative stability and its ability to cross membranes through aquaporins (Moller and Sweetlove 2010). Hydrogen peroxide forms a very important player in plants response to cadmium during plants growth as shown in the table below. Several advanced research data indicated that Cd promotes H<sub>2</sub>O<sub>2</sub> generation in plants and plant cell cultures (Arasimowicz-Jelonek et al. 2012; Zhao et al. 2012). It is believed that, Cd induced  $H_2O_2$  might be produced by plasma membrane NADPH oxidase or originates in mitochondria and in peroxisomes which then diffuses to other parts of cells and apoplastic space (De Michele et al. 2009; Arasimowicz-Jeloneketal.2012). Research has shown that extracellular NADPH oxidase is dependent on generation of H<sub>2</sub>O<sub>2</sub> and may also be followed by increased production of superoxideanion (O<sup>2-</sup>) in mitochondria, which causes accumulation of fatty acid hydron peroxide (Garnier et al.2006). NADPH oxidase produce  $O^{2-}$  by generating superoxide during transferring electrons from NADPH to molecular oxygen, that is subsequently dismutated to  $H_2O_2$  and  $O_2$  by superoxide dismutase enzymes(SOD). Strong superoxide accumulation correlated with SOD activity was found in plants treated with Cd (Hsu and Kao, 2007; Yeh et al. 2007; Zhao et al. 2012; Maksymiec and Krupa 2006). In responding to Cd reactive oxygen species, ROS can be formed in non-enzymatic and enzymatic reactions for e.g., catalyzed by NADPH oxidase, super oxide dismutase enzymes in different cell compartments like the plasma membrane, mitochondria, or peroxisomes.

# 4.3.2 Antioxidant enzymes

Rice plants contain catalyzed enzymes cleaning up system known as defense system. This system ensures the normal cellular functioning of the rice plant by avoiding active oxygen damage (Horváth et al. 2007). Hence, equilibrium between activities of antioxidant enzymes and Reactive Oxygen Species production ensures the possibility of oxidative signaling and/or damage occurring in the rice plants cell (I.M. Møller et al. 2007). Rice plants have a composite enzymatic and non enzymatic antioxidant system for minimizing oxidative stress effects, these include; Catalase (CAT), Peroxides (POD), Ascorbate peroxidase (APX), low molecular mass antioxidants (glutathione reductase (GR), Carotenoids, Ascorbate and ROS scavenging enzymes, superoxide dismutase (SOD) (Apel and Hirt 2004). Cadmium concentration is dependent on variations in the antioxidant defenses of rice plants which stimulates oxidative stress (Dixit et al. 2001) Photosynthetic membranes maintained under oxidative stress by the cooperation of non enzymatic antioxidant plays an eminent role in rice plants survival under Cd stress (R. Mittler 2002). The enzymatic apparatus do act by the production of a non enzymatic antioxidant or directly scavenge the ROS.

#### 4.3.3 Effects on gene regulation and expression

Rice exposure to Cd toxicity experience changes in the expression of a number of genes. Microarray analyses have revealed that Cd modulates expression of more than 1700 genes in rice, and cadmium exposure leads to increased expression of several gene families in Arabidopsis (Kovalchuketal et al. 2005). Cd dependent induction of some genes in Arabidopsis has also been proven by the real time PCR technique. Cd toxicity increases the regulation of genes encoding pathogens related to proteins, TFs, antioxidant enzymes, transporters, as well as proteins associated with glutathione metabolism. Studies have revealed that genes encoding proteins connected with photosynthesis were down regulated in response to short term Cd stress. Long term impact of Cd on gene activity requires an inter play of different gene regulating mechanisms. Results from the different studies showed that Cd dependent regulation of genes expression is being mediated by the modifications in chromatin, changes in the activity of TFs and modulation of micro RNA levels. Wheat and rice plants subjected to cadmium stress were characterized by an elevated expression of the *HsfA4* gene, and plants over expressing TF were more tolerant to cadmium stress, while those with hampered HsfA4 expression showed reduced resistance to Cd.

# V. Amendment strategies

Considering the effects of Cd in rice and its subsequent consumption by humans especially in Asia, mitigating Cd toxicity in rice fields and translocation to grains becomes a major issue among researchers in the agricultural field. Apart from the "Itai-itai disease" experienced in Japan, other areas in China and Thailand, also have reported case of Cd contamination in paddy soils and rice grains causing renal malfunctioning in the

population (Nordberg et al. 1997; Jin et al. 2002; Honda et al. 2010). Below are some practices that have proven to help mitigate Cd toxicity in contaminated soils.

#### 5.1 Application of signaling molecules and plant growth regulators

For alleviation heavy metal toxicity, some researchers mainly focused on salicylic acid (SA) (Metwally et al. 2003; Guo et al. 2007a, b), with the belief that, the mode of SA signaling pathway is associated with increased H<sub>2</sub>O<sub>2</sub> levels. However, much has not been done to prove that H<sub>2</sub>O<sub>2</sub> pretreatment can increase Cd tolerance in plants. Cd toxicity is mostly in part related to oxidative stress, that is caused by the generation of free radicals and reactive oxygen species (ROS), including superoxide radical (O<sup>2-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radical (OH) (Sandalio et al. 2001; Romero-Puertas et al. 2004; Shah et al. 2001). Even though details of Cd stress mechanism have still not been fully known, unlike other heavy metals like iron (Fe) or copper (Cu), Cd does not directly catalyze reactive oxygen species (ROS) generation; instead it causes disturbances in the balance of other essential metals such as Fe and Cu (Stohs and Bagchi 1995). Further evidences continue to show that Cd toxicity in plants is as a result of the H<sub>2</sub>O<sub>2</sub> accumulation (Cho and Seo 2005; Hsu and Kao 2007), accelerating lipid peroxidation affects cell membrane permeability and fluidity as a result of the alteration composition of membrane lipids (Shah, et al, 2001; Ammar et al. 2007; Molina et al. 2008). To repair Cd induced oxidative stress in plants, antioxidants such as Glutathione, Ascorbic acid (AsA), a-tocopherol, carotenoids and antioxidant enzymes such as; Catalase (CAT), Superoxide dismutase (SOD), ascorbate peroxidase (APX), Guaiacol peroxidase (GPX), glutathione peroxidase (GPOX) and Glutathione reductase (GR)) (Noctor and Foyer 1998) have proven to be effective components. Cd accumulation processes in rice grain can also be influenced by several mechanisms such as; root uptake, xylem transport from the roots to the shoots, transfer from the xylem to the phloem, and phloem transportation through sources and sinks (Riesen and Feller 2005). Another effective mechanism Cd tolerance is being ensured in plants is by chelating with sulfhydryl containing peptides such as phytochelatins (PCs) and reduced glutathione (GSH) (Metwally et al. 2003) and subsequent vacuolar compartmenting, which may be effective in inhibiting long distance transporting of proteins within the plant. It may be therefore, possible to enhance Cd tolerance and control its translocation from the root to the shoot through sequestering in vacuole of root cells. The exogenous application of varying signaling molecules like nitric oxide (NO) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as well as plant growth regulators such as auxins (indole-3-acetic acid, IAA), SA, ABA, and GA has proven effective for alleviation Cd toxicity in rice seedlings (Wu et al. 2015; He et al. 2010, 2014). During He et al. (2014) studies, in which the influence of increasing doses of sodium nitroprusside (SNP), used as exogenous NO donor, on rice seed germination and growth as well as activities of antioxidant enzymes subjected to 100 µM Cd stress for 7 days were investigated. From the results, Cd accumulation by plants, seed germination and seedling growth in a dose dependent manner were decreased. SNP application decreased oxidative stress,  $H_2O_2$ production as well as MDA, whereas, the activities of antioxidant enzymes, APX, SOD, POD, and CAT, and proline contents in rice shoots and roots were increased. Other studies on exogenous application of SNP have reported that, stress metabolism in rice seedlings under Cd stress were regulated (Panda et al. 2011). Singh and Shah (2014b) further reported that SNP exogenous application decreased Cd uptake and reversed the Cd induced toxic effects on rice seedlings through restoring the membrane functioning ability. Rice has an internal defense system to cope with Cd stress through the production of phyto-hormones. However, endogenous defense system efficiency decreased with increased or higher levels of Cd stress in the soil. Hence, the exogenous application of phyto-hormones further enhanced rice tolerance to Cd stress (Farooq et al. 2016; He et al. 2014). Research has widely proven that exogenous SA application significantly decreased Cd toxicity and uptake in rice by enhancing antioxidant enzymes activities (Fatima et al. 2014; Singh and Shah 2015). Pretreatment with Salicylic acid increased rice seed germination, seedling growth, and amylase contents in the rice (He et al. 2014). Furthermore, Fatima et al. (2014) reported that the application of SA improved mineral nutrition in rice under Cd stress with rice response been varied with genotypes. In addition to plant growth regulator application during rice growth, Singh and Shah (2014a) studies concluded that methyl jasmonate improved antioxidant enzymes (CAT, SOD, POD, and GR) activities in rice under Cd stressed. L-TRP (an auxin precursor) exogenous application increased rice growth and yield under Cd toxic soil. Eventhough, L-TRP increased the concentration of Cd in rice straw, it however, decreased the translocation of Cd to the rice grains when compared to treatments without L-TRP (Farooq et al. 2015). In other experiment conducted by Cai et al. (2010), rice growth, biomass, photosynthesis, mineral nutrient uptake, Zn, Cu, as well as manganese (Mn) were increased and Cd uptake reduced after exogenous application of GSH

#### **5.2 Application of Biochar (BC)**

Biochar forms a carbon rich compound that is produced through combustion of organic materials under limited oxygen supply (Lehmann and Joseph 2009). As heavy metals remediating means, biochar increase water holding capacity, improves soil structure and increases the activities of soil microbial activities in Cd contaminated soils. Several studies have reported that BC application reduced metal uptake in rice (Bian et al.

2016; Rizwan et al. 2016; Suksabye et al. 2016). The application of BC during rice growth decreased Cd accumulation and uptake in rice and increased rice performance. Chen et al. (2014a, b) concluded that application of BC in historically Cd contaminated soil do increased rice yield and thus decreased grain Cd concentrations when compared to control. Suksabye et al. (2016) also compared the effect of three different types of biochars (sawdust fly ash, bagasse fly ash, and rice husk ash) on Cd accumulation in rice grains grown in Cd toxic soil. The results revealed that sawdust fly ash decreased the maximum Cd concentration in rice grains when compared to other BCs subjected in the same conditions. Zheng et al. (2012) similarly reported that biochar from rice straw was more significant in reducing Cd concentration in rice seedlings when compared to BC from rice bran and husk. It has also been reported in several research that the application of rice straw BC decreased the uptake of Cd in japonica rice variety as well as decreasing free proline, MDA and antioxidant enzymes (SOD, POD, and CAT) activities in rice flag leaves subjected to Cd stress (Zhang et al. 2014). The application of biochar in combination with organic and inorganic amendments might likely be of tremendous significance to amending Cd toxic soils.

#### 5.3 The use of organic materials like compost.

The use of compost has proven in several eminent researches to have an effect in mitigating Cd in the soil. The application of compost decreased the bioavailable Cd in contaminated soils t and reduced uptake by rice plants when compared to the control treatment (Wu et al. 2011). Research has reported that the use of compost significantly reduced Cd uptake in rice grown under low and high pH soils during 45 and 75 days growth period respectively. The authors concluded that the decrease in Cd concentration in low pH soil was faster compared to the high pH which might be as a result of the fast fractionation and redistribution processes of Cd after compost amendment. Report has also shown that, the use of vermicompost as amendment in toxic soils increased the concentration of Cd in Indian rice cultivars (MO 16 and MTU 7029), and decreased photosynthetic pigments Sebastian and Prasad (2013). The authors further suggested that increased root growth with applied vermicompost might be responsible for higher Cd accumulation in rice plants. Cattani et al. (2008) reported that combining two or more amendments (compost and lime) proved more effective in reducing Cd accumulation in rice grains when compared to the use of compost or lime amendment alone. This showed that combined ammendments using different amendments might be an effective means of reducing Cd toxic effects in rice plants. However, there exist wide gaps in understanding the efficiency of different combined amendments in mitigating Cd toxicity in rice plants. Rapeseed residue applied as a green manure has also proven to decrease the concentration of Cd in growing rice plants, this is due to the ability of rapeseed in transferring the Cd to more stable fractions and by improving soil quality and fertility (Ok et al. 2011).

# 5.4 Irrigating the field

Water management in rice fields has proven to be a cost effective means for reducing Cd accumulation and uptake in rice plants (Pan et al. 2016). Several studies have reported that, that constant flooding of rice fields during rice growth reduced Cd uptake by rice plants. In field studies conducted by Hu et al. (2015), Cd accumulation in rice husk were relatively lower in intermittent and flooding treatments compared to those in aerobic intermittent treatments. Cd contents in rice straw were decreased from 1.76 to 0.35 mg kg<sup>-1</sup> with increased irrigation from aerobic to flooding, respectively. Furthermore, rice grain yields were found increased with intermittent and conventional water treatments when compared to aerobic treatments. The Ministry of Agriculture, Forestry, and Fisheries of Japan have over the years encouraged rice growers to irrigate their rice fields flooded before and after rice heading to minimize rice Cd uptake in toxic areas during growth. This is because, under flooded condition, the soil is subjected to a reductive condition and a large proportion of Cd in the soil forms CdS with low solubility (Iimura and Ito 1978). But once the field is drained, an oxidative condition becomes obvious in the soil and CdS in the soil is then changed to Cd2+ which becomes readily available to the rice plants (Ito and limura 1976). Flooding drastically reduces grain Cd concentrations, but however, increases grains arsenic concentration (Arao, et al., 2009). Fulda et al. (2013) studies revealed the formation of CdS in highly Cd toxic paddy soils and concluded that reduction in Cd concentration under flooded paddy conditions might be as a result of insoluble CdS formation in the soil. The application of sepiolite combined with phosphate fertilizer was also reported to decrease exchangeable Cd in brown rice Cd under continuous flooding, conventional irrigation, and wetting irrigation, when compared to the control treatments.

# 5.5 Phyto-extraction

Several approaches to clean up paddy soils moderately polluted with Cd have been suggested. Phyto extraction using high Cd accumulating cultivars (Murakami et al. 2009), and soils chemical washing with the use of ferric chloride have been possible methods in terms of efficiency, cost and environmental friendliness (Makino et al. 2008).

# 5.6 Breeding schemes

In addition to the above mentioned soil mitigation approaches, establishing low Cd rice based on molecular and genetic findings seemed to be producing promising measures to reduce Cd uptake in rice. Transgenic technique has been successfully used as regulating means of Cd transporters to reduce Cd accumulation in rice plants. Functional allele of OsHMA3Over expression in Nipponbare background under soil with higher concentration of Cd (1.5 mg/kg) resulted in reduction of grain Cd concentration without reducing the zinc and iron concentration in the rice grains (Ueno et al. 2010). This phenotype becomes significant, since, zinc and iron are very important minerals for human health. It has also been shown that, knocking down of OsLCT1 also reduced Cd in grain by 50% under soil containing 0.2 mg Cd/kg (Uraguchi et al. 2011). Mineral contents and growth in grains were observed not to have negatively affected OsLCT1 knocked down in rice plants. These findings showed good molecular approach examples of Cd mechanisms based on physiological characters. Cao et al. (2014) reported variation in Cd accumulation among rice genotypic during field trials. The research revealed genotypic variations in grain Cd accumulation among the nine rice genotypes for the six different locations during the two successive years of experimentation.

# 5.7 Mineral fertilization

The application of mineral fertilizers has been proven in several researches to have an effect on mitigating cadmium toxicity under paddy conditions. Nitrogen for instance is a macronutrient and is largely required by plants for growth and development as well as yield increase. Several studies carried out on N application have reported that timely and required dose of N application can mitigate Cd toxicity in growing rice (Lin et al. 2011). N deficiency in growing rice plant increased Cd uptake in different rice parts which affects rice morphological parameters (Lin et al. 2011). This showed that N application at the required dose and time might alleviate Cd toxicity in growing rice seedling under Cd toxic soil, however, the reduction in Cd toxicity varies with N forms applied during rice growth. For instance, in Yang et al. (2016) hydroponic experiment, found that excessive application of  $NO_3^-$  in nutrient solution (2.86 and 5.72 mM) did not affect the rice growth but rather increased the concentrations of Cd in shoot, root, and grains of rice when compared to the control treatment. In another experiment carried out by Hassan et al. (2005c), the results showed a decrease in Cd content in rice when treated with ammonium-N when compared to nitrate-N which had an increased result in plant growth and dry matter content. In Hassan et al. (2005b) conclusion, the plant growth and biomass increase might possibly be due to the nitrate reductase activity reduction in  $(NH_4)_2SO_4$ - applied to rice plants compared to other forms of N. Other studies have shown that Cd toxicity in rice plant might be reduced with N application during the growth process and it's sometimes been influenced by genotype variability. Hence, selecting suitable N and cultivars might yield great dividend in growing rice on Cd-contaminated soils with minimum risk of Cd entry into the food chain. Zinc is another micronutrient largely required by rice plant for normal growth, development as well as yield formation. However, the physical and chemical properties found in Zn are comparatively similar with those found in Cd and thus, forms a possible means reducing Cd toxicity in growing rice plants through Zn application (Hassan et al. 2006). In general, two types of interactions interplay between metals during plant uptake, that is, either antagonistic (adding one metal decreases the uptake of the other) or synergistic (adding one metal increases uptake of the other). Several studies have reported the interaction of Zn and Cd during rice growth (Fahad et al. 2015; Basnet et al. 2014). Zn application increased plant growth and photosynthetic pigments as well as decreased MDA contents and antioxidant enzyme activities in rice grown on Cd stress condition. In Liu et al. (2007) research, the findings revealed that Zn application decreased the concentration of Cd in rice roots and increased Cd concentrations in rice shoots. In another study conducted by Basnet et al. (2014), increased plant growth and biomass as well as reduction in oxidative stress were observed, this might be due to compartmentalization of Cd in plant tissues as a result Zn-Cd binding. Thus, detailed studies are further needed to determine the influence of Zn on Cd uptake and translocation in the different vegetative rice parts. Iron is subsumed among the various strategies use to reduce Cd uptake and translocation in rice especially based on formation of iron plaque (IP) on rice roots surfaces. Sebastian and Prasad (2015) and Zhou et al. (2015) studies reported that Fe application reduced Cd content in rice plants and increased plant growth as well as yield components. Zhou et al. (2015) reported that rice forms iron plaque under field conditions and Cd contents in rice roots were significantly lowered in treatments with IP than in roots without iron plaque.

Furthermore, the use of selenium (Se) has also been seen as an amendment mechanism to Cd stress, it's positive effect on decreasing Cd effects has been shown by many researchers (Lin et al. 2012; Chen et al. 2014a, b). In Lin et al. (2012) experiment, it was shown that the application of 3  $\mu$ M Se in 50  $\mu$ M Cd level conducted in the nutrient solution relatively increased rice growth and decreased uptake and concentration of Cd as well as oxidative stress in rice. The application of Se improved the nutrient status and inhibited the Cd uptake by rice seedlings but further research is needed to extend understanding on uptake and translocation at different parts.

#### VI. Conclusion

Since Cd has been observed to be one of the most harmful heavy metals to rice plant and humans, its presence in the soil creates harmful threats to agro-ecosystem and human health. Soil Cd toxicity alters morpho-physiological and biochemical features of rice plants and hence, raises global concern for crop productivity and food safety. Understanding the varying processes involved in soils accumulated Cd and translocation mechanisms in rice plant during growth can be of tremendous significance for ameliorating toxicity in rice. The use of various management strategies, like bio-char amendments, exogenous application of signaling molecules and plant growth regulators, irrigation and several other means are amongst the numerous approaches widely adopted and highly acknowledged for mitigating Cd-contaminated paddy soils. Further research is needed in several aspects including molecular engineering and marker assisted breeding schemes. The progress made in understanding and application in other food crop for regarding Cd toxicity can be extended to rice research, further eminent studies are needed to evaluate the long term effects of various amendments on Cd immobilization and reduction in rice under varying environmental conditions, identifying a transporters for phloem Cd transport in rice plants as well as identifying proteins that play vital roles in transporting essential nutrients during plant growth, in which iron concentration from roots to leaves is signaled and the amount of iron from the soil needed by the plant regulated, to ensure that rice plant partition Cd away from the edible part (grains).

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