# AMELIORATION OF CADMIUM TOXICITY IN RICE BY UTILIZATION OF ORGANIC MANURES

**NAFISA NAWAL**

**DEPARTMENT OF AGRONOMY**

**SHER-E-BANGLA AGRICULTURAL UNIVERSITY**

**DHAKA-1207**

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# AMELIORATION OF CADMIUM TOXICITY IN RICE BY UTILIZATION OF ORGANIC MANURES

**BY**

**NAFISA NAWAL**

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**APPROVED BY**

| | |
|---|---|
| **Anisur Rahman, PhD** | **Dr. Parimal Kanti Biswas** |
| **Associate Professor** | **Professor** |
| **Supervisor** | **Co-supervisor** |

**Prof. Dr. Md. Shahidul Islam**
**Chairman**
**Examination Committee**

*Dedicated to*

*My beloved parents*

**DEPARTMENT OF AGRONOMY**
Sher-e-Bangla Agricultural University
**Sher-e-Bangla Nagar, Dhaka-1207**

## CERTIFICATE

*This is to certify that thesis entitled, **"Amelioration of Cadmium Toxicity in Rice by Utilization Of Organic Manures"** submitted to the faculty of Agriculture, Sher-e- Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE in AGRONOMY,** embodies the result of a piece of bonafide research work carried out by **Nafisa Nawal,** Registration No.: **13-05739** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

*I further certify that such help or source of information, as has been availed of during the course of this investigation has been fully acknowledged.*

**Dated:**
**Place:** Dhaka, Bangladesh

**Anisur Rahman, PhD**
Associate Professor
Supervisor

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***The Author***

# AMELIORATION OF CADMIUM TOXICITY IN RICE BY UTILIZATION OF ORGANIC MANURE


## ABSTRACT

A pot experiment was conducted at Sher-e-Bangla Agricultural University during T. *aman* season 2018, to examine the effect of Cadmium (Cd) on rice and role of organic manure in reducing Cd toxicity. This experiment consisted of two factors, Factor A: Different levels of Cd stress and Factor B: Different types of organic manures. Factor A consisted of 3 stresses *viz*. i. $CdCl_2$ 0 $mgkg^{-1}$ soil ($Cd_0$) ii. $CdCl_2$ 10 $mgkg^{-1}$ soil ($Cd_1$) iii. $CdCl_2$ 25 $mgkg^{-1}$ soil ($Cd_2$) and Factor B consisted of 4 types of manures *viz*. i. Control ($M_0$) ii. Cowdung 5 t $ha^{-1}$ ($M_1$) iii. Wood-ash 2.5 t $ha^{-1}$ ($M_2$) and iv. Vermicompost 4 t $ha^{-1}$ ($M_3$). Results revealed that exposure of Cd in rice decreased plant growth, yield and yield contributing parameters. Grain yield decreased by 22 and 35% by exposure of $Cd_1$ and $Cd_2$ stress, respectively. Cadmium exposure also increased Cd accumulation in grain. Application of different organic manures influenced growth and yield of rice and played positive role under Cd stress conditions. Vermicompost gave highest grain (9.77g $pot^{-1}$) and straw (44.14 g $pot^{-1}$) yields. In case of combined effect, vermicompost gave highest grain and straw yield in all stresses *viz*. $Cd_0$ ($Cd_0M_3$), $Cd_1$ ($Cd_1M_3$) and $Cd_2$ ($Cd_2M_3$). So, it might be concluded that, the growth and yield of rice decreased with the increasing dose of Cd and vermicompost can be a reasonable approach to reduce the Cd stress in rice.

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# Chapter I

# INTRODUCTION

Soil contamination is a vital problem in this world, because soil is the major plant growth medium. It also provides plants with water and the nutrients which they need to be healthy. In recent decades, contamination of soil by toxic metals has become a serious problem in crop production worldwide. The rapid increase in population together with fast industrialization has caused serious environmental problems, including the production and release of considerable amounts of toxic metals into the environment (Sarma, 2011; Hasanuzzaman and Fujita, 2012). Heavy metals are released into the environment by both natural and anthropogenic sources. Some soils may have higher levels of heavy metals due to volcanic activity or weathering of parent materials. Anthropogenic activities, including smelting, mining, use of pesticides, fertilizers, sludges, and emissions from industries are also responsible for heavy metals accumulations in the soils. Heavy metals cannot be degraded and hence accumulate in the environment, having the tendency to contaminate the food chain. This pollution threatens soil quality, plant survival, and human health (Ferreiro *et al.,* 2013). Bangladesh is not exception from this situation and poses a new challenge to combat health hazards associated with heavy metal toxicity in soil.

Bangladesh is going to face unpredictable situation of heavy metal exposure with the increase of unplanned and socially and environmentally degraded industries (Ahmed *et al.*, 2018). Heavy metal exposure from various means is become a burning issue in Bangladesh.The rapid increase in population together with fast industrialization causes serious environmentalproblems, including the production and release of considerable amounts of heavy metals in the environment (Sarma, 2011). We have more than 30,000 industrial units of which about 900 are large polluting industries, which discharging heavily toxic effluents directly into the adjacent soils (Nuruzzaman *et al.,* 1998). Approximately 30 million gallons of untreated industrial waste water effluent are mixing daily with our environment mainly with water and soil (Jolly *et al*., 2012). Among them, around 600-700 textile, dyeing and glass industries have been set up around Dhaka at Narayangonj, Tejgaon, Savar, Tongi, Gazipur and Tangail areas during the last few years (Khan, 2006). There are 1184 industries in Tangail from which a large amount of industrial effluents is discharged into the surrounding agricultural lands daily. As a result, agricultural land is exposed to large amount of heavy metal concentrations (Ahmed *et al.*, 2018). In addition, Farmers are using fertilizers in large amount for their crop production and perhaps this also leads to metal contamination of soil.

However, Heavy metals are the metals with atomic masses over 20 and having a specific gravity of above 5 g $cm^{-3}$ or more. Nevertheless, some of these metals such as zinc, copper and nickel are essential micronutrients and are required in trace amounts as they act as cofactors for various enzymes. While other metals such as cadmium (Cd) and lead present in pesticides do not have any beneficial role and become toxic if their concentration exceeds a certain limit (Ali *et al*., 2011, 2012). Cadmium is ranked seventh on the list published by the Agency for Toxic Substances and Disease Registry (ATSDR, 2005) of potentially toxic elements that are considered hazardous to the environment. Cadmium is generally released into the soil through various industrial processes, and due to its phytotoxic nature, it causes severe damage to plants. Cadmium is easily taken up by plant roots and transported to different plant parts, causing various phytotoxic symptoms (Bernard, 2008). It also obstructs almost all of the physiological and metabolic processes of the plants. Cadmium also diminishes water and nutrient uptake (Li *et al.*, 2008), as well as causes chlorosis and necrosis of the leaves and roots with reduced growth. It inhibits biosynthesis of photosynthetic pigments, can decrease electron transport efficiency, inhibits the enzymes involved in photosynthesis, reduces photosynthetic carbon assimilation, and causes oxidative damage to sub-organelles (Maksymiec *et al.*, 2007). The Cd-induced oxidative stress also causes various complications in plant physiological processes. As a consequence, the growth and development of plant are impeded, which is evidenced by Cd-induced reduced and abnormal seed germination, reduced and irregular growth, chlorosis and necrosis, anomalous development of reproductive organs and yield components, and reduced yield (Gill and Tuteja, 2011). Moreover, the phyto-toxicity of Cd is so high that higher Cd accumulation than 5–10 μg Cd $g^{-1}$ leaf dry weight leads to plant death, except in Cd-hyperaccumulators (White and Brown, 2010).

Cadmium is highly toxic to humans and other living organisms even at low concentrations (Smolders and Mertens, 2013), and it is greatly mobile in soils, leaching into groundwater and contaminating aquifers. One of the main routes by which humans are exposed to Cd is the ingestion of plants grown in areas with high contents of this metal (ATSDR, 2005).

Studies on provisional tolerable weekly intake (PTWI) indicate that major food sources that contribute Cd to human diet are rice, wheat, starchy roots/tubers, mollusks, and shellfish. Among a wide range of Cd polluting sources, repeated application of Cd-containing phosphate fertilizers and irrigation using Cd-contaminated water releasing from factories are causing massive contamination of Cd in rice fields (Sandalio *et al.*, 2001). Since rice grain contributes a diet rich in vitamins, minerals, and complex carbohydrates for half the world population, there is persistent demand for Cd minimization in rice. The search for reducing Cd in rice opens the need to explore scientific approaches to minimize Cd in rice plants and hence reduction in the amount of Cd in the grain. Morpho-physiological features of rice are in the direction of favoring uptake of Cd. For example, rice being a monocot with fibrous root system that increases surface area for mineral absorption is favoring the chance of uptake of Cd (Coudert *et al.,* 2010). Solubilization of Cd by a cell-wall-mediated process is also described as a factor that increases the chance of Cd uptake by root cells (Clemens, 2006).

The addition of organic matter amendments, such as compost, cow dung, wood-ash, vermicompost and farm wastes is a common practice for immobilization of heavy metals and amelioration of contaminated soils (Angelova *et al.,* 2010). The basic principles of bioremediation by organic substances involve reducing the solubility of these environmental contaminants by changing pH, acidic soil was found to have more plant-exchangeable Cd (Appel *et al.,* 2008; Sauvé *et al.,* 2003), the redox reactions and adsorption of contaminants from polluted environment and make them unavailable for the plant. Cow-dung is an eco-friendly and low-cost adsorbent. It is a bio-organic waste that contains 12.48% calcium oxide, 0.9% magnesium oxide, 0.312% calcium sulphate, 20% aluminum oxide, 20% iron oxide and 61% silica (Miller *et al.,* 2003). The presence of maximum percentage of silica makes it to exhibits considerable affinity for metal ions. According to Shi *et al.,* (2008), silica possibly plays a significant role in decreasing Cd uptake in plants by changing pH and increasing the adsorption of Cd ion. Wood ash is the inorganic and organic residue remaining after combustion of wood or unbleached wood fiber. Wood-ash supply essential nutrients and decrease the soil acidity, mostly of the organic layer and mineral topsoil layers (Greger *et al.,* 1995). Vermicompost is a by-product of earthworm mediated recycled organic waste which is rich in plant nutrients and growth promoting substances. Vermicompost removes excessive amounts of heavy metals and there by served as a means of detoxification (Kumaresan *et al.*, 1984).

Perceiving the above discussion, the present study was designed

1) To understand the plants response to Cd toxicity.

2) To know the role of organic manures on rice plants.

3) To know the impact of organic manure in Cd-induced rice plants.

# Chapter II

# REVIEW OF LITERATURE

Plant grown under environmental stress condition is a major issue and a major constrains for the crop production. Environmental stress such as salinity, drought, high temperature, heavy metal stress generating great problem. Heavy metal contamination especially Cd contamination is now a considerable issue for the rice production and also for health hazard. Organic manure can be a way to overcome this situation. Different organic manure such as cowdung, wood-ash and vermicompost play different roles for mitigating the Cd stress. This chapter includes different reviews related to Cd stress and role of organic manure. But the available relevant reviews related to Cd stress and role of organic manure is very limited in context to Bangladesh. Some of the recent and past information have been reviewed under the following headings.

## 2.1 Abiotic Stress

Stress can be defined as any external factor that negatively influences plant growth, productivity, reproductive capacity or survival of plant. Plants are subjected to a wide range of environmental stresses which reduces and limits the productivity of agricultural crops. Beside biotic stress, abiotic stress causes the loss of major crop plants worldwide. Plant abiotic stresses are defined as the negative impact of non-living factors on plant in a specific environment (Hasanuzzaman *et al.,* 2012). Abiotic stress is the negative impact of non-living factors on the living organisms in a specific environment. This stress has been becoming a major threat to food security due to the constant changes of climate and deterioration of environment caused by human activity. In the recent decades, plants exposed to different abiotic stresses, such as salinity, drought, flood, low temperature, high temperature, toxic metals, nutrient deficiency, UV radiation etc. These abiotic stresses may create toxic chemical components such as Reactive oxygen species (ROS) that contain hydrogen per oxide ($H_2O_2$), superoxide ($O^{2-}$), hydroxyl ($OH^-$) and many more. Abiotic stresses change the metabolic processes of plant and alter the growth developmental process, the growth and procreation of the plant is prohibited, and the plant died (Wei and Zhou, 2008).

### 2.1.1 Heavy metal stress

Plants in nature are constantly faced with abiotic and biotic stresses. Plants grown under natural conditions are continuously subjected to various environmental stressors, which are the major constraints to crop production, because they are unpredictable, unavoidable, complex in nature, and change gradually (Gill *et al.*, 2011). Heavy metals such as Fe, Mn, Cu, Ni, Co, Cd, Zn, Hg and arsenic are for long being accumulated in soils through anthropogenic activities such as industrial waste, fertilizer applications, smelting and sewage disposal (Aydinalp and Marinova, 2009). These activities cause leaching of metals into groundwater or accumulate them on soil surface (Gupta and Ali 2002; Ali *et al.*, 2009).

Industrial discharge of untreated or partially treated effluents directly into surrounding agricultural lands is considered as the most significant anthropogenic activity responsible for soil pollution by various pollutants like heavy metals such as Cd, Cu, Zn, Cr, Ni, Pb and Mn (Islam *et al.*, 2012; Jolly *et al.*, 2012). All heavy metals are non-biodegradable, that is, they cannot be purged out naturally from the environment via any possible natural means, although some of them are reported to be immobile, that is, they cannot move from the place where they are accumulated, while there are others that are termed as mobile, that is, they can be taken up by plants root system via diffusion, endocytosis or through metal transporters (Ozturk, 1989; Ali and Jain, 2004) Nevertheless, some of these metals such as Zn, Cu and Ni are essential micronutrients and are required in trace amounts as they act as cofactors for various enzymes. While other metals such as Cd and Pb present in pesticides do not have any beneficial role and become toxic if their concentration exceeds a certain limit (Gough *et al.*, 1979; Sharma and Ali, 2011).

Toxicity of heavy metals develops various stress symptoms in plants. Ultimate result is growth inhibition and furthers the death of plant. Ullah *et al.* (1999) reported higher metal concentrations in soils of different sites around Dhaka, Bangladesh, are of growing concern and there is a strong need for remediation of these sites. Heavy metals show its toxic effects in plants connected with water relations, especially inthe early stage of plant growth, it influences membrane transport and inhibit root growth andenzyme activities (Appenroth, 2010).

#### 2.1.1.1 Cadmium Stress

The rapid increase in population together with fast industrialization has caused serious environmental problems, including the production and release of toxic metals into the environment (Sarma, 2011; Hasanuzzaman and Fujita, 2012; Hasanuzzaman and Fujita, 2013). Of all heavy metals (HMs), cadmium (Cd) ranks the highest in terms of damage to plant growth and productivity (Nouairi *et al*., 2009). It has been reported that human activities contribute a major share, about 13,000 out of the total 30,000 tonnes, of annual Cd addition to the environment (Gallego *et al*., 2012). Atmospheric deposition is considered as a major source of Cd input in agricultural soils. Contamination of agricultural soils with Cd has become one of the most toxic and widespread environmental problems (Du *et al.*, 2013).

It is well known that food is the major source of Cd intake for humans (Yu *et al*., 2006) Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population. In rice fields, application of phosphate fertilizers and water irrigation is the major anthropogenic Cd polluting sources (Kosolsaksakul *et al.,* 2014). It was reported that Cd can be readily taken up by rice and translocated to shoot and then to grains (Wang *et al*., 2014; Song *et al.,* 2015). Thus, Cd can enter into the food chain through rice consumption, even at low Cd concentrations in the soils, and cause toxicities to humans (Du *et al*., 2013). It has been extensively reported that Cd stress caused a reduction in rice growth and biomass (Ahsan *et al.* 2007; Li *et al*., 2012a, b; Wang *et al.,* 2014). Excessive Cd (between 0.2 and 1.0mM) in the growth medium reduced rice seed germination (Ahsan *et al.,* 2007). In addition, excessive Cd exposure (100 mg Cd $kg^{-1}$ of soil) caused leaf chlorosis and necrosis (Liu *et al.,* 2003a, b). Exposure to Cd caused chlorosis, leaf rolling symptoms, and growth inhibition (Rahman *et al.,* 2015).

Cadmium toxicity restricted rice growth as estimated in terms of root and shoot length, leaf and root area, and number of leaves and roots per plant (Yu *et al.,* 2006; Song *et al*., 2015). The accumulation of cadmium (Cd) alters different physiological and biochemical attributes that affect plant growth and yield (Rahman *et al*., 2016). Under Cd stress, photosynthesis and gas exchange characteristics (chlorophyll a, chlorophyll b, carotenoids, net photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency) decreased in rice (Rascio *et al.,* 2008;

Wang *et al.*, 2014). In general, Cd stress inhibited rice growth and biomass as well as it is a disastrous element for human world.

## 2.2 Organic Manure

Organic manures may be defined as materials which are organic in origin, bulky and concentrated in nature, capable of supplying plant nutrients and improving soil physical environment having no definite chemical composition with low analytical value. Organic manures have been shown to help preserve natural resources and reduce degradation of ecosystem (Mader *et al.,* 2002), hence play a great to plant growth and yield development. With external organic manure application initially improved soil properties and promoted plant growth, which resulted in the increase of plant nutrient uptake, growth, and yield Angelova *et al.* (2010) investigated organic amendments, resulting in an increase in the starch yield, absolute dry substance and quantity, and a decrease of reducing sugars in potatoes. Applications of both chemical and organic fertilizers need to be applied for the improvement of soil physical properties which supply of essential plant nutrients for higher yield. A suitable combination of organic and inorganic sources of nutrients is necessary for sustainable agriculture that can ensure food production with high quality, Organic manures produced from animal, plant and other organic wastes and by products. Those are included well rotten farm yard manure (FYM), cow-dung, compost, wood-ash, green manures etc. Organic manure helps to enhance bioremediation of metal in soil and plant. Bioremediation is gradually being accepted as the standard practice for the restoration of heavy metal contaminated soils since it is eco-friendlier and cost effective compared to the conventional chemical and physical methods, which are often very expensive and ineffective when metal concentrations are low (Ekperusi *et al.,* 2015; Ayangbenro *et al.,* 2017).

The basic principles of bioremediation by different organic amendments involve reducing the solubility of environmental contaminants by changing pH, the redox reactions and adsorption of contaminants from polluted environment (Jain *et al*., 2016). A number of amendments are used either to mobilize or immobilize heavy metals in soils. Different kinds of organic amendments that have been used for the treatment of contaminated soil include manures, biosolids, sawdust, and wood-ash, composts obtained from different source materials, sewage sludge, bark chips, and woodchips. Different organic materials such as crop residues, animal manures, green manures etc. play direct role to the soils such as soil organic matter content such as, soil fertility, soil physical characteristics, and augmentation of microbial activities, amelioration of metal toxicity (Escobar *et al*., 2008).Over a longer period of time, applications of organic materials such as livestock manure and crop residues have been found to bring about a gradual improvement in soil productivity and crop performance and create a positive environment for the plant. However, in fields under intensive monoculture which receive heavy applications of chemical fertilizers alone, there is a chance of slow decline in productivity. This decline occurs even in irrigated paddy fields (FFTC, 1998). An important benefit was found that, the increased use of organic materials is that it can help to solve pollution problems caused by agro-industrial wastes. In today’s era, heavy doses of chemical fertilizers and pesticides are being used by the farmers to get better yield of various field crops. These chemical fertilizers and pesticides decreased soil fertility and caused health problems to the consumers. Due to adverse effects of chemical fertilizers, interest has been stimulated for the use of organic manure. The organic production system aims at supporting and sustaining healthy ecosystems, soil, farmers, food production, the community, and the economy. Reduction and elimination of the adverse effects of synthetic fertilizers and pesticides on human health and the environment is a strong indicator that organic agriculture is gaining worldwide attention (Aksoy, 2001; Chowdhury, 2014).

### 2.2.1 Cowdung

Cowdung is an organic matter, mostly derived from animal feces , which can be used as organic fertilizer in agriculture. Cowdung contribute to the fertility of the soil by adding organic matter and nutrients, such as nitrogen, that are utilized by bacteria, fungi and other organisms in the soil.Cow dung has long been recognized as perhaps the most desirable animal manures because of its high nutrient and organic matter content which contains a high proportion of humified organic matter (OM). The animal manure (such as cattle manure) is another source of nitrogen with other nutrients which can decrease the demand of chemical fertilizer. It has been used for centuries to increase soil fertility (Darzi *et al.*, 2012).

It is a bio-organic waste that contains 12.48% calcium oxide, 0.9% magnesium oxide, 0.312% calcium sulphate, 20% aluminum oxide, 20% iron oxide and 61% silica (Vasanthakumar and Bhagavanalu, 2003). Cowdung significantly increased the growth and yield of plants.Addition of cowdung increases the organic carbon content of degraded soil which may lead to the increasing activity of beneficial soil microorganisms as well as the fertility status of soil by increasing the availability of nutrients for the plants from soil.

### Cowdung as a problem-solving agent of heavy metal stress

It is hypothesized that organic matter like cow-dung in the soil will increase organic acids (like humic acid and fulvic acids) and proteins which will provide binding sites to excess heavy metal ions in the soil and this will reduce heavy metal accumulation in vegetable crops (Sharma *et al.,* 2012). The soil pH was increased more in cow dung amended soil. This may be due to sorption of toxic heavy metal by the protein, carbohydrate and phenolic compounds contained in cowdung. Cowdung have metal binding functional group such as carboxyl, hydroxyl and amino groups which helps to bind metals and make them unavailable for the plant. Besides, the presence of cation exchange capacity in cowdung would have played the roles in the nutrient balance via supply of essential macronutrients (NPK) and micronutrients (Fe, Mn, Cu, B etc.) and exchange those nutrients with metal cations. Cowdung can decrease the bioavailability of heavy metals in soil (Tordoff *et al.,* 2000). Several studies have shown that the application of OM (farmyard manure, compost, peat soil) resulted in a decrease in Cd concentration in some crop plants (corn, wheat, radish) (Ram *et al.,* 1985; Pichtel *et al*., 2008).The OM forms strong complexes with Cd which results in immobilization of Cd in the soil. The presence of maximum percentage of silica makes it to exhibits considerable affinity for metal ions.

### 2.2.2 Wood ash

Wood ash is the inorganic and organic residue remaining after combustion of wood or unbleached wood fiber. The physical and chemical properties of wood ash vary significantly depending on many factors. Wood ash produced through combustion is particularly promising as it increases soil pH and provides nutrients such as calcium (Ca), magnesium (Mg), potassium (K), and phosphorus (P) (Augusto *et al*., 2008).Wood ash is composed of many major and minor elements needed by the tree for plant growth. Since most of these elements are extracted from the soil and atmosphere during the tree's growth cycle, they are elements that are common in our environment and are also essential elements in the production of crops and forages. Calcium is the most abundant element in wood ash and gives ash properties similar to agricultural lime. Ash is also a good source of K, P and Mg. In terms of commercial fertilizer, average wood ash would be about 0-1-3 (N-P-K). In addition to these macro-nutrients, wood ash is a good source of many micronutrients needed in trace amounts for adequate plant growth. Wood ash contains few elements that pose environmental problems.

Field and greenhouse research have confirmed the safety and practicality of recycling wood ash on agricultural lands. It has shown that wood ash has a liming effect of between 8 and 90 percent of the total neutralizing power of lime and can increase plant growth up to 45 percent over traditional limestone.

### Effect of wood-ash on heavy metal stress

Wood ash contains varying amounts of toxic heavy metals (Hakkila, 1986; Greger *et al*., 1995; Mahmood, 2000). Heavy metals originate mostly from wood fuel but the impact of other contaminants (due to various combustion and storage conditions, impregnated wood, etc.) is also evident (Steenari *et al*., 1999). But essential nutrients of wood ash decrease the soil acidity, mostly of the organic layer and mineral topsoil layers (Greger *et al.*, 1995; Eriksson, 1998). Such pH changes reduce the solubility of the metals.

### 2.2.3 Vermicompost

Vermicompost, a by-product of earthworm mediated recycled organic waste which is rich in plant nutrients and growth promoting substances. Vermicompost is an organic amendment produced by biodegradation of organic material through interactions between earthworms and microorganisms. It is a stabilized, finely divided peat-like material with a low C: N ratio. It has high porosity and high water-holding capacity. It is a stable, fine granular organic manure, which increases soil quality by improving soil physical, chemical and biological properties. It is highly useful in raising seedling and for crop production. Vermicomposting is a process of bio-transforming and stabilizing organic materials (often waste) into humus by the combined activity of earthworms and microorganisms. Application of vermicompost in combination with NPK fertilizers resulted in higher content of total nitrogen compared to FYM. It also resulted in higher content of P significantly (Kale *et al.,* 1992).In terms of plant growth, it encourages the growth of many plant species including vegetables, fruits, cereals and also beenfound to have positive effects on some aromatic and medicinal plants (Domínguez *et al*., 2010). Vermicompost have a positive effect on physicochemical properties of soil hence improving growth and yield of tomato (Atiyeh *et al.,* 2000). Global movement for the second "Green revolution" ought to emphasize on composting, particularly vermicomposting (Buchanan *et al.,* 1988). The vermicompost promotes growth from 50-100% over conventional compost and 30-40% over chemical fertilizers (Sinha *et al.,* 2010). Vermicompost play a significant role on growth and yield of cabbage (Chaudhary *et al*., 2004). Investigations show that vermicompost permanently enhances biological activity and can be used for the improvement of seed germination, flowering, growth and yield compared to commercial culture media which lack applicable nutrition (Atiyeh *et al.,* 2000). The presence of nutrients such as nitrates, phosphates and exchangeable Ca and soluble K remained in vermicompost. Vermicompost contains plant growth influencing materials produced by microorganisms. Vermicompost contains plant growth influencing materials produced by microorganisms. Organic amendmentslike vermicompost promote humification increased microbial activity and enzyme production, which, in turn, increases the aggregate stability of soil particles, resulting in better aeration (Perucci *et al*., 1990). Beside these, earthworm castings arereported to contain plant growth promoters, such as auxins and cytokines (Krishnamoorthy *et al*., 1986).

## Effect of vermicompost on heavy metal stress

The composted organic wastes exert variety of physical, chemical and biochemical influences to the soil. It maintains the soil in a proper homeostatic state. It also removes excessive amounts of heavy metals and there by served as a means of detoxification (Kumaresan *et al.,* 1984). Vermicompost has an extensive potential for use on agricultural crops to replace inorganic fertilizers. Most of the researchers have evaluated that the effects of vermicompost on the growth of other plants through various greenhouse and field studies. Peat, compost, and vermicompost application led to the effective immobilization of phyto accessible forms of Pb, Cu, Zn, and Cd in soil. As most studies focused on this topic reported, vermicomposting significantly reduced the exchangeable fraction of initial raw materials, greatly sequestered the water-soluble ions and transformed them into the residual fraction (Lv *et al.,* 2016; Singh and Kalamdhad, 2013). The mechanism for this sequestration was due to mineralization and humification effect of earthworms and microorganisms brought the heavy metals to an inert fraction. Organic amendments decreased heavy metal contents in potato peel and tubers, and this decrease was best expressed with 10% compost and 10% vermicompost, separately.

# Chapter III

# MATERIALS AND METHODS

## 3.1 Experimental details of the site

### 3.1.1 Study Area

The experiment was conducted in the Sher-e-Bangla Agricultural University farm, Dhaka, under the agro-ecological zone of Modhupur Tract, AEZ-28 during the T. *aman* season of 2018.

This chapter presents a brief description of the crop, experimental design, treatments, and cultural operations, collection of soil and plant samples and analytical methods followed in the experiment.

### 3.1.2 Climate

The climate of the experimental area is characterized by sub-tropical accompanied by moderate high rainfall associated with relatively high temperature during T. *aman* season. Sub-tropical climate consisted with three 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 (Edris *et al*., 1979).

## 3.2 Plant material

BRRI dhan62, a high yielding variety of rice, was used as a test crop. The variety was developed by the Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur, as a short duration T. *aman* rice. BRRI dhan62 is a Zn enriched rice variety with 19 $mgkg^{-1}$ Zn and 9% protein. The life duration of this rice variety is 100 days and yields about 3.5-4.5 $tha^{-1}$.

## 3.3 Raising of seedling

Healthy seeds were selected by specific gravity method and then immersed in water bucket for 24 hours. The seeds started sprouting after 48 hours and were sown after 72 hours. The uniformly germinated seeds were then transferred to the nursery bed and were taken about 20-25 days to produce seedling.

## 3.4 Pot preparation

Soil were collected from the field and exposed to the sun for 12 hours. Soils were mixed and turned twice a day for uniform drying. The soils were then being cleared of rocks, coarse organic materials, weeds, stubbles etc. forty eight pots were taken for the experiment. Size of the pot was 35 cm′ height and 30cm diameter. 10kg soil was taken for each pot. Required amount of Organic manures and chemical fertilizers were added to each pot. Experimental pots were laid down as per treatment and design.

## 3.5 Experimental design

The experiment was laid out in a randomized complete block design with four replications. Each replication is sub divided into 12 unit of pot. The treatments were randomly distributed to the unit pots with each replication.

## 3.6 Fertilizer application

All the fertilizers, except urea were applied in soil during pot preparation. Urea was applied in two equal splits. The first split was applied during transplanting of seedling, the second split after 30 days of transplanting i.e. at active vegetative stage. The fertilizer was thoroughly mixed with the soil by hand.

### 3.7.1 Organic manure application

Organic manures (cowdung, wood-ash, vermicompost) was mixed with soil during pot preparation on 31stJuly, 2018.

**Table 1. Rates of different organic manures**

| Organic Manure | Rate (t $ha^{-1}$) |
|---|---|
| Cowdung | 5 |
| Wood-ash | 2.5 |
| Vermicompost | 4 |

### 3.7.2 Chemical fertilizer application

Chemical fertilizers were mixed with soil on 2nd August, 2018.

**Table 2. Sources and rates of different chemical fertilizer**

| Chemical Fertilizer | Rate (kg $ha^{-1}$) | Nutrient Element |
|---|---|---|
| Urea | 124 | N |
| TSP | 42 | $P_2O_5$ |
| MP | 68 | $K_2O$ |
| Gypsum | 50 | S |

### 3.8 Transplanting of seedling

Seedling of 25 days old was transplanted into the pots on 9th August 2018. The seedlings were carefully uprooted from the field. There were three hills per pot and one seedling was used per hill.

## 3.9 Treatments

There were 12 treatments per replication including rate of organic manure and varying concentration of cadmium. The treatments were as follows-

**Factor A:** Cadmium (Cd) concentration

1. $CdCl_2$ 0 mg $kg^{-1}$ soil ($Cd_0$)
2. $CdCl_2$ 10 mg $kg^{-1}$ soil ($Cd_1$)
3. $CdCl_2$ 25 mg $kg^{-1}$ soil ($Cd_2$)

**Factor B:** Organic manure management

1. Control ($M_0$)
2. Cowdung 5 t $ha^{-1}$ ($M_1$)
3. Woodash2.5 t $ha^{-1}$ ($M_2$)
4. Vermicompost 4 t $ha^{-1}$ ($M_3$)

## 3.10 Intercultural operations

The following intercultural operations were done for ensuring the normal growth of the crop. Top dressing of Urea was done as per schedule and the normal cultural practices including weeding and insecticides spray were followed as and when necessary. There was some incidence of insects especially for rice stem borer, rice bug, which was controlled by spraying Diazinon 50EC. Irrigation was also provided as per requirement.

## 3.11 Data collection and sampling procedure

### 3.11.1 Growth contributing parameter

The data of growth contributing characters of crop were calculated as follows:

I. Plant height
II. No. of tiller
III. No. of leaves
IV. Leaf area index
V. Root length
VI. Root weight

## Plant height

The plant height was measured from the ground level to the top of the panicle. Plants of 3 hills were measured and averaged for each pot.

## Number of tillers per hill

Number of tillers were counted from 3 hills of each pot and their average was calculated as well.

## Number of leaves per hill

Number of leaves were counted from 3 hills of each pot and their average was also calculated.

## Leaf area index

Length and breadth of a leaf from each hills following by 3 hills from each pot were calculated. In this way LAI were calculated by using the following equation-

$$\text{LAI} = \frac{\text{Surface area of leaf sample (m2)}}{\text{Ground area from where the sample was collected (m2)}}$$

## Root length

After harvesting, root stalks were collected and washed several times to clean the dirt and soil from the root. Then root length was calculated from the collar region in a wet condition.

## Root weight

Roots from each pot were collected carefully. Washed them several times with fresh water and dried. After that they were weighted in an electrical balance.

### 3.11.2 Yield contributing parameter

The data of yield contributing characters of crop were calculated as follows:

I. Effective tiller
II. Panicle length
III. Filled grain
IV. Straw weight
V. Grain weight
VI. 1000 grain weight

#### Effective tillers per hill

After harvesting of plant, tillers from each pot were observed, among them tillers with panicle and grain considered as effective tillers. Effective tillers were counted and their average was calculated.

#### Panicle length

Measurement was taken from basal node of the rachis to apex of each panicle. Each observation was an average of 3 hills from each pot.

#### Filled and unfilled grain

Ten panicles were taken at random and the unfilled and filled grains per panicle were counted and averaged.

#### Grain weight

Grains from panicle from each pot were collected, dried from some days and then weighted.

### 1000 grain weight

The weight of 1000-grains from each plot was measured after sun drying by an electrical balance.

### Straw weight

After harvesting of plant, rice straw were collected and dried in a field. Dried straw were then weighted and their average was calculated.

## 3.12 Chemical analysis of grain

The level of Cadmium were analyzed in rice grains by using Atomic Absorption Spectrometer in the laboratory of Soil and Environmental Research Section, Biological Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR).Before chemical analysis samples had to gone through following digestion process-

Before going to digestion process, Firstly, grains were oven dried at 70°C for 48 h. Dried grains were then ground and about 0.5g samples were taken for the digestion process. 5ml 70% $HNO_3$ and 5ml $H_2O_2$ chemicals were used for the digestion process. In the experiment Nitric Acid Digestion method was followed.

This approach was partly modified from that of Zheljazkov and Nielson (1996). 0.5g sample (with rice husk and without rice husk) was taken in a 25ml beaker. 5ml 70% $HNO_3$ was added for predigestion process cover them and it was predigested for about 24 hour. After that, again 5ml $HNO_3$ was added to the sample and heated them for 30 minute with 80°C and then finally temperature was increased up to 150°C at which sample was started to boil for at least 2 hour until clear solution was obtained. Those sample was cooled at room temperature and the interior walls of the beaker were washed down with a little distilled water and the beaker was swirled throughout the digestion to keep the wall clean and prevent the loss of the sample. Then 5ml 30% $H_2O_2$ was added and heat them at 220°C for 1 hour until the volume was reduced to 1ml. After cooling the samples, those were volume in a 25ml volumetric flask with distilled water. Each sample was then filtrate with Whatman No. 42 filter paper.

The concentrations of Cd in the final solutions were determined by an Atomic Absorption Spectrometer (AAS).

## 3.13 Statistical analysis

The plant software STATISTIX 10 was used to analyze the data. The mean differences among the treatments were compared by the Least Significant Difference Test (LSD) at 5% level of probability.

# Chapter IV

# RESULTS AND DISCUSSION

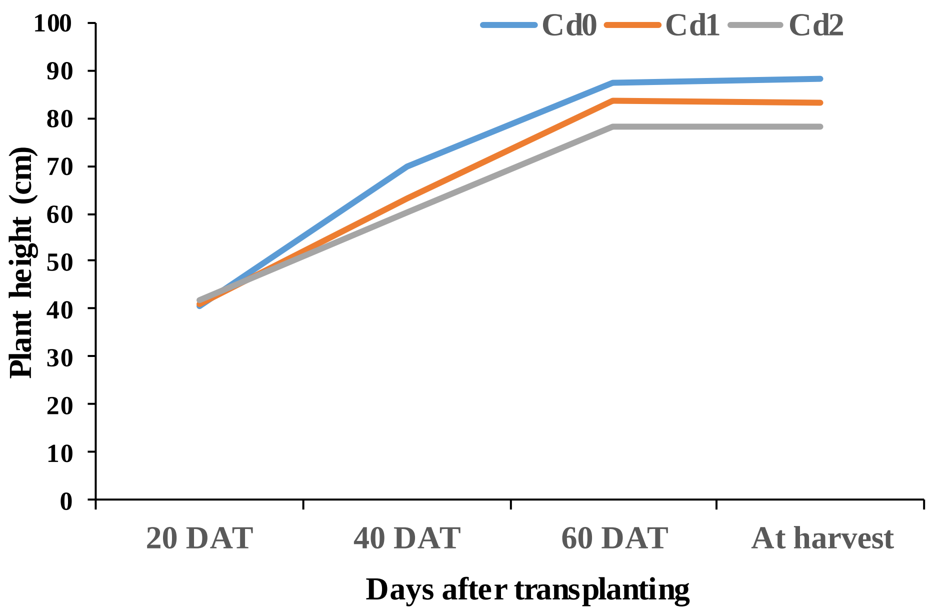
The present experiment was conducted to ameliorate Cadmium toxicity in rice plant by using organic manure. Data on plant growth characters, yield contributing characters and concentration of Cd in rice grain were recorded to assess the trend of growth, development and yield of crops under Cd stress condition. The analysis of variance (ANOVA) of data is given in Appendices. The results have been presented and discussed under the following headings:

## 4.1 Growth Parameter

### 4.1.1 Plant height

#### 4.1.1.1 Effect of Cadmium

Plant height was recorded at 40DAT, 60DAT and at harvest. There is a variation in plant height with the application of Cd (Figure: 3). The lowest plant height (60.37 cm, 78.42 cm and 78.06 cm at 40 DAT, 60 DAT and at harvest, respectively) was observed with the exposure of $Cd_2$($CdCl_2$25 mg $kg^{-1}$ soil) which is 13.37%, 10.37% and 11.74% lower compared to control. Cadmium decreased plant height as it is one of the most toxic HMs and its higher rate causes serious problems in growth and developmental processes of plants (Prasad, 1995). Cadmium is not essential for plant growth. It is readily taken up and accumulates in traces in plants but due to its high mobility and hydrophilic nature it poses a source of potential damage to plant growth((Pinto *et al*., 2004; Amani, 2008). It was observed that plant height reduced highly with the higher dose of Cd.

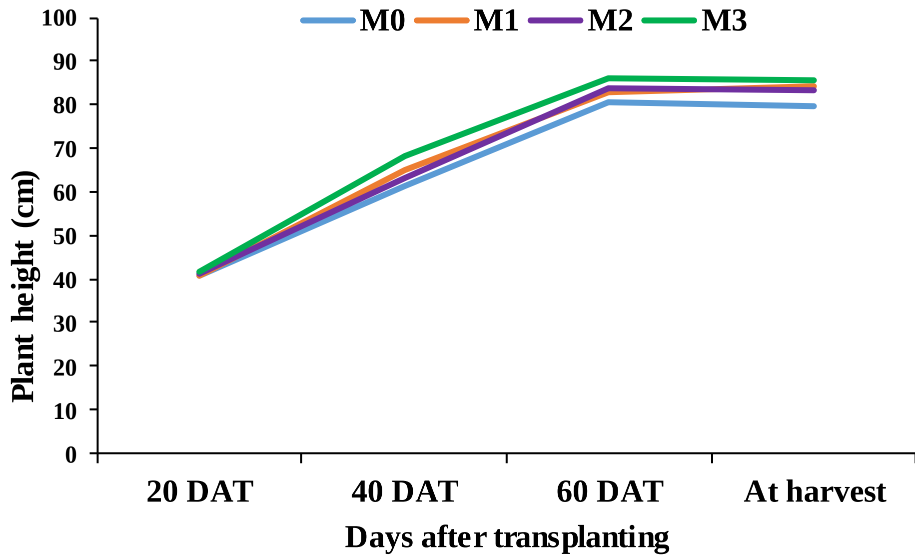



Here, $Cd_0$= $CdCl_2$ 0 $mgkg^{-1}$ soil, $Cd_1$= $CdCl_2$ 10 $mgkg^{-1}$ soil and $Cd_2$= $CdCl_2$ 25 $mgkg^{-1}$ soil

**Figure 3. Effect of Cadmium on plant height of rice at different days of transplanting [LSD $_{(0.05)}$ = NS, 3.16, 2.73 and 2.18 at 20, 40, 60 DAT and at harvest, respectively]**

### 4.1.1.2 Effect of organic manure

Application of different categories of organic manure showed significant variation in plant height (Figure 4). At 40 DAT, 60 DAT and at harvest (68.25, 85.82 and 85.71cm, respectively) the highest plant height was recorded by the application of Vermicompost ($M_3$) which is 11.61%, 7.65% and 7.40% higher compared to control. Vermicompost has a positive effect on plant growth and development. Vermicompost encourages the growth of many plant species including vegetables, fruits, cereals and also been found to have positive effects on some aromatic and medicinal plants (Domínguez *et al.*, 2010). In this experiment vermicompost play a key role for plant height development but second-best result (64.84cm, 82.94cm and 84.09 cm) was found from Cowdung application ($M_1$).




Here, $M_0$ = Control, $M_1$ = 5 t $ha^{-1}$ Cowdung, $M_2$ = 2.5 t $ha^{-1}$ Wood ash and $M_3$ = 4 t $ha^{-1}$ Vermicompost

**Figure 4. Effect of organic manure on plant height of rice at different days of transplanting [LSD $_{(0.05)}$ = NS,3.65, 3.15 and 2.51 at 20, 40, 60 DAT and at harvest, respectively]**

### 4.1.1.3 Combined effect of Cadmium and organic manure

Cadmium-induced stress resulted excessive accumulation of Cd in plant parts with phenotypic damaging symptoms, which can be cured by the exogenous application of organic manure. Combined application of Cd and organic manure showed non-significant differences on plant height (Table 3). Highest plant height was recorded (74.89, 90.82and 92.37cm at 40 DAT, 60 DAT and at harvest, respectively) with the combination of$Cd_0M_3$which is 12.76%, 4.45% and 5.3% higher compared to control. The lowest plant height (57.91, 74.80 and 73.78cm at 40, 60 DAT and at harvest, respectively) was observed with the combination of $CdCl_2$25 mgkg$^{-1}$ Soil and control ($Cd_2M_0$)which is 12.79%, 13.97% and 15.89% lower compared to control.

**Table 3. Plant height of rice as influenced by cadmium and organic manure at different growth stage of rice plant**

| Treatments | Plant height (cm) | | | |
|---|---|---|---|---|
| | 20 DAT | 40 DAT | 60 DAT | At harvest |
| $Cd_0M_0$ | 40.12 | 66.41 | 86.95 | 87.72 |
| $Cd_0M_1$ | 41.12 | 68.54 | 85.28 | 87.06 |
| $Cd_0M_2$ | 41.03 | 68.91 | 86.97 | 86.65 |
| $Cd_0M_3$ | 40.16 | 74.89 | 90.82 | 92.37 |
| $Cd_1M_0$ | 39.36 | 59.10 | 79.55 | 77.90 |
| $Cd_1M_1$ | 41.00 | 64.65 | 84.55 | 85.50 |
| $Cd_1M_2$ | 42.56 | 61.71 | 84.59 | 84.08 |
| $Cd_1M_3$ | 41.17 | 66.97 | 85.96 | 84.77 |
| $Cd_2M_0$ | 42.24 | 57.91 | 74.80 | 73.78 |
| $Cd_2M_1$ | 40.36 | 61.34 | 78.99 | 79.72 |
| $Cd_2M_2$ | 40.49 | 59.33 | 79.23 | 78.75 |
| $Cd_2M_3$ | 43.72 | 62.88 | 80.67 | 80.00 |
| $LSD_{0.05}$ | NS | NS | NS | NS |
| CV (%) | 8.15 | 6.83 | 4.56 | 3.64 |

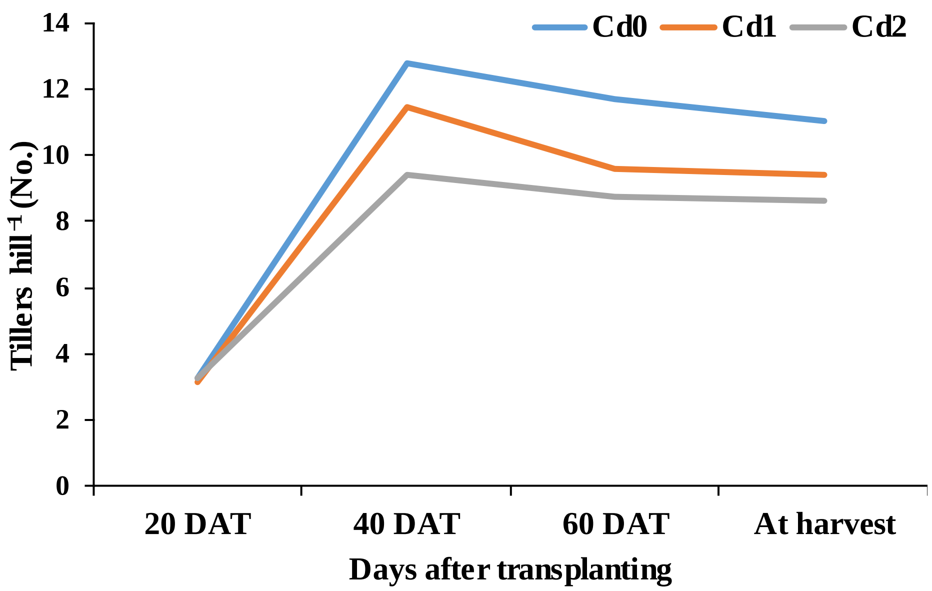
$Cd_0$ = $CdCl_2$ 0 $mgkg^{-1}$ soil
$Cd_1$ = $CdCl_2$ 10 $mgkg^{-1}$ soil
$Cd_2$ = $CdCl_2$ 25 $mgkg^{-1}$ soil

$M_0$= Control
$M_1$= 5 t $ha^{-1}$ Cowdung
$M_2$= 2.5 t $ha^{-1}$ Wood ash
$M_3$= 4 t $ha^{-1}$ Vermicompost

## 4.1.2 Number of tillers $hill^{-1}$

## 4.1.2.1 Effect of Cadmium

External application of Cd reduced tiller number in *aman* rice. Exposure of Cd decreased the number of tiller $hill^{-1}$throughout the life cycle. The lowest number of tillers were confirmed at 40DAT (9.43), 60DAT (8.71) and at harvest (8.62) by the application of 25 $mgkg^{-1}$ soil of Cd which is 26.38%, 25.42% and 22.06% lower compared to control. Tiller number decreased because Cd causes disorders in biochemical and physiological processes and ultimately it affects plant growth, morphology, and development (Sgherri *et al*., 2002).

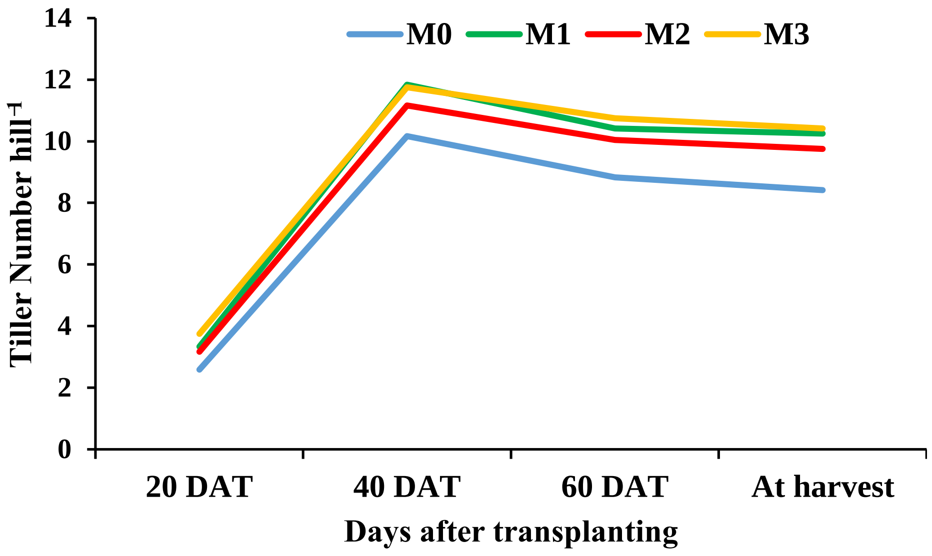



Here, $Cd_0 = CdCl_2$ 0 mgkg$^{-1}$ soil, $Cd_1 = CdCl_2$ 10 mgkg$^{-1}$ soil and $Cd_2 = CdCl_2$ 25 mgkg$^{-1}$ soil

**Figure 5. Effect of Cadmium on number of tiller hill$^{-1}$ at different days of transplanting [LSD $_{(0.05)}$ = NS, 0.94, 0.78 and 0.60 at 20, 40, 60 DAT and at harvest, respectively]**

### 4.1.2.2 Effect of organic manure

Organic manure acts as a growth developmental agent for the plant growth. Application of different type's organic manure showed variation in tiller production. The highest number of tillers (3.75, 11.75, 10.75 and 10.41 at 20, 40, 60 DAT and at harvest, respectively) was recorded in entire life cycle of rice for the application of vermicompost ($M_3$) (Figure 6).




Here, $M_0$ = Control, $M_1$= 5 t $ha^{-1}$ Cowdung, $M_2$ = 2.5 t $ha^{-1}$ Wood ash and $M_3$= 4 t $ha^{-1}$ Vermicompost

**Figure 6. Effect of Organic manure on number of tillers per hill of rice at different days of transplanting [LSD $_{(0.05)}$ = 0.59, 1.08, 0.91 and 0.69 at 20, 40, 60 DAT and at harvest, respectively]**

### 4.1.2.3 Combined effect of Cd and organic manure

Application of organic manure in presence of Cd showed remarkable variation in tiller production (Table 5). The highest number of tillers (13.75, 12.25 and 12.25 at 40, 60 DAT and at harvest, respectively) were produced from the $Cd_0M_3$treatment. Alternatively, Lowest number of tillers (7.75, 7.00 and 7.00) at 40, 60 DAT and at harvest, respectively) were recorded from $Cd_2M_0$.Bioremediation by organic manure is gradually being accepted as the standard practice for the restoration of heavy metal contaminated soils (Ekperusi *et al.*, 2015; Ayangbenro and Babalola, 2017), hence helped in plant growth.

**Table 4. Tiller number of rice as influenced by cadmium and organic manure at different stages of rice growth**

| Treatments | Number of tillers hill$^{-1}$ | | | |
|---|---|---|---|---|
| | 20 DAT | 40 DAT | 60 DAT | At harvest |
| $Cd_0M_0$ | 2.50 | 12.25 a-c | 11.57 a-c | 9.75 bc |
| $Cd_0M_1$ | 3.00 | 13.00 ab | 12.25 a | 11.75 a |
| $Cd_0M_2$ | 3.25 | 12.25 a-c | 11.25 ab | 10.50 b |
| $Cd_0M_3$ | 4.25 | 13.75 a | 12.25 a | 12.25 a |
| $Cd_1M_0$ | 2.50 | 10.50 cd | 8.50 ef | 8.50 d |
| $Cd_1M_1$ | 3.50 | 12.50 ab | 9.75 b-e | 9.75 bc |
| $Cd_1M_2$ | 3.25 | 11.25 b-d | 9.75 b-e | 9.75 bc |
| $Cd_1M_3$ | 3.25 | 11.50 b-d | 10.50 b-d | 9.75 bc |
| $Cd_2M_0$ | 2.75 | 7.75 e | 7.00 f | 7.00 e |
| $Cd_2M_1$ | 3.50 | 10.00 d | 9.25 de | 9.25 cd |
| $Cd_2M_2$ | 3.00 | 10.00 d | 9.12 de | 9.00 cd |
| $Cd_2M_3$ | 3.75 | 10.00 d | 9.50 c-e | 9.25 cd |
| $LSD_{0.05}$ | NS | 1.87 | 0.91 | 1.21 |
| CV (%) | 22.21 | 11.60 | 10.90 | 8.65 |

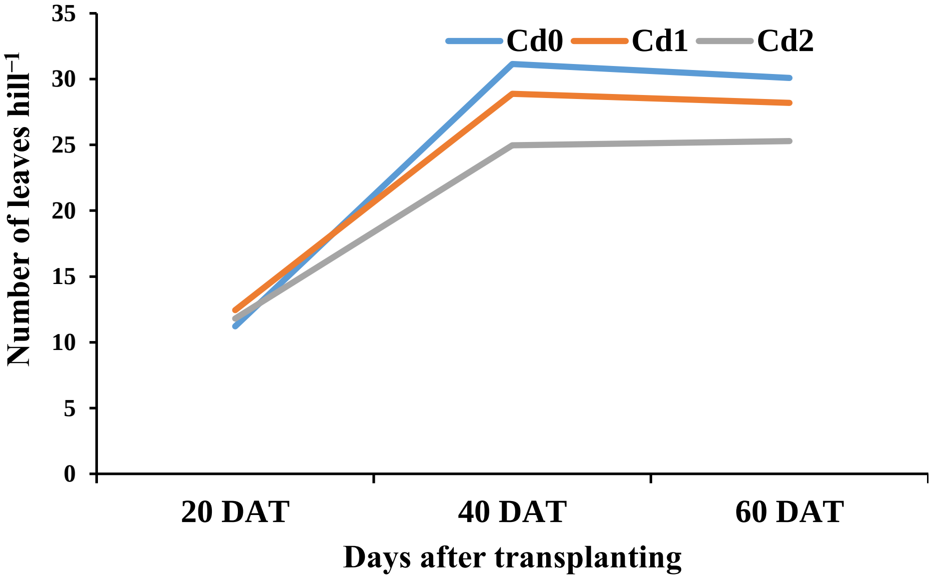
$Cd_0$= $CdCl_2$ 0 mgkg$^{-1}$ soil
$Cd_1$= $CdCl_2$ 10 mgkg$^{-1}$ soil
$Cd_2$= $CdCl_2$ 25 mgkg$^{-1}$ soil

$M_0$= Control
$M_1$= 5 t ha$^{-1}$ Cowdung
$M_2$= 2.5 t ha$^{-1}$ Wood ash
$M_3$= 4 t ha$^{-1}$ Vermicompost

## 4.1.3 Number of leaves hill$^{-1}$

## 4.1.3.1 Effect of cadmium

Leaf number greatly affects the plant growth. Exposure of different level of Cd showed different number of leaves hill$^{-1}$ (Figure 7). The Lowest number of leaves hill$^{-1}$at 40 DAT (24.97) and at 60 DAT (25.27) was recorded by the exposure of 25 mg kg$^{-1}$of Cd which is 19.81% and 16.04% lower compared to control. Excess amounts of Cd in plants cause chlorosis, necrosis, leaf rolling, altered stomatal action, decreased water potential, cation efflux, alterations in membrane functions, photosynthesis inhibition, altered metabolism, altered activities of several key enzymes, and even death (Anjum *et al.,* 2008; Dubey, 2011; Gill *et al.*, 2011). Cadmium also diminishes water and nutrient uptake (Li et al., 2008), as well as causes chlorosis and necrosis of the leaves and roots with reduced growth.

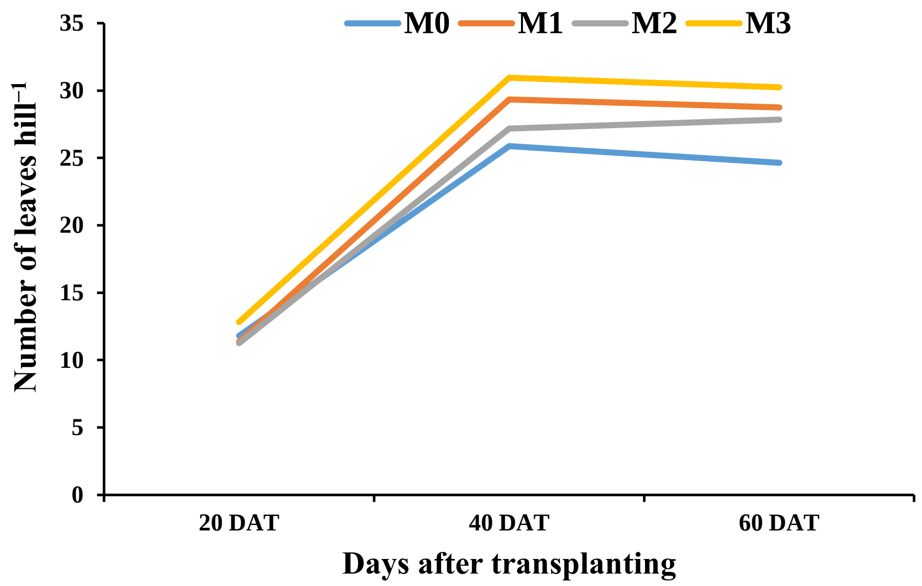



Here, $Cd_0$ = $CdCl_2$ 0 mgkg$^{-1}$ soil, $Cd_1$= $CdCl_2$ 10 mgkg$^{-1}$ soil and $Cd_2$ = $CdCl_2$ 25 mgkg$^{-1}$ soil

**Figure 7. Leaf number of rice as influenced by cadmium at different days of transplanting [LSD $_{(0.05)}$ = 0.75, 1.70 and 1.50 at 20DAT, 40DAT and 60DAT, respectively]**

## 4.1.3.2 Effect of organic manure

Organic manure greatly affects the number of leaves of plant. Among the different types of organic manure applied, highest number of leaves (12.81, 30.95 and 30.23) (Figure: 8) was observed with Vermicompost ($M_3$) which is 8.55%, 19.68% and 22.78% higher compared to control. Addition organic manure initially improved soil properties and promoted plant growth（Angelova *et al.*, 2010).




Here, $M_0$= Control, $M_1$= 5 t $ha^{-1}$ Cowdung, $M_2$= 2.5 t $ha^{-1}$ Wood ash and $M_3$= 4 t $ha^{-1}$ Vermicompost

**Figure 8. Leaf number of rice as influenced by organic manure at different days after transplanting [LSD $_{(0.05)}$ = 0.87, 1.96 and 1.72 at 20, 40, 60 DAT respectively]**

### 4.1.3.3 Combined effect of Cd and organic manure

Interaction of Cd and organic manure significantly influenced number of leaves per hill (Table 6). The highest number of leaves at 40 DAT (32.25) and at 60 DAT (32.25) was recorded in $Cd_0M_3$ (0 $mgkg^{-1}$ Cd and 4 t $ha^{-1}$Vermicompost) treatment. Reversely, Lowest number of leaves at 40 DAT (21.5) and at 60 DAT (22.58) was recorded from $Cd_2M_0$ (25 $mgkg^{-1}$ Soil of Cd and control). Different organic materials such as crop residues, animal manures, green manures etc. play direct role to the soils such as amelioration of metal toxicity and enhance plant growth in abiotic stress condition (Escobar *et al*., 2008).

**Table 5. Leaf number and leaf area index of rice as influenced by cadmium and organic manure**

| Treatments | Number of Leaves $pot^{-1}$ (No.) | | | Leaf area index | |
|---|---|---|---|---|---|
| | 20 DAT | 40 DAT | 60 DAT | 40 DAT | At harvest |
| $Cd_0M_0$ | 11.47 b | 29.79 ab | 28.10 bc | 0.073 cd | 0.087 de |
| $Cd_0M_1$ | 11.14 b | 31.75 a | 29.30 a-c | 0.069 d | 0.095 cd |
| $Cd_0M_2$ | 10.75 b | 30.77 ab | 30.74 ab | 0.064 de | 0.082 d-f |
| $Cd_0M_3$ | 11.43 b | 32.25 a | 32.25 a | 0.115 b | 0.125 b |
| $Cd_1M_0$ | 11.75 b | 26.23 c | 23.19 d | 0.052 ef | 0.069 f |
| $Cd_1M_1$ | 11.19 b | 31.14 ab | 30.00 ab | 0.077 cd | 0.087 de |
| $Cd_1M_2$ | 11.32 b | 28.11 bc | 29.60 a-c | 0.086 c | 0.105 c |
| $Cd_1M_3$ | 15.50 a | 30.08 ab | 29.97 ab | 0.145 a | 0.155 a |
| $Cd_2M_0$ | 12.17 b | 21.55 e | 22.58 c | 0.042 f | 0.052 g |
| $Cd_2M_1$ | 11.77 b | 25.13 cd | 26.89 d | 0.076 cd | 0.075 ef |
| $Cd_2M_2$ | 11.70 b | 22.67 de | 23.14 d | 0.072 cd | 0.095 cd |
| $Cd_2M_3$ | 11.50 b | 30.52 ab | 28.49 bc | 0.067 d | 0.090 c-f |
| $LSD_{0.5}$ | 1.50 | 3.40 | 2.98 | 0.01 | 0.02 |
| CV (%) | 8.85 | 8.34 | 7.43 | 12.32 | 11.43 |

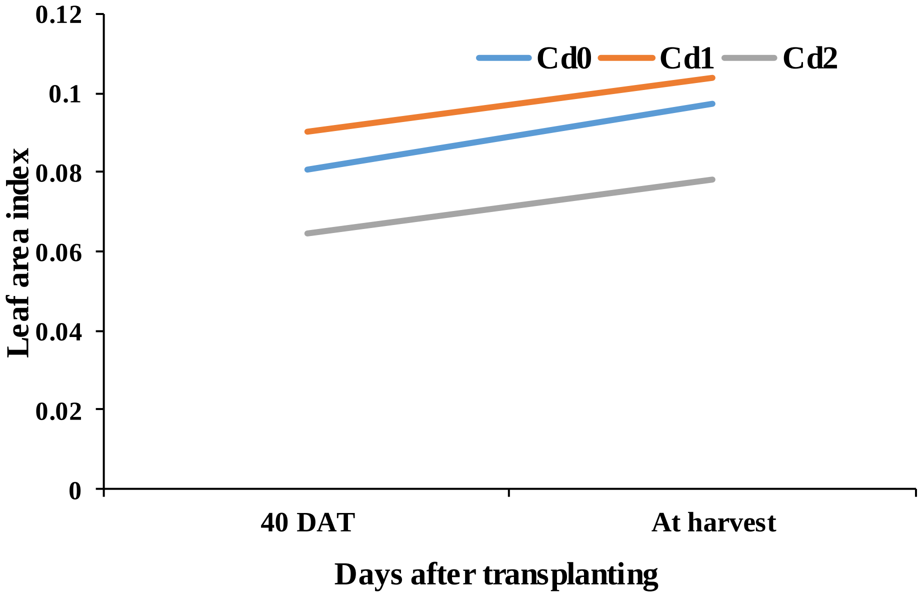
$Cd_0$= $CdCl_2$ 0 $mgkg^{-1}$ soil
$Cd_1$= $CdCl_2$ 10 $mgkg^{-1}$ soil
$Cd_2$= $CdCl_2$ 25 $mgkg^{-1}$ soil

$M_0$= Control
$M_1$= 5 t $ha^{-1}$ Cowdung
$M_2$= 2.5 t $ha^{-1}$ Wood ash
$M_3$= 4 t $ha^{-1}$ Vermicompost

## 4.1.4 Leaf area index

### 4.1.4.1 Effect of cadmium

Leaf area of the plant reduced by the exposure of cadmium (Figure 9). The lowest leaf area index at 40 DAT (0.064) and at harvest (0.078) was observed for the highest dose of Cd ($Cd_2$) which is 19.72 and19.89 % lower compared to control. However, satisfied LAI (0.09 and 0.10) was observed under 10 $mgkg^{-1}$ soil of Cd at 40 DAT and at harvest. Cadmium can hamper photosynthesis by reducing leaf area, impairing the biosynthesis of photosynthetic pigment, damaging the thylakoid or chloroplast membrane through oxidative stress, impairing the pigment system, disrupting the electron transport system, altering stomatal function, and inhibiting the enzymes of pigment biosynthesis, the Calvin cycle, and other sub-phases (Mysliwa-Kurdziel *et al*., 2004; Appenroth, 2010).

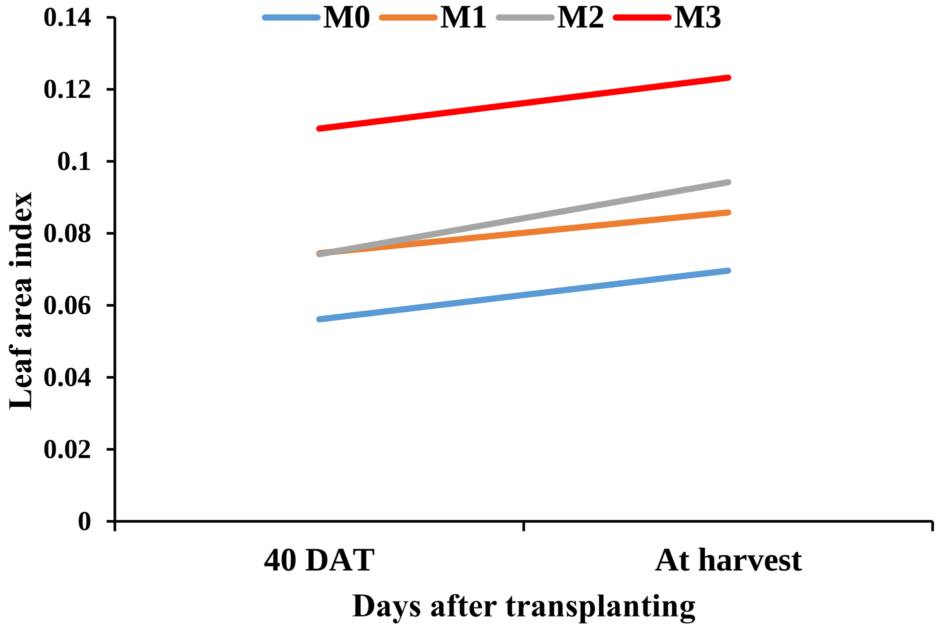



Here, $Cd_0$= $CdCl_2$ 0 $mgkg^{-1}$ soil, $Cd_1$= $CdCl_2$ 10 $mgkg^{-1}$ soil and $Cd_2$= $CdCl_2$ 25 $mgkg^{-1}$ soil

**Figure 9. Effect of cadmium on leaf area index of rice at different days after transplanting [LSD $_{(0.05)}$ =0.01 and 0.01 at 40DAT and at harvest, respectively]**

### 4.1.4.2 Effect of organic manure

Leaf area index varied in T. *aman* rice for the application of different types organic manures. Among the organic manures vermicompost gave good result in case of leaf area index (Figure 10). At 40DAT and at harvest higher LAI (0.10 and 0.12) was recorded for the application of vermicompost ($M_3$). Organic fertilizers contain macro nutrients, essential crop micro nutrients, vitamins, growth promoting substances and beneficial micro-organisms (Olatunji *et al.*, 2006) which helps to promote growth of plant.




Here, $M_0$ = Control, $M_1$= 5 t ha$^{-1}$ Cowdung, $M_2$ = 2.5 t ha$^{-1}$ Wood ash and $M_3$= 4 t ha$^{-1}$ Vermicompost

**Figure 10. Effect of organic manure on leaf area index of rice at different days of transplanting [LSD $_{(0.05)}$ = 0.01 and 0.01 at 40 and at harvest respectively]**

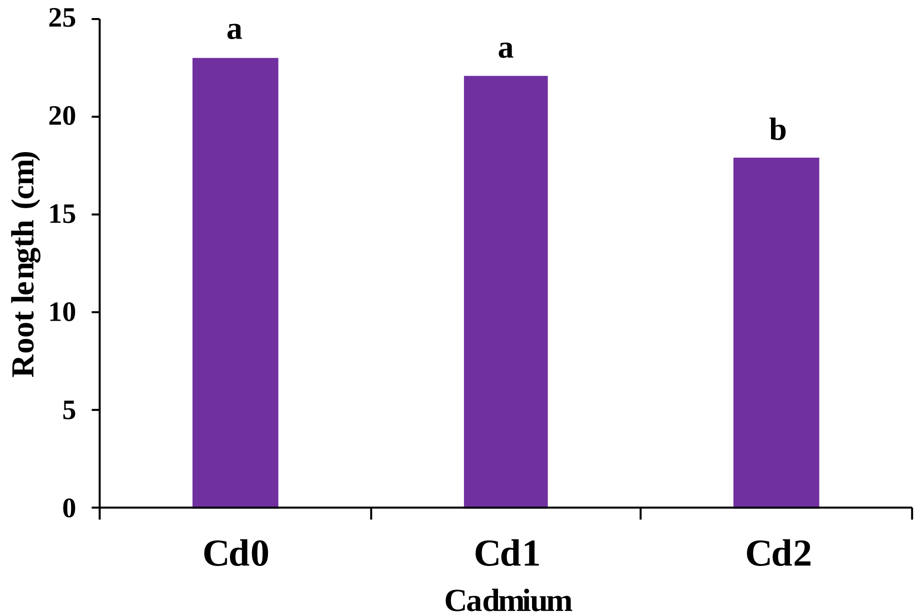
## 4.1.4.3 Combined effect of Cd and organic manure

Organic manure can minimize the damages of metal-induced stress. Highest LAI (0.14 and 0.15 at 40 DAT and at harvest, respectively) was recorded in $Cd_1M_3$. On the other hand, at 40 DAT the lowest LAI (0.04 and) was recorded in $Cd_2M_0$ treatment and at harvest the lowest LAI (0.06) in $Cd_1M_0$ treatment (Table 5).

### 4.1.5 Root length

### 4.1.5.1 Effect of cadmium

Cadmium has high mobility and hydrophilic nature (Pinto *et al*., 2004), so it can be easily taken up by plants by roots. The Shortest root length (17.87 cm) was found by the exogenous application of 25 mg $kg^{-1}$ soil of Cd ($Cd_2$) (Figure 11 and 12) which is 22.37% lower compared to control. Rice being a monocot with fibrous root system that increases surface area for mineral absorption is favoring the chance of uptake of Cd (Coudert *et al*., 2010). Cadmium toxicity restricted rice growth as estimated in terms of root and shoot length, leaf and root area, and number of leaves and roots per plant (Yu *et al*. 2006; Song *et al*., 2015). Cadmium is easily taken up by plant roots and transported to different plant parts, causing various phytotoxic symptoms (Bernard, 2008).




Here, $Cd_0$= $CdCl_2$ 0 $mgkg^{-1}$ soil, $Cd_1$= $CdCl_2$ 10 $mgkg^{-1}$ soil and $Cd_2$= $CdCl_2$ 25 $mgkg^{-1}$ soil

**Figure 11. Effect of cadmium on root length of rice at harvest [LSD $_{(0.05)}$ = 0.98 at harvest]**

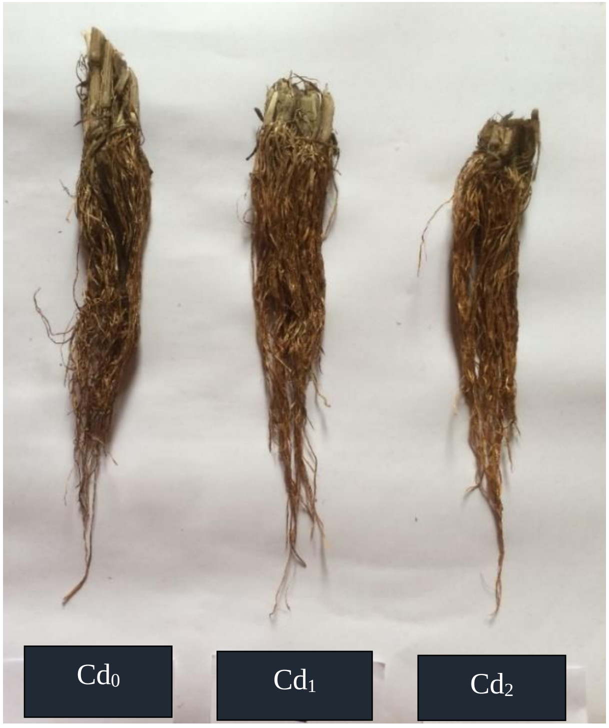
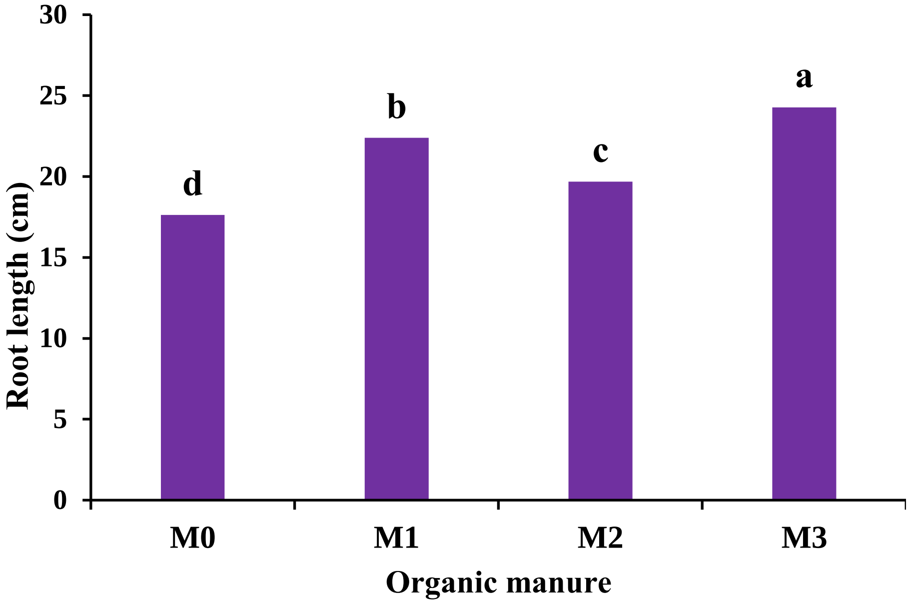



**Figure 12. Phenotypic appearance of root length and root weight of rice for the effect of cadmium at harvest**

## 4.1.5.2 Effect of organic manure

Organic manures create favorable condition for the plant root to uptake nutrients from the soil. In that case Vermicompost played a major role for root development of rice. The longest root (24.27cm) was found from Vermicompost applied pot ($M_3$) which is 22.37% lower compared to control (Figure 13). Plants take nutrients mostly from the soil by using their roots. The optimal growth of plants is not only caused by the total amount of nutrients in the soil but also influenced by physico-chemical-biological properties of soil such as: soil texture, organic matter, cation exchange capacity, pH, electrical conductivity and activity of soil microbes (Bell and Dell, 2008) which helps to develop plant root growth.




Here, $M_0$ = Control, $M_1$= 5 t $ha^{-1}$ Cowdung, $M_2$ = 2.5 t $ha^{-1}$ Wood ash and $M_3$= 4 t $ha^{-1}$ Vermicompost

**Figure 13. Effect of organic manure on root length of rice at harvest [LSD $_{(0.05)}$ = 1.14 at harvest]**


**Figure 14. Phenotypic appearance of root length and root weight of rice under the effect of cadmium at harvest**

### 4.1.5.3 Combined effect of Cd and organic manure

Organic manure can be a way to minimize the Cd toxicity to a certain level. Exogenous application of Cd created stress condition to the plant but was considerably recovered by the application of organic manure (Table 6). Vermicompost played a good role for the reduction of toxic effect of Cd on root length. It gives longest root length (28.14cm) in $Cd_1M_3$ (10 $mgkg^{-1}$ soil of Cd with 4 t $ha^{-1}$ Vermicompost) treatment, which is 36.60 % higher than the control condition. On the other hand, shortest root length (13.43cm) was found in $Cd_1M_0$ (25 $mgkg^{-1}$ soil of Cd with control) which is 34.32% lower compared to control.

**Table 6. Root length and root weight as influenced by Cd and organic manure**

| Treatments | Root length (cm) | Root weight(g) |
|---|---|---|
| $Cd_0M_0$ | 20.45 e-g | 13.68 de |
| $Cd_0M_1$ | 23.85 bc | 15.33 c |
| $Cd_0M_2$ | 22.68 cd | 11.63 fg |
| $Cd_0M_3$ | 25.10 b | 19.03 a |
| $Cd_1M_0$ | 18.94 g | 10.86 gh |
| $Cd_1M_1$ | 21.78 de | 17.56 b |
| $Cd_1M_2$ | 19.38 g | 12.95 ef |
| $Cd_1M_3$ | 28.14 a | 14.67 cd |
| $Cd_2M_0$ | 13.45 i | 9.76 h |
| $Cd_2M_1$ | 21.51 d-f | 13.68 de |
| $Cd_2M_2$ | 16.96 h | 10.97 gh |
| $Cd_2M_3$ | 19.58 fg | 12.55 ef |
| $LSD_{0.5}$ | 1.97 | 1.32 |
| CV (%) | 6.55 | 6.78 |

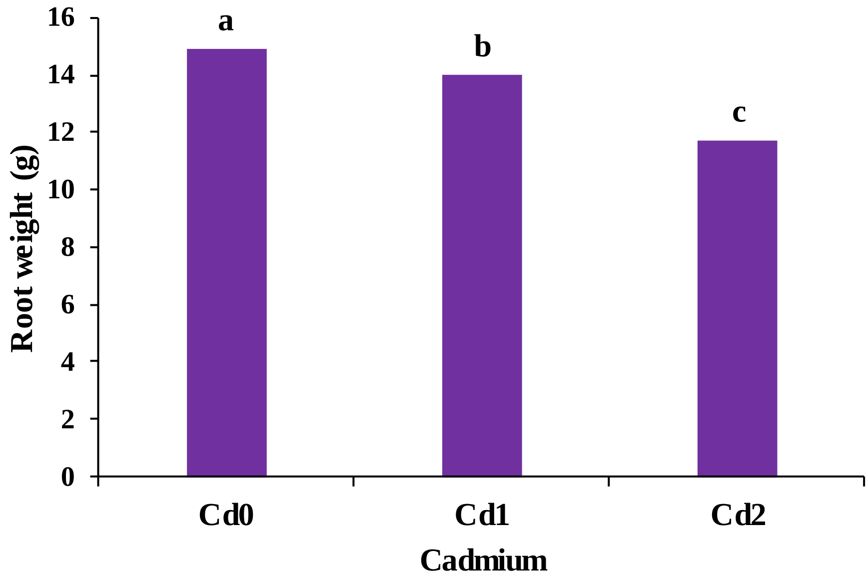
$Cd_0$= $CdCl_2$ 0 $mgkg^{-1}$ soil
$Cd_1$= $CdCl_2$ 10 $mgkg^{-1}$ soil
$Cd_2$= $CdCl_2$ 25 $mgkg^{-1}$ soil

$M_0$= Control
$M_1$= 5 t $ha^{-1}$ Cowdung
$M_2$= 2.5 t $ha^{-1}$ Wood ash
$M_3$= 4 t $ha^{-1}$ Vermicompost

## 4.1.6 Root Weight

### 4.1.6.1 Effect of cadmium

Cadmium affects the dry matter content of plant. The lowest root weight (11.74g) was recorded with 25 $mgkg^{-1}$ soil of Cd ($Cd_2$) (Figure 15) which is 21.31% lower than control. Cadmium toxicity restricted rice growth as estimated in terms of root and shoot length, leaf and root area, and number of leaves and roots per plant (Yu *et al.*, 2006; Song *et al.*, 2015).

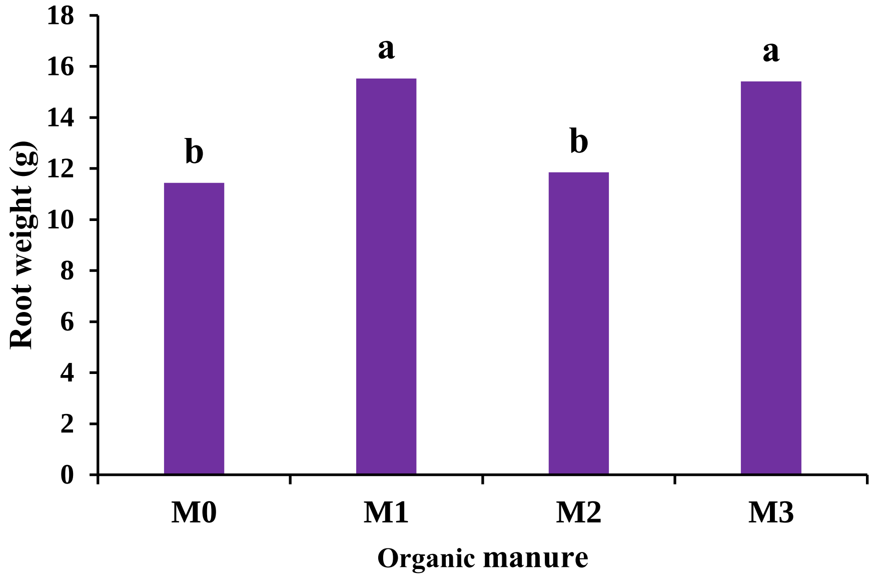



Here, $Cd_0$ = $CdCl_2$ 0 $mgkg^{-1}$ soil, $Cd_1$ = $CdCl_2$ 10 $mgkg^{-1}$ soil and $Cd_2$ = $CdCl_2$ 25 $mgkg^{-1}$ soil

**Figure 15. Effect of cadmium on root weight of rice at harvest [LSD $_{(0.05)}$ = 0.66 at harvest]**

### 4.1.5.2 Effect of organic manure

The highest root weight of rice (15.52 g) was recorded with Cowdung ($M_1$) and similar result (15.41g) with Vermicompost ($M_3$) which is 35.78% and 34.82% higher compared to control. On the contrary, the lowest root weight (11.43g) was recorded in control condition following 11.85g with Wood-ash (Figure 16). The optimal growth of plants is not only caused by the total amount of nutrients in the soil but also influenced by physico-chemical-biological properties of soil such as: soil texture, organic matter, cation exchange capacity, pH, electrical conductivity and activity of soil microbes (Bell and Dell, 2008) which helps to develop plant root growth.




Here, $M_0$ = Control, $M_1$= 5 t $ha^{-1}$ Cowdung, $M_2$ = 2.5 t $ha^{-1}$ Wood ash and $M_3$= 4 t $ha^{-1}$ Vermicompost

**Figure 16. Effect of organic manure on root weight of rice at harvest [LSD $_{(0.05)}$ = 0.76 at harvest]**

### 4.1.5.3 Combined effect of Cd and organic manure

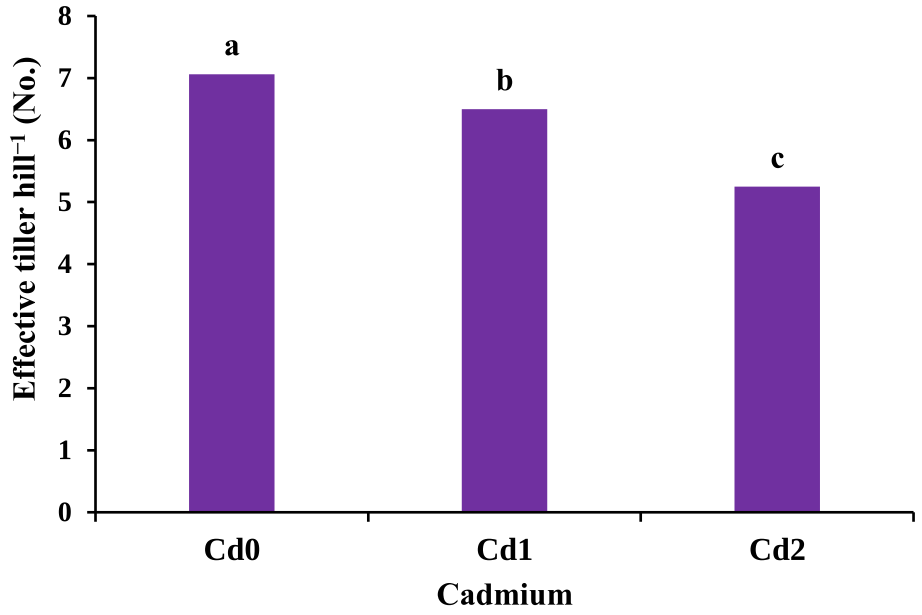
The highest root weight (19.03g) was recorded in the combination of $Cd_0M_3$ (0 $mgkg^{-1}$ soil of Cd with 4 t $ha^{-1}$of Vermicompost) which is 39.07% higher compared to control.The lowest root weight (9.76g) was found in the combination of $Cd_2M_0$ (25 $mgkg^{-1}$ soil of Cd without any organic manure) treatment (Table 6) which is 28.65% lower than the control condition.

## 4.2 Yield parameter

### 4.2.1 Effective tillers $hill^{-1}$

#### 4.2.1.1 Effect of cadmium

Exogenous application of Cd decreased effective tillers in rice. The lowest number of effective tillers per hill (5.25) was recorded by the application of Cd ($Cd_2$) which is 25.63% lower than control. Cadmium accumulates differentially in various parts of the plant (Gao *et al*., 2011) and hampers the yield of crop.

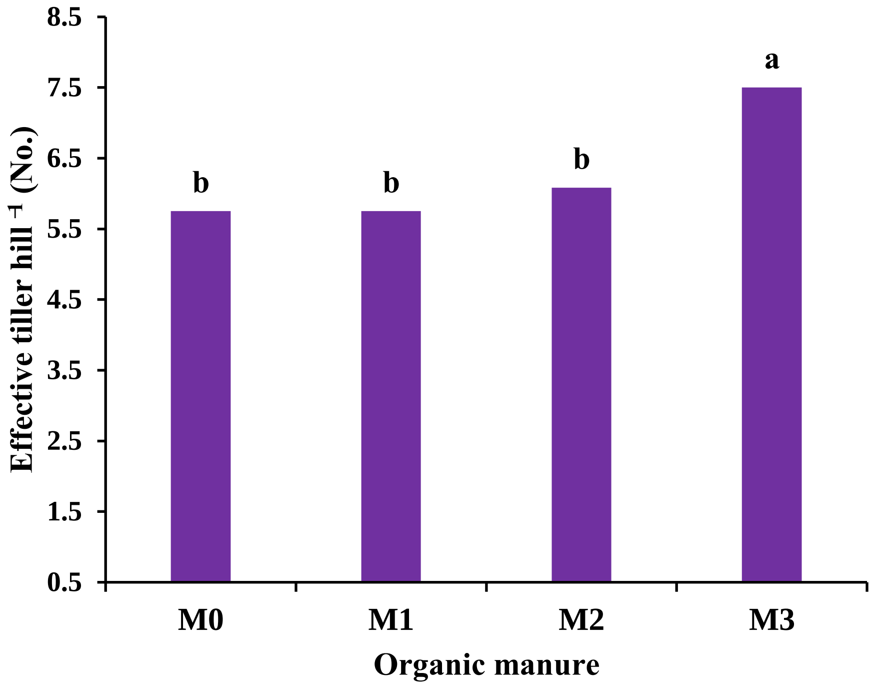



Here, $Cd_0$= $CdCl_2$ 0 $mgkg^{-1}$ soil, $Cd_1$= $CdCl_2$ 10 $mgkg^{-1}$ soil and $Cd_2$= $CdCl_2$ 25 $mgkg^{-1}$ soil

**Figure 17. Effect of cadmium on effective tillers of rice at harvest [LSD $_{(0.05)}$ = 0.52 at harvest]**

#### 4.2.1.2 Effect of organic manure

Application of different types of organic manure significantly influenced the number of effective tillers per hill. The highest number of effective tillers per hill (7.50) was observed in Vermicompost applied in pots. On the other hand, the lowest number of effective tillers (5.75) was recorded in control plant which was statistically similar with$M_1$ and $M_2$ treatments (Figure 14). The vermicompost promotes growth from 50-100% over conventional compost and 30-40% over chemical fertilizers (Sinha *et al.*, 2010)




Here, $M_0$= Control, $M_1$= 5 t $ha^{-1}$ Cowdung, $M_2$= 2.5 t $ha^{-1}$ Wood ash and $M_3$= 4 t $ha^{-1}$ Vermicompost

**Figure 18. Effect of organic manure on effective tillers of rice at harvest [LSD $_{(0.05)}$ = 0.60 at harvest]**

### 4.2.1.3 Combined effect of Cd and organic manure

Interaction of Cd and organic manures affected the number of effective tillers per hill. In the combination of Cd and organic manure the highest number of effective tillers (8.75) was found in $Cd_0M_3$ (0 $mgkg^{-1}$ soil of Cd with 4 t $ha^{-1}$of Vermicompost) treatment which is 29.62% higher than the control condition. Alternatively, the lowest number of effective tillers (4.75) was recorded in $Cd_2M_0$ (25 $mgkg^{-1}$ soil of Cd without any organic manure) which is 29.62% lower compared to control (Table 7).

**Table 7. Yield contributing parameter of rice as influenced by cadmium and organic manure**

| Treatments | Effective tillers hill$^{-1}$ (No.) | Panicle length (cm) | Filled grains pot$^{-1}$ (No.) | Total grains pot$^{-1}$ (No.) | 1000-grain wt. (g) |
|---|---|---|---|---|---|
| $Cd_0M_0$ | 6.75 b-d | 20.14 b-d | 455.75 ab | 515.50 a | 20.80 |
| $Cd_0M_1$ | 6.50 c-e | 20.65 a-c | 460.50 a | 515.75 a | 21.47 |
| $Cd_0M_2$ | 6.25 c-f | 20.14 b-d | 461.00 a | 510.50 a | 21.80 |
| $Cd_0M_3$ | 8.75 a | 21.09 ab | 462.50 a | 517.00 a | 22.09 |
| $Cd_1M_0$ | 5.75 d-g | 17.81 ef | 329.25 e | 408.75 cd | 18.33 |
| $Cd_1M_1$ | 5.25 f-g | 21.93 a | 402.00 cd | 456.50 b | 20.02 |
| $Cd_1M_2$ | 7.75 ab | 19.81 b-d | 389.75 d | 454.50 b | 20.09 |
| $Cd_1M_3$ | 7.25 bc | 20.40 a-c | 427.00 bc | 467.25 b | 20.07 |
| $Cd_2M_0$ | 4.75 gh | 16.94 f | 214.00 f | 307.25 e | 17.96 |
| $Cd_2M_1$ | 5.50 e-g | 18.45 d-f | 329.00 e | 378.75 d | 19.75 |
| $Cd_2M_2$ | 4.25 h | 19.06 c-e | 329.50 e | 407.00 cd | 19.76 |
| $Cd_2M_3$ | 6.50 c-e | 20.13 b-d | 352.00 e | 416.50 c | 19.60 |
| $LSD_{0.5}$ | 1.04 | 1.72 | 29.64 | 33.02 | NS |
| CV (%) | 11.56 | 7.43 | 6.07 | 5.14 | 3.90 |

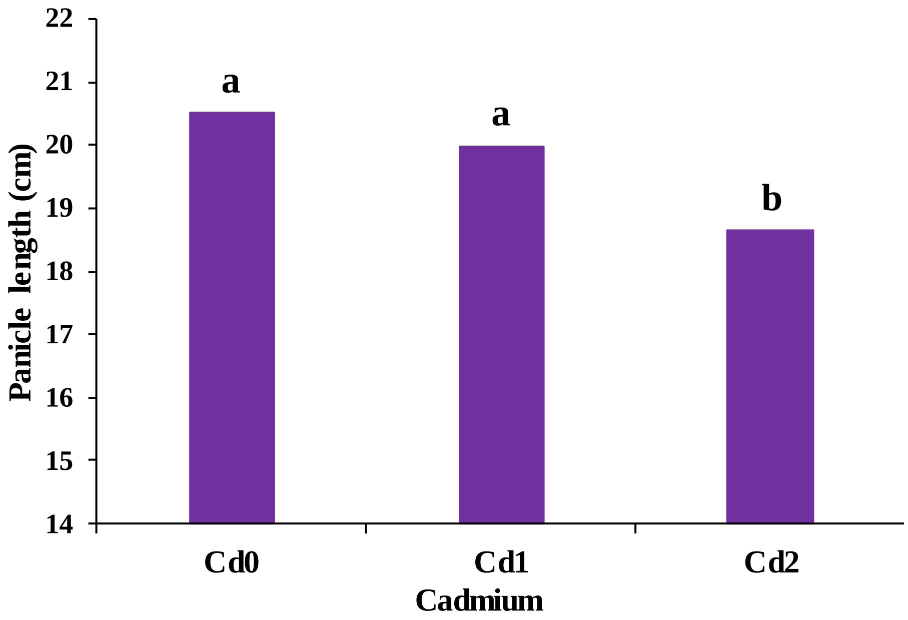
$Cd_0$= $CdCl_2$ 0 mgkg$^{-1}$ soil
$Cd_1$= $CdCl_2$ 10 mgkg$^{-1}$ soil
$Cd_2$= $CdCl_2$ 25 mgkg$^{-1}$ soil

$M_0$= Control
$M_1$= 5 t ha$^{-1}$ Cowdung
$M_2$= 2.5 t ha$^{-1}$ Wood ash
$M_3$= 4 t ha$^{-1}$ Vermicompost

## 4.2.2 Panicle length

### 4.2.2.1 Effect of Cadmium

Panicle with filled grain indicates the higher yield of the plant. In this experiment, the shortest panicle length (18.64cm) was recorded in $Cd_2$ treatment (Figure 15) which is 9.07% lower compared to control. Cadmium greatly affects the ultimate yield of a crop and the crop yield declines due to inhibition of metabolic processes (Singh *et al.,* 2010).

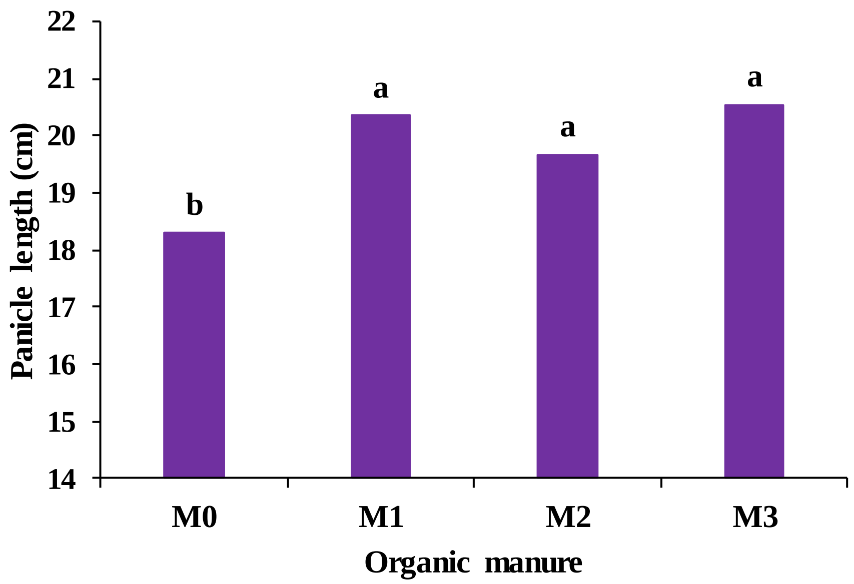



Here, $Cd_0$= $CdCl_2$ 0 $mgkg^{-1}$ soil, $Cd_1$= $CdCl_2$ 10 $mgkg^{-1}$ soil and $Cd_2$= $CdCl_2$ 25 $mgkg^{-1}$ soil

**Figure 19. Effect of cadmium on panicle length of rice at harvest [LSD $_{(0.05)}$ = 0.86 at harvest]**

### 4.2.2.2 Effect of organic manure

Organic manure is a positive element for the crop yield development. In this experiment, Vermicompost played a good role for the development of panicle length. The longest panicle (20.54cm) recorded from Vermicompost ($M_3$) applied pot compared to other organic manure such as wood ash and cowdung (Figure 20). Wood ash and cowdung gives almost similar result as control condition. Vermicompost showed 12.24% longer panicle length compared to control condition.




Here, $M_0$= Control, $M_1$= 5 t ha$^{-1}$ Cowdung, $M_2$= 2.5 t ha$^{-1}$ Wood ash and $M_3$= 4 t ha$^{-1}$ Vermicompost

**Figure 20. Effect of organic manure on panicle length of rice at harvest [LSD $_{(0.05)}$ = 1.0 at harvest]**

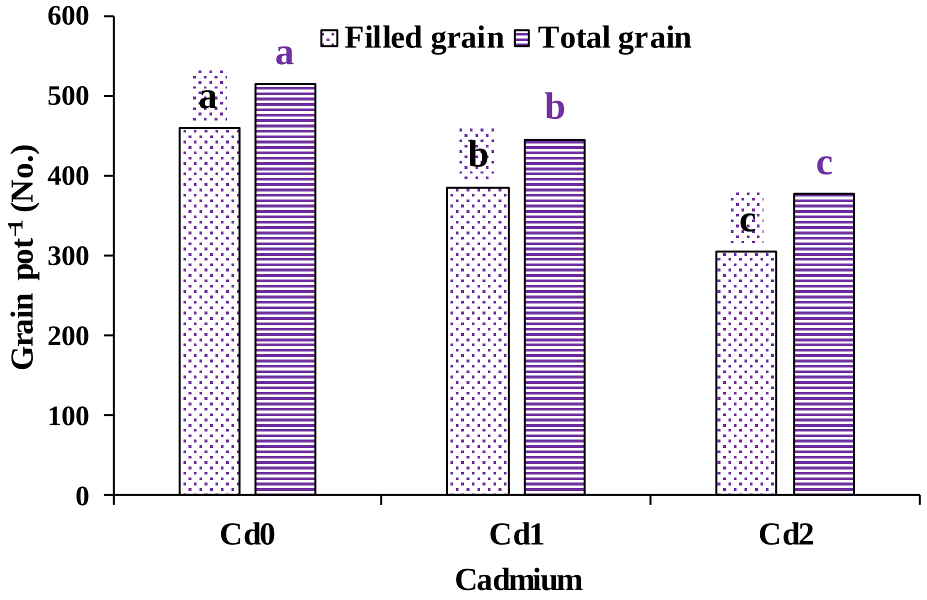
### 4.2.2.3 Combined effect of Cd and organic manure

Interaction of Cd and organic manures showed variation in panicle length (Table 5). The longest panicle length (21.93cm) was recorded from$Cd_1M_1$ treatment which is 8.88% higher than control condition ($Cd_0M_0$). The shortest panicle length (16.94cm) was also recorded in $Cd_2M_0$ (Table 7) which is 15.88% lower than control ($Cd_0M_0$).

### 4.2.3 Filled grains pot$^{-1}$

#### 4.2.3.1 Effect of cadmium

Cadmium accumulation into the plant affect severely to the yield contributing parameters. Severe exposure of Cd (25 mgkg$^{-1}$ soil of Cd or $Cd_2$) caused severe damage and gave the lowest number of filled grain per pot (306.13) which is 33.44% lower than the control condition ($Cd_0M_0$) (Figure 21). Impairments in reproductive development were identified in reducing the yield components and yield, which included reduced pollen germination, fruit set, and seed set (Sabrine *et al.*, 2010).

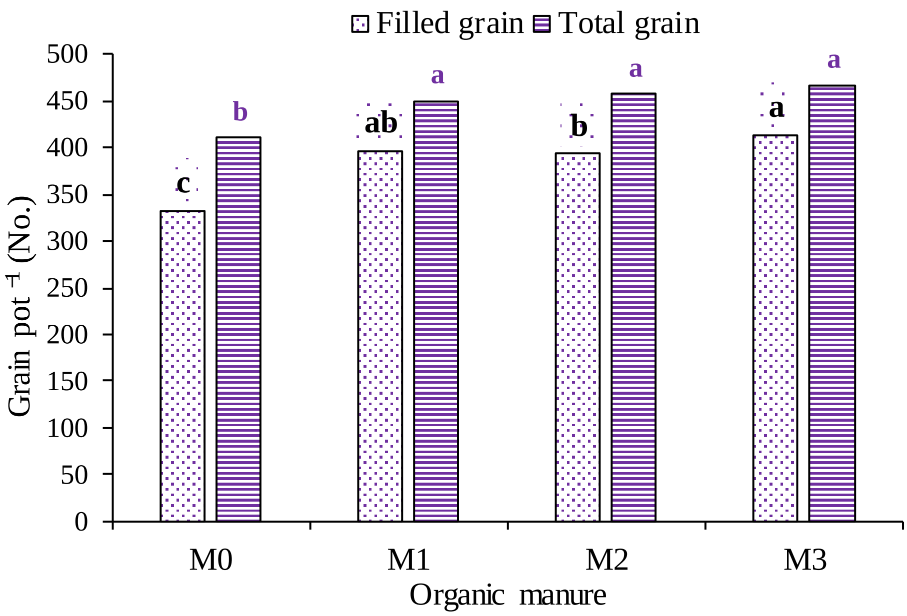



Here, $Cd_0$= $CdCl_2$ 0 mgkg$^{-1}$ soil, $Cd_1$= $CdCl_2$ 10 mgkg$^{-1}$ soil and $Cd_2$= $CdCl_2$ 25 mgkg$^{-1}$ soil

**Figure 21. Effect of cadmium on number of grains of rice at harvest [LSD $_{(0.05)}$ = 14.82 and 16.51 at harvest]**

#### 4.2.3.2 Effect of organic manure

The number of filled grain per pot varied by the application of different types of organic manures. The highest number of filled grain (413.83) was found in Vermicompost ($M_3$) applied pot which is 24.27% highest than control. Whereas the lowest number of filled grain per pot (333) was recorded in control condition (Figure 22). Vermicompost have a positive effect on physicochemical properties of soil hence improving growth and yield of crop by upgrading yield contributing parameters (Atiyeh *et al.*, 2000).




Here, $M_0$= Control, $M_1$= 5 t $ha^{-1}$ Cowdung, $M_2$= 2.5 t $ha^{-1}$ Wood ash and $M_3$= 4 t $ha^{-1}$ Vermicompost

**Figure 22. Effect of organic manure on number of grain of rice at harvest [LSD $_{(0.05)}$ = 17.11 at harvest]**

### 4.2.3.3 Combined effect of Cd and organic manure

Simultaneous application of Cd and organic manures affect the number of filled grain per pot of rice. The highest number of filled grain per pot (462.50) was recorded from $Cd_0M_3$ treatment which is similar with $Cd_0M_1$, and $Cd_0M_2$ treatments (Table 8). The lowest number of filled grain per pot (214) was recorded in $Cd_2M_0$ treatment which is 53.04% lower compared to control condition ($Cd_0M_0$).

### 4.2.4 Total grains hill$^{-1}$

### 4.2.4.1 Effect of cadmium

Severe dose of Cd confirmed lower grain development to the crop. The highest number of total grain per pot (514.69) was recorded in control condition ($Cd_0$) and the lowest number of grain per pot (377.38) was observed in 25 mg $kg^{-1}$soil of Cd-treated pot ($Cd_2$) which is 26.67% lower than the control (Figure 21).

#### 4.2.4.2 Effect of organic manure

Application of organic manures increased the total number of grains per pot compared with control. Vermicompost treated pot gave highest number of grains per pot (466.92) which is 13.74% higher than control (Figure 22). However, vermicompost, cowdung and wood-ash applied pot showed similar results.

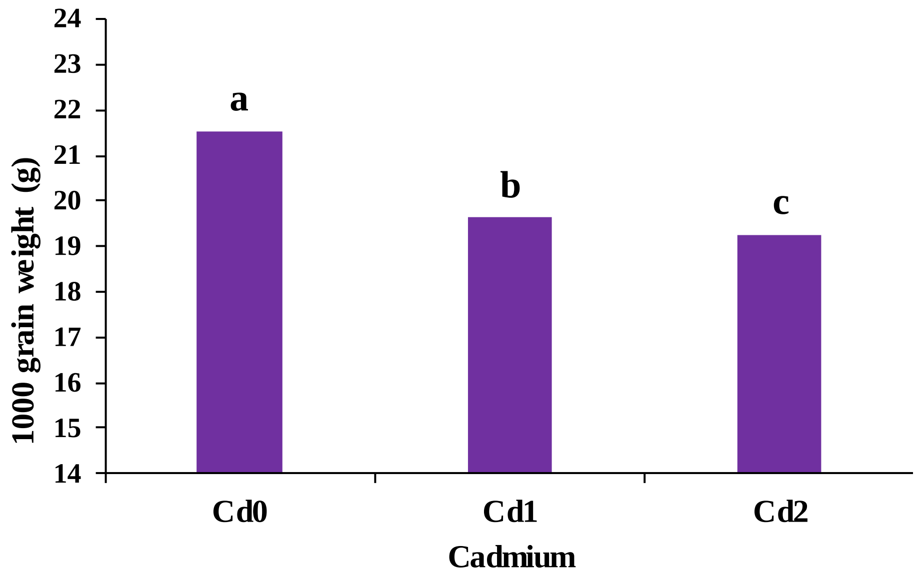
#### 4.2.4.3 Combined effect of Cd and organic manure

Combination of Cd and organic manures significantly influenced the total number of grains per pot. The highest number of total grains per pot were recorded from $Cd_0M_0$, $Cd_0M_1$, $Cd_0M_2$ and $Cd_0M_3$ (Table 7) treatments. The lowest number of total grains per pot (307.25) was recorded from $Cd_2M_0$ (25 mgkg$^{-1}$ soil of Cd with no organic manure) treatment.

### 4.2.5 1000-grain wt.

#### 4.2.5.1 Effect of cadmium

Cadmium adversely affect the grain yield, as in this experiment, higher dose of Cd ($Cd_2$) resulted the lowest 1000-grain weight which is 19.27g and it is 10.53% lower than the control condition (Figure 23). Seed production stage is highly sensitive to Cd, so due to high dose of Cd exposure Cd could transfer to rice grains and decreased grain yield, quality, and nutrient uptake (Arao and Ae, 2003; He *et al*., 2006; Liu *et al*., 2007; Rodda *et al*., 2011; Li *et al*., 2012a, b)

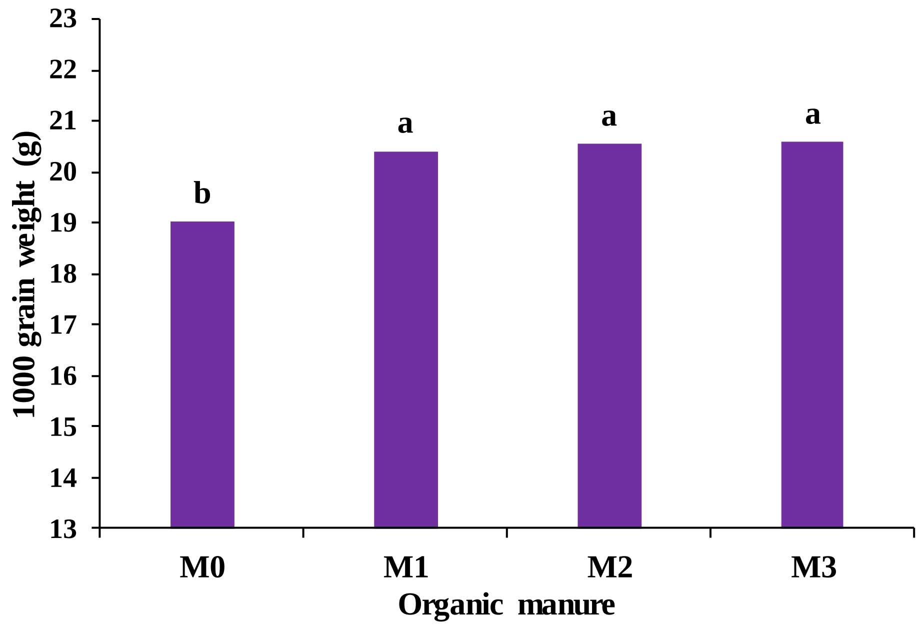



Here, $Cd_0$ = $CdCl_2$ 0 $mgkg^{-1}$ soil, $Cd_1$= $CdCl_2$ 10 $mgkg^{-1}$ soil and $Cd_2$ = $CdCl_2$ 25 $mgkg^{-1}$ soil

**Figure 23. Effect of cadmium on 1000 grain weight of rice at harvest [LSD $_{(0.05)}$ = 0.56 at harvest]**

### 4.2.5.2 Effect of organic manure

Addition of different types of organic manures influenced 1000-grain weight of rice. Almost similar results were found by the application of Cowdung (20.41 g), Woodash (20.55 g) and Vermicompost (20.59) which are 7.25%, 7.98% and 8.19% higher, respectively, compared to control condition (Figure 24).




Here, $M_0$= Control, $M_1$= 5 t $ha^{-1}$ Cowdung, $M_2$= 2.5 t $ha^{-1}$ Wood ash and $M_3$= 4 t $ha^{-1}$ Vermicompost

**Figure 24. Effect of organic manure on 1000 grain weight of rice at harvest [LSD $_{(0.05)}$ = 0.65 at harvest]**

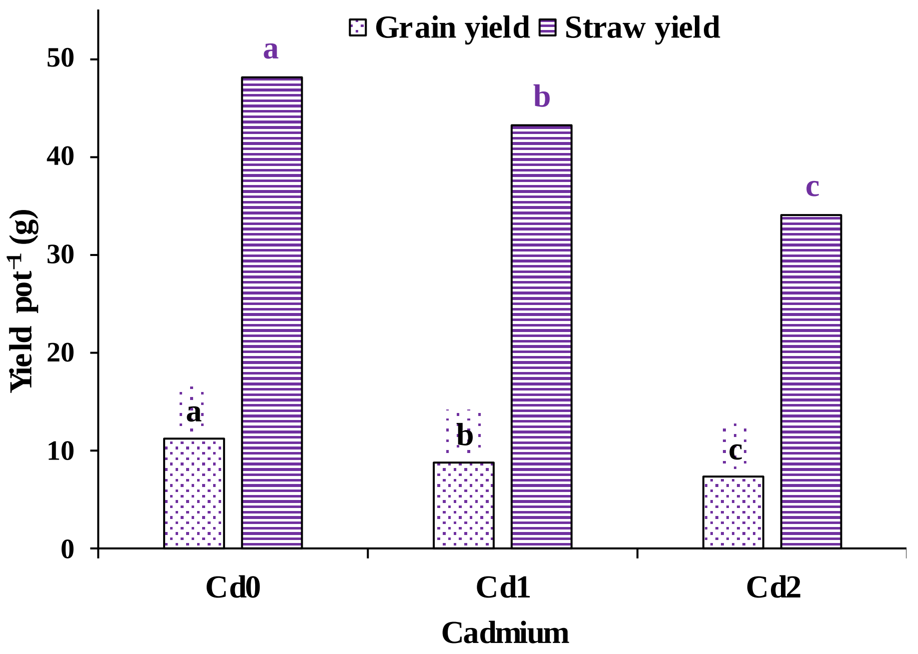
### 4.2.5.3 Combined effect of cadmium and organic manure

In case of combination of Cd dose and organic manure, higher amount of 1000-grain weight was observed in $Cd_0M_3$ treatment which is 0 $mgkg^{-1}$ soil of Cd withvermicompost which is 6.20% higher compared to control ($Cd_0M_0$). On the other hand, two treatments ($Cd_2M_0$ and $Cd_1M_0$) showed lower amount of 1000-grain weight which is 17.96g and 18.33g (Table 7) and show 13.65% and 11.87% lower, respectively, compared to control.

### 4.2.6 Grain yield $pot^{-1}$

### 4.2.6.1 Effect of cadmium

Higher levels of Cd create unfavorable condition to the crop. The highest grain yield per pot (11.21g) was observed in control pot (0 $mgkg^{-1}$ soil of Cd) whereas the lowest grain yield (7.34g) was recorded in $Cd_2$(25$mgkg^{-1}$ soil of Cd) (Figure 25) which is 34.52% lower grain weight compared to control. The contents of starch, protein, fat in grain and kernel, as well as their contents vary differentially by crop, including *O. sativa* due to Cd stress. (Wang *et al*., 1993).

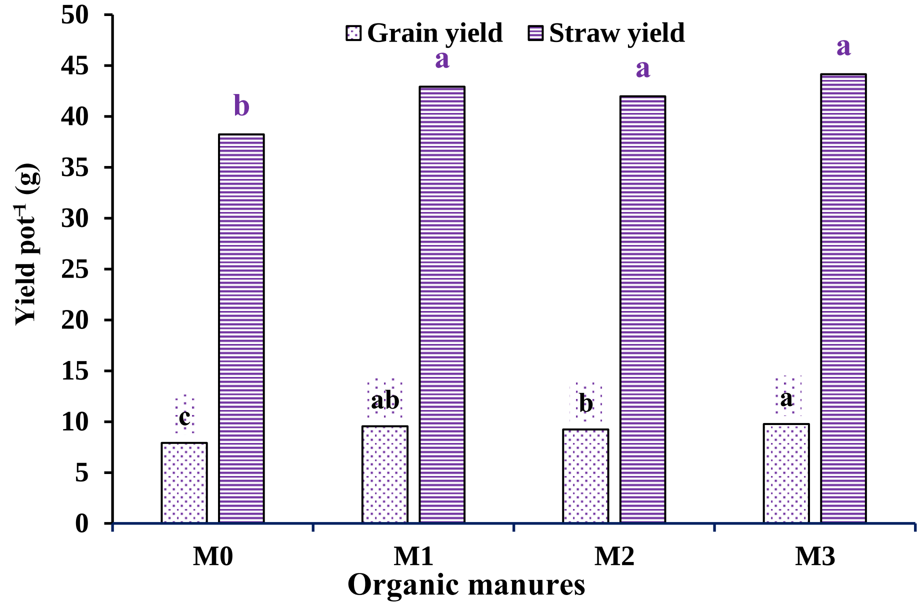



Here, $Cd_0$= $CdCl_2$ 0 $mgkg^{-1}$ soil, $Cd_1$= $CdCl_2$ 10 $mgkg^{-1}$ soil and $Cd_2$= $CdCl_2$ 25 $mgkg^{-1}$ soil

**Figure 25. Effect of cadmium on yield of rice at harvest [LSD $_{(0.05)}$ = 0.38 and 2.19 at harvest]**

### 4.2.6.2 Effect of organic manure

Higher grain yield (9.77g) was recorded in vermicompost ($M_3$) applied pot which is 23.67% higher than the control (Figure 26). Vermicompost permanently enhances biological activity which can be used for the improvement of seed germination, flowering, growth and yield (Atiyeh *et al*. 2000).




Here, $M_0$= Control, $M_1$= 5 t $ha^{-1}$ Cowdung, $M_2$= 2.5 t $ha^{-1}$ Wood ash and $M_3$= 4 t $ha^{-1}$ Vermicompost

**Figure 26. Effect of organic manure on yield of rice at harvest [LSD (0.5) = 0.45 and 2.54 at harvest]**

### 4.2.6.3 Combined effect of cadmium and organic manure

Statistically non-significant result was observed in the interaction effect between Cd and organic manure. However, higher grain yield (11.62g) was attained in $Cd_0M_3$ (0 $mgkg^{-1}$ soil of Cd with 4 t $ha^{-1}$ of vermicompost) which is 8.49% higher than control ($Cd_0M_0$).The lowest grain yield per pot (5.52g) was observed in $Cd_2M_0$ (25 $mgkg^{-1}$ soil of Cd with no organic manures) which is 48.45% lower compared to control($Cd_0M_0$) (Table 8).

**Table 8. Yield of rice as influenced by Cadmium and organic manure**

| Treatments | Grain yield $pot^{-1}$ (g) | Straw yield $pot^{-1}$ (g) |
|---|---|---|
| $Cd_0M_0$ | 10.71 b | 46.46 |
| $Cd_0M_1$ | 11.42 ab | 49.35 |
| $Cd_0M_2$ | 11.08 ab | 46.13 |
| $Cd_0M_3$ | 11.62 a | 50.56 |
| $Cd_1M_0$ | 7.48 e | 41.08 |
| $Cd_1M_1$ | 9.24 c | 43.94 |
| $Cd_1M_2$ | 8.90 cd | 43.57 |
| $Cd_1M_3$ | 9.52 c | 44.27 |
| $Cd_2M_0$ | 5.52 f | 27.19 |
| $Cd_2M_1$ | 7.95 e | 35.44 |
| $Cd_2M_2$ | 7.72 e | 36.27 |
| $Cd_2M_3$ | 8.16 de | 37.60 |
| $LSD_{0.5}$ | 0.77 | NS |
| CV (%) | 5.90 | 7.31 |

$Cd_0$= $CdCl_2$ 0 $mgkg^{-1}$ soil
$Cd_1$= $CdCl_2$ 10 $mgkg^{-1}$ soil
$Cd_2$= $CdCl_2$ 25 $mgkg^{-1}$ soil

$M_0$= Control
$M_1$= 5 t $ha^{-1}$ Cowdung
$M_2$= 2.5 t $ha^{-1}$ Wood ash
$M_3$= 4 t $ha^{-1}$ Vermicompost

## 4.2.7 Straw yield $pot^{-1}$

### 4.2.7.1 Effect of cadmium

The higheststraw yield per pot (48.12g) was recorded in $Cd_0$ (0 $mgkg^{-1}$ soil of Cd) and the lowest straw yield (34.12g) was recorded in $Cd_2$ (25 $mgkg^{-1}$ soil of Cd) which is 29.09% lower compared to control (Figure 26). Cadmium-induced stress confirmed accumulation of Cdin different parts of plant which decrease growth and yield (Gao *et al.*, 2011).

### 4.2.7.2 Effect of organic manure

Application of organic manures increased straw yield of rice (Figure 26). Application of Cowdung, Wood-ash and Vermicompost gave straw yield per pot42.91, 41.99 and 44.14g, respectively which are statistically similar. However control pot provided the lowest straw yield (38.24 g) which is 12, 9.80 and 15.4% lower compared to Cowdung, woodash and vermicompost, respectively (Figure 26).

### 4.2.7.3 Combined effect of cadmium and organic manure

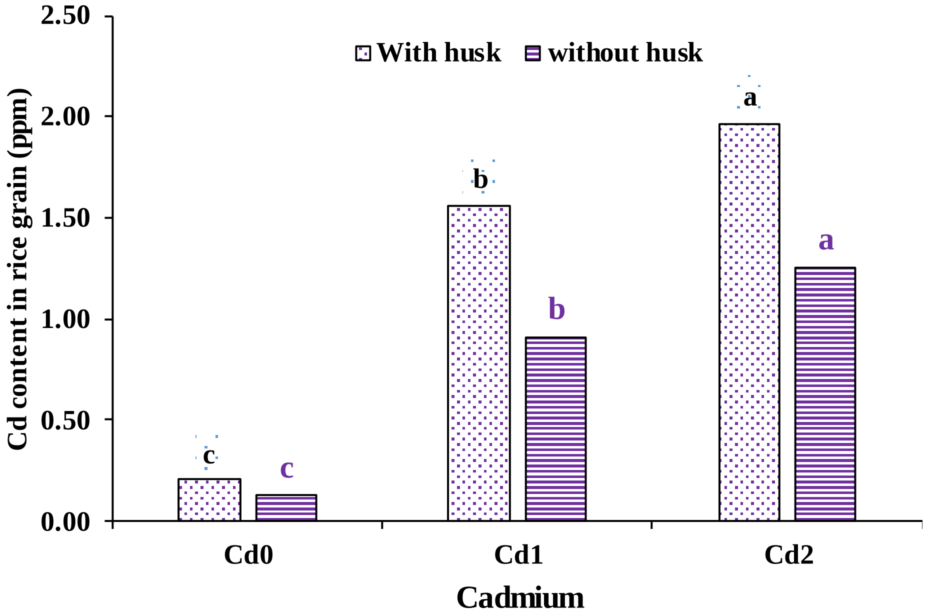
Combination of Cd and organic manures showed variation in straw yield of rice. In the combination of Cd and Vermicompost, the highest straw yield per pot (50.56g) was recorded in $Cd_0M_3$ treatment which is 8.82% higher than the control ($Cd_0M_0$). The lowest straw yield 35.44 and 36.27g was recorded from $Cd_2M_1$ and $Cd_2M_2$ treatment, respectively (Table 9).

## 4.3 Chemical Analysis

### 4.3.1 Cadmium content in rice grain (With Husk)

### 4.3.1.1 Effect of cadmium

Exposure of Cd caused Cd accumulation in rice grains. The highest Cd content (1.96 ppm) was recorded by the application of 25mgkg$^{-1}$ soil of Cd ($Cd_2$) which is 89.79% higher compared to control (Figure 27). Cadmium accumulates differentially in various parts of the plant (Shekar *et al.*, 2011).

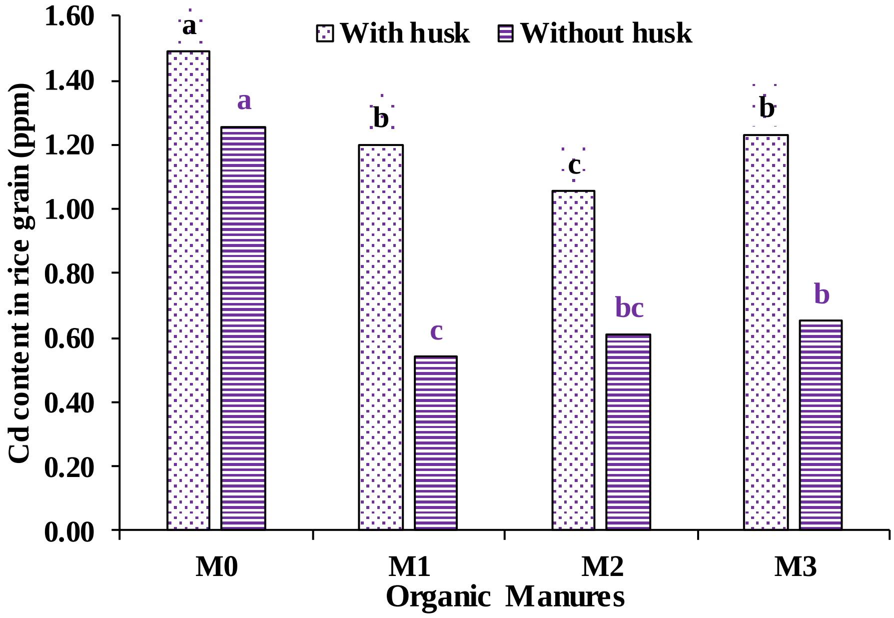



Here, $Cd_0$= $CdCl_2$ 0 mgkg$^{-1}$ soil, $Cd_1$= $CdCl_2$ 10 mgkg$^{-1}$ soil and $Cd_2$= $CdCl_2$ 25 mgkg$^{-1}$ soil

**Figure 27. Effect of cadmium on cadmium content of rice grain [LSD $_{(0.05)}$ = 0.07 and 0.06 at harvest]**

### 4.3.1.2 Effect of organic manure

The highest amount of Cd content (1.49ppm) was recorded in $M_0$ (Control) and on the other hand lower amount of Cd content (1.05 ppm) was recorded in wood ash- treated pot ($M_2$) which is 29.53% lower compared to control (Figure 28).




Here, $M_0$= Control, $M_1$= 5 t $ha^{-1}$ Cowdung, $M_2$= 2.5 t $ha^{-1}$ Wood ash and $M_3$= 4 t $ha^{-1}$ Vermicompost

**Figure 28. Effect of organic manure on cadmium content of rice grain [$LSD_{(0.05)}$ = 0.08 and 0.07 at harvest]**

### 4.3.1.3 Combined effect of cadmium and organic manure

Interaction of Cd and organic manures showed variation in Cd accumulation in rice grains. In the interaction, as expectedly the highest amount of Cd (2.29 ppm) was found in $Cd_2M_0$ (25 $mgkg^{-1}$ soil of Cd with control). In severe Cd-stressed condition ($Cd_2$) application of cowdung, wood ash and vermicompost decreased Cd accumulation by 60, 61 and 60%, respectively, compared to control (Table 9).

**Table 9. Interaction effect of Cadmium and organic manure on Cd content of rice grain**

| Treatments | Cd content in rice grain (ppm) | |
|---|---|---|
| | Rice grain with husk | Rice grain Without husk |
| $Cd_0M_0$ | 0.00 g | 0.00 i |
| $Cd_0M_1$ | 0.24 f | 0.08 hi |
| $Cd_0M_2$ | 0.26 f | 0.14 h |
| $Cd_0M_3$ | 0.31f | 0.28 g |
| $Cd_1M_0$ | 1.89 b | 1.47 b |
| $Cd_1M_1$ | 1.46 d | 0.63 f |
| $Cd_1M_2$ | 1.22 e | 0.79 de |
| $Cd_1M_3$ | 1.66 c | 0.76 e |
| $Cd_2M_0$ | 2.57 a | 2.29 a |
| $Cd_2M_1$ | 1.90 b | 0.91 c |
| $Cd_2M_2$ | 1.68 c | 0.90 cd |
| $Cd_2M_3$ | 1.72 c | 0.91 c |
| $LSD_{0.5}$ | 0.14 | 0.12 |
| CV (%) | 8.07 | 11.10 |

$Cd_0$= $CdCl_2$ 0 $mgkg^{-1}$ soil
$Cd_1$= $CdCl_2$ 10 $mgkg^{-1}$ soil
$Cd_2$= $CdCl_2$ 25 $mgkg^{-1}$ soil

$M_0$= Control
$M_1$= 5 t $ha^{-1}$ Cowdung
$M_2$= 2.5 t $ha^{-1}$ Wood ash
$M_3$= 4 t $ha^{-1}$ Vermicompost

## 4.3.2 Cd content in rice grain (Without husk)

### 4.3.2.1 Effect of cadmium

The highest amount of Cd content in rice (without husk) (1.25 ppm) was recorded in $Cd_2$and the lowest Cd content (0.13 ppm) was recorded in $Cd_0$ (Figure 27). Due to Cd toxicity, the contents of starch, protein, fat in grain and kernel, as well as their contents vary differentially by crop, including *O. sativa* (Wang *et al.,* 1993)

### 4.3.2.2 Effect of organic manure

The highest amount of Cd content (1.25 ppm) in rice grain (without husk) was found in $M_0$ (Control). On the other hand, the lowest amount of Cd content (0.54 ppm) was recorded in $M_1$ (Figure 28).

#### 4.3.2.3 Combined effect of cadmium and organic manure

The highest amount of Cd (2.29 ppm) in rice grain (without husk) was recorded in $Cd_2M_0$. Alternatively, the lowest amount of Cd (0.0075 ppm) in $Cd_0M_0$ (Table 9) treatments. Organic manure and its amendments play a key role to ameliorate the metal toxicity (Escobar *et al*. 2008).

# Chapter V

# SUMMARY AND CONCLUSION

## 5.1 Summery

The pot experiment was assessed at Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period of July 2018 to December 2018 to evaluate the amelioration of Cd toxicity in rice by using Organic manure. This experiment was composed of two factors. Factor A: Cadmium (Cd) concentration: 1. $CdCl_2$ 0 $mgkg^{-1}$ soil ($Cd_0$) 2. $CdCl_2$ 10 $mgkg^{-1}$ soil ($Cd_1$) 3. $CdCl_2$ 25 $mgkg^{-1}$ soil ($Cd_2$). Factor B: Organic manure management: 1. Control ($M_0$) 2. Cowdung 5 $tha^{-1}$ ($M_1$) 3. Woodash 2.5$tha^{-1}$ ($M_2$) 4. Vermicompost 4 $tha^{-1}$ ($M_3$).

In case of Cd exposure, plant growth decrease with the increasing dose of Cd. The shortest plant height (60.37, 78.42 and 78.06 cm at 40, 60 DAT and at harvest, respectively) was observed in $Cd_2$. In similar way, the lowest number of tillers and leaves, and leaf area index were recorded for the exposure of 25 $mgkg^{-1}$ soil of $CdCl_2$.

Exposure of Cd greatly reduced the yield and yield contributing parameters of of rice. The lowest number of effective tiller per hill (5.25) was recorded in $Cd_2$. At harvest lower number of filled grain per pot (306.13) was recorded in $Cd_2$.The lowest 1000-grain weight was observed with highest dose of Cd which is 19.27g. Lowest grain yield per pot (7.34g) was recorded in $Cd_2$. Lower straw yield (34.12g) was recorded in $Cd_2$.

Organic manure helps to develop plant growth. Highest plant height (68.25, 85.82 and 85.71cm at 40, 60 DAT and at harvest, respectively) was recorded in $M_3$. The highest number of tillers (3.75, 11.75, 10.75 and 10.41 at 20, 40, 60 DAT and at harvest, respectively) was recorded in $M_3$ .The highest number of leaves per pot (12.81, 30.95 and 30.23) was observed in $M_3$. The highest LAI (0.10 and 0.12) was recorded in $M_3$. The longestroot (24.27cm) of root was found in $M_3$ and highest root weight (15.52g) was recorded in $M_1$(15.41g) and in $M_3$.

All organic manures don't have same ability to contribute to yield development of rice plant. In this experiment vermicompost ($M_3$) played a major role for the development of yield of rice. The highest number of effective tiller per hill (7.50) was observed for the application of vermicompost ($M_3$). The longest panicle length (20.54cm) was recorded in $M_3$. The highest number of filled grain per pot (413.83) was found in $M_3$. The highest 1000-grain weight was found from $M_1$(20.41g), $M_2$(20.55g) and $M_3$(20.59g). The highest grain yield per pot (9.77g) was recorded in $M_3$. The highest, straw yield was found in $M_1$ (2.91g), $M_2$ (41.99g) and ($M_3$)44.14g

In terms of interaction effect, exposure of Cd interacts with organic manures and gave the better results for plant growth and yield development of rice.

The highest plant growth and development was seen by the application of vermicompost with the absence of Cd. The lowest plant height (57.91, 74.80 and 73.78cmat 40 and 60 DAT and at harvest, respectively) was observed in $Cd_2M_0$. Similarly, the lowest value of other growth parameters such as tiller number, leaf area index and root length was recorded in $Cd_2M_0$.

The lowest filled grain per pot (214), 1000-grain weight (17.96 g) lowest grain yield per pot (5.52g) was observed in $Cd_2M_0$.

Cadmium accumulation in rice grain increased with increasing Cd stress. Cadmium content in rice grain (without husk), the highest Cd content (1.25 ppm) was recorded in $Cd_2$ (25 $mgkg^{-1}$ soil of Cd) and the lowest Cd content (0.13 ppm) was recorded in $Cd_0$ (0 $mgkg^{-1}$ soil of Cd). In case of interaction, of the highest Cd content (2.29 ppm) was recorded in $Cd_2M_0$ (25 $mgkg^{-1}$ soil of Cd with control). Alternatively, lower amount of Cd (0.0075 ppm) in $Cd_0M_0$.

## 5.2 Conclusion

Cadmium is widely distributed heavy metal and can be a disastrous pollutant to the rice cultivation. However, plants follow different and organized way to minimize the toxic effect of this pollutant at physiological and organelle level. But in case of higher concentration of Cd into the plant cell, may become impossible to minimize its toxicity. We need to give a solution and try to make possible ways to minimize this problem. So possible ways to minimize this toxic effect according to this experiment is concluded here-

1. Exposure of Cd decreased plant growth and yield and increased Cd accumulation in rice grain
2. The growth and yield of rice decreased with increasing the rate of Cd exposure
3. In terms of growth parameter, Vermicompost played an excellent role to overcome and help crop to tolerate the toxicity of Cd.
4. In terms of yield parameter, Vermicompost showed its ability for increasing yield.
5. In the chemical analysis of grains, lower amount of Cd was found with the treatment of wood-ash.

# RECOMMENDATIONS

Soil contamination by Cadmium is a prime environmental concern, especially to the area which is insecure by the heavy industrialization. This hazardous element needs to be control by any unfolding solution. So following recommendations are asked to explore-

1) This experiment need to be done to the industrialized areas where there is a high risk of Cd contamination to the soil.
2) Chemical composition of organic manures should be done to understand the variation among them.
3) Biochemical parameters of plant should be done to understand the mechanism.

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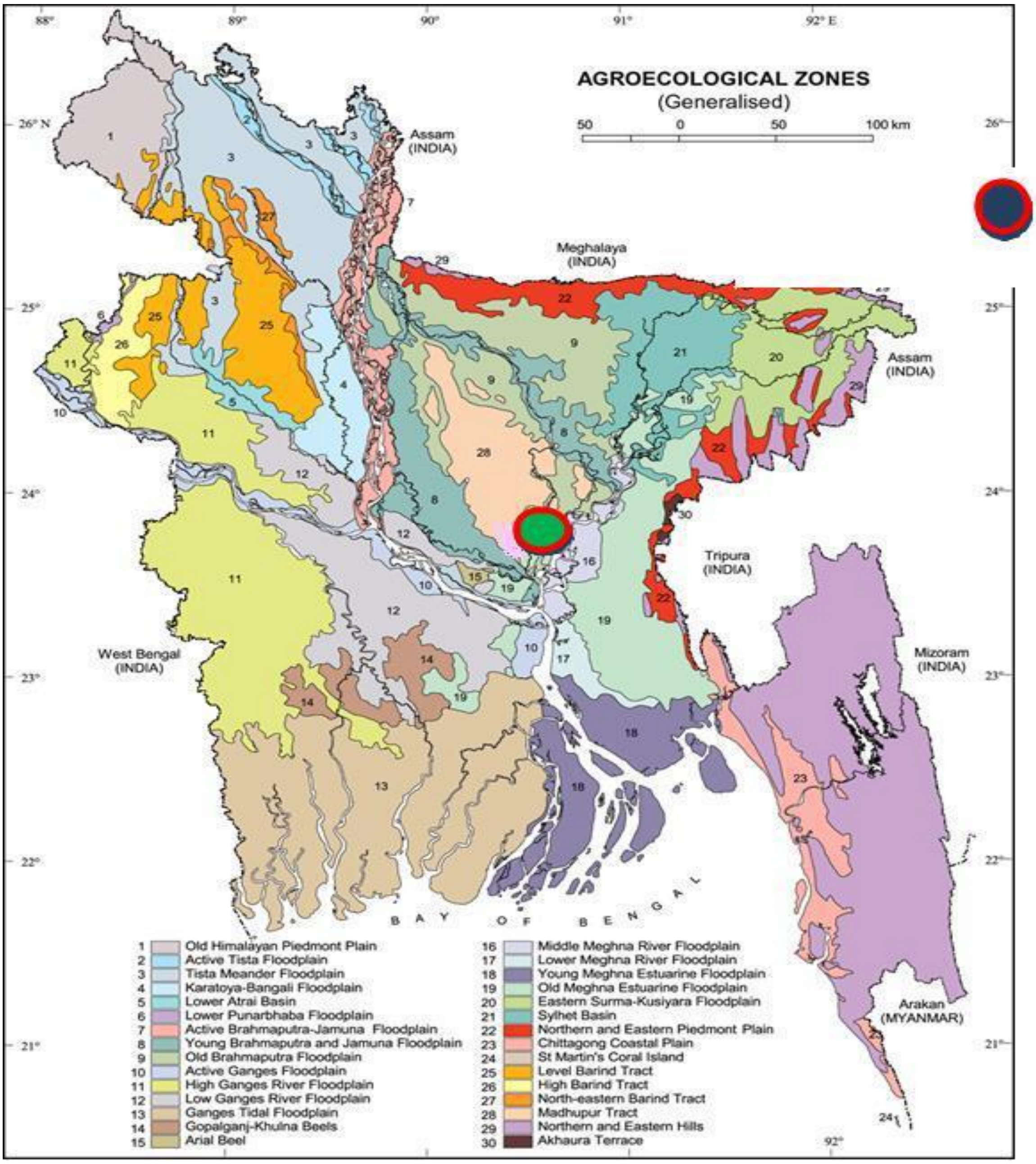
# APPENDICES

## Appendix I- Map showing the experimental site under the study



**Appendix II- Monthly average temperature, average relative humidity and total rainfall and average sunshine of the experimental site during the period from July- December, 2018**

| Month | Average Temperature (ºC) | | Average Relative Humidity (%) | Rainfall (mm) | Average sunshine (hr) |
|---|---|---|---|---|---|
| | Minimum | Maximum | | | |
| June, 2018 | 23.2 | 35.5 | 78 | 312 | 5.4 |
| July, 2018 | 24.5 | 36.0 | 83 | 563 | 5.1 |
| August, 2018 | 23.5 | 36.0 | 81 | 319 | 5.0 |
| September, 2018 | 24.4 | 34.5 | 81 | 279 | 4.4 |
| October, 2018 | 25 | 32 | 79 | 175 | 6 |
| November, 2018 | 21 | 30 | 65 | 35 | 8 |
| December, 2018 | 15 | 29 | 74 | 15 | 9 |

**Source:** Bangladesh Meteorological Department (Climate & Weather Division), Agargoan, Dhaka-1212.

**Appendix III- Mean sum square values of the data for plant height at different days after transplanting**

| Source of Variation | DF | Mean Sum square values of plant height | | | |
|---|---|---|---|---|---|
| | | 20 DAT | 40 DAT | 60 DAT | At harvest |
| Replication | 3 | 3.8222 | 26.727 | 18.998 | 3.299 |
| Cadmium | 2 | 4.9084 NS | 367.255** | 332.528** | 431.751** |
| Organic manure | 3 | 3.0394 NS | 107.121** | 58.898** | 74.703** |
| Cadmium×Organic manure | 6 | 7.5802 NS | 6.033 NS | 10.247 NS | 17.949 NS |
| Error | 33 | 11.2171 | 19.343 | 14.390 | 9.148 |
| Total | 47 | | | | |

**Appendix IV-Mean sum square values of the data for tiller number at different days after transplanting**

| Source of Variation | DF | Mean Sum square values of tiller number | | | |
|---|---|---|---|---|---|
| | | 20 DAT | 40 DAT | 60 DAT | At harvest |
| Replication | 3 | 0.58333 | 2.2431 | 3.4774 | 0.9167 |
| Cadmium | 2 | 0.08333 NS | 46.0833 ** | 37.0365 ** | 24.6458** |
| Organic manure | 3 | 2.80556* | 7.0764** | 8.3941** | 9.8611** |
| Cadmium×Organic manure | 6 | 0.47222 NS | 1.3889 NS | 0.7170 NS | 0.8403 NS |
| Error | 33 | 0.50758 | 1.6976 | 1.1896 | 0.7045 |
| Total | 47 | | | | |

**Appendix V- Mean sum square values of the data for leaf number at different days after transplanting**

| Source of Variation | DF | Mean Sum square values of leaf number | | |
|---|---|---|---|---|
| | | 20 DAT | 40 DAT | 60 DAT |
| Replication | 3 | 0.20785 | 5.765 | 4.3367 |
| Cadmium | 2 | 6.19919 ** | 156.033 ** | 94.3312 ** |
| Organic manure | 3 | 6.00856 ** | 61.268** | 67.4879 ** |
| Cadmium×Organic manure | 6 | 5.81966 ** | 13.044* | 11.4750 * |
| Error | 33 | 1.09341 | 5.579 | 4.2871 |
| Total | 47 | | | |

**Appendix VI- Mean sum square values of the data for leaf area index at different days after transplanting**

| Source of Variation | DF | Mean Sum square values of leaf area index | |
|---|---|---|---|
| | | 40 DAT | 60 DAT |
| Replication | 3 | 6.834 | 8.272 |
| Cadmium | 2 | 2.659 ** | 2.921** |
| Organic manure | 3 | 5.868 ** | 6.068** |
| Cadmium×Organic manure | 6 | 1.695 ** | 1.152** |
| Error | 33 | 9.362 | 1.135 |
| Total | 47 | | |

**Appendix VII- Mean sum square values of the data for root length and root weight at harvest**

| Source of Variation | DF | Mean Sum square values of root | |
|---|---|---|---|
| | | Root length | Root weight |
| Replication | 3 | 1.383 | 1.3438 |
| Cadmium | 2 | 119.804 ** | 42.8295 ** |
| Organic manure | 3 | 103.471** | 58.9887 ** |
| Cadmium×Organic manure | 6 | 16.455** | 12.1236 ** |
| Error | 33 | 1.891 | 0.8464 |
| Total | 47 | | |

**Appendix VIII- Mean sum square values of the data for yield contributing parameters**

| Source of Variation | DF | Mean Sum square values of yield contributing parameters | | | | |
|---|---|---|---|---|---|---|
| | | Effective tiller hill$^{-1}$ | Panicle length | Filled grain pot$^{-1}$ | Total grain pot$^{-1}$ | 1000-grain wt. |
| Replication | 3 | 0.13 | 1.46 | 344.7 | 192.0 | 2.04 |
| Cadmium | 2 | 13.77 ** | 14.74** | 94717.1** | 75421.6 ** | 23.84** |
| Organic manure | 3 | 8.35** | 12.33** | 15010.2** | 7378.7** | 6.69** |
| Cadmium×Organic manure | 6 | 3.19** | 3.59* | 3731.1** | 2558.5** | 0.29 NS |
| Error | 33 | 0.53 | 1.43 | 424.5 | 526.8 | 0.62 |
| Total | 47 | | | | | |

**Appendix IX-Mean sum square values of the data for grain and straw yield of rice**

| Source of Variation | DF | Mean Sum square values of grain and straw yield | |
|---|---|---|---|
| | | Grain yield | Straw yield |
| Replication | 3 | 0.5417 | 10.273 |
| Cadmium | 2 | 61.2101 ** | 806.883** |
| Organic manure | 3 | 8.3352** | 77.625** |
| Cadmium×Organic manure | 6 | 0.7965* | 19.158 NS |
| Error | 33 | 0.2894 | 9.352 |
| Total | 47 | | |

**Appendix X- Mean sum square values of the data for cadmium content in rice grain**

| Source of Variation | DF | Mean Sum square values of Cd content | |
|---|---|---|---|
| | | Rice grain with husk | Rice grain without husk |
| Replication | 3 | 0.0182 | 0.02154 |
| Cadmium | 2 | 13.6706 ** | 5.32426 ** |
| Organic manure | 3 | 0.3888** | 1.30695** |
| Cadmium×Organic manure | 6 | 0.3510** | 0.61558** |
| Error | 33 | 0.0101 | 0.00726 |
| Total | 47 | | |

Plate 1. Field view of experiment in Agronomy Net House, SAU

Plate 2. Performance of different treatments during maturity stage