The potential transfer of Cadmium to food crops where organic matter is applied to soil

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Abstract

Cadmium (Cd) is a non-essential element that can be absorbed by plant roots and accumulate in plant tissues. The presence of Cd in plants potentially poses a serious risk for human health through the food chain. This risk is increasing because of the direct contact between agriculture and urban land use which leads to Cd contamination of agricultural land through disposal of urban and industrial waste. Some widely grown leafy vegetables are a high Cd accumulator, such as Bok Choy (*Brassica chinensis.* L). The application of organic matter to soil has been considered as a mitigation strategy to reduce Cd availability in the soil due to the ability of this amendment to immobilise Cd in soil solution. In developing countries, where the agriculture sector is mostly run by smallholder farmers, compost application is therefore a low-cost approach to reduce Cd toxicity. However, as organic matter experiences rapid decomposition, particularly in tropical regions, there is a possibility that Cd bound to the soil could potentially be released back to the soil solution. The effect of organic matter decomposition over time on Cd availability and Cd plant uptake has been poorly studied. The research in this thesis aimed to observe the effect of compost application over time in Cd availability in the soil and Cd uptake in Bok Choy as a model crop plant. Such information will establish proper agricultural practices in Cd enriched soils in the Indonesian agriculture systems.

In an incubation experiment (Chapter 3), two soils with contrasting properties (Recent Soil and Allophanic Soil) were spiked with five levels of Cd (0, 1,2,5 and 10 mg Cd/kg) and amended with four rates of compost (0%, 5%, 10% and 15%). Subsamples were incubated at 25^0C under dark conditions with a moisture content of 70% field capacity for 90 days. Every 30 days a soil sample (20 g) was removed from each experimental unit for soil analysis. The results showed that greater compost application reduced Cd availability in the soil and an increased Cd availability as a function of time. The results varied between the two soils: changes were significant in the Recent Soil and insignificant in the Allophanic Soil. The greatest magnitude of change was observed for the Recent Soil between Day 30 and Day 60.

In a subsequent pot trial experiment, Bok Choy (*Brassica chinensis* L) was grown in Recent and Allophanic Soil spiked with 10 mg/kg Cd and four levels of compost application (0%, 5%, 10%

and 15%). Biomass was destructively sampled from separate pots at Day 30 and Day 60. The results showed that Cd content increased proportional to dry weight biomass. Greater application of compost decreased the magnitude of the Cd content difference between the two sampling times. For 0% compost application, the Cd root content increased with time, ranging from 515% to 850% for the Recent and Allophanic Soil, while for the 15% compost application this range was between 12% to 248%. For shoots, the increase for the 0% compost treatment was 34.3% in the Recent Soil and 149% in Allophanic Soil. Plant roots experienced a more prominent Cd content increase from Day 30 to Day 60 compared to shoots.

Overall, there was evidence of organic matter decomposition in soil that caused a slight decrease in organic matter percentage with time in both experiments. This led to an increase in Cd availability over time in the soils; a change that was significant in the Recent Soil but insignificant in the Allophanic Soil. Higher compost rates reduced the increase in Cd content over time for Bok Choy (*Brassica chinensis.* L). compared to no compost application. Based on the results of this thesis, good agricultural practices such as reapplying compost regularly, at a certain application rate, could be followed by farmers to reduce the risk of Cd transfer to the food chain where leafy vegetables are commonly grown in Cd-enriched soils. Further work is needed to define the optimal application rate and period.

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1. Introduction

Agriculture is an important sector for livelihoods across Indonesia. Generally, the Indonesian agriculture sector is run by smallholder farmers who produce horticulture products. However, as urbanisation is growing rapidly, some land that can grow crops is being used to establish urban settlements. Urban waste contamination of agricultural land is therefore becoming common, and the primary vector for contamination is irrigation water (Ferina et al., 2020). Of the many contaminants that can be introduced into Indonesian agricultural systems, an important one is the trace metal cadmium which is associated with the excessive use of phosphate fertilisers (Ferina et al., 2020; Pradika et al., 2019).

Cadmium is a non-essential trace element that occurs in soils due to pedogenic processes and anthropogenic activities (Kabata-Pendias & Mukherjee, 2007; Loganathan et al., 2012). Cadmium is toxic and the element can be absorbed by plant crops and enter human bodies through the food chain (Loganathan et al., 2012). The presence of Cd can pose a risk to the agricultural sector; however, risk is not always directly associated with the cadmium concentration in the soil. Even when the cadmium concentration is low, there is potential risk that the element is phytoavailable. Cadmium bioavailability, which controls Cd phytoavailability, is therefore a critically important parameter and this is influenced by soil and plant factors.

Options to reduce Cd bioavailability and plant uptake using soil amendments have been extensively studied and is considered a potential mitigation strategy (Al Mamun et al., 2016). The presence of soil amendments in agricultural soils can bind Cd, reducing bioavailability. Compost from animal manure is the most common soil amendment that small-scall farmers use in tropical and developing countries due to the affordable price (Agegnehu et al., 2017). According to Welikala et al. (2018), the bond between Cd and dissolved organic matter (DOM) causes Cd immobilisation in soil solution. Therefore, compost as a soil amendment could potentially reduce the risk of Cd toxicity through the food chain.

Due to the humid climate and high rainfall intensity, organic matter decomposition rates in tropical areas are faster than in temperate areas (Coleman et al., 1989). As organic matter decomposes, the organic matter content reduces, decreasing the effectiveness of the soil

amendment ability to complex Cd in soil solution. This process could influence the ability of organic matter to bind Cd in soil. Therefore, to better understand the potential for risk reduction, the decomposition rate of organic matter in tropical areas should be considered.

The impact of organic matter decomposition over time on its ability to bind Cd in soil has been rarely studied. Furthermore, it is important to know how long the organic matter can retain Cd in the soil to be able to quantify the potential timeframe for risk reduction. Therefore, the current study was developed to better understand possible mechanisms that can reduce the risk of Cd toxicity in the Indonesian agricultural system. An expected outcome of this work is information that can encourage agriculture practitioners to plan compost application rates and schedules. This research will also provide practical and applicable frameworks based on an increased understanding of organic matter decomposition over time.

1.1 Thesis structure

The thesis comprises five chapters, outlined as follows:

Chapter 1 introduces the topic of interest, providing a brief introduction to cadmium toxicity in the soil and the mechanism by which organic matter application and decomposition in soil can control cadmium mobility, sequentially binding and then potentially returning Cd to the soil solution.

Chapter 2 reviews the relevant knowledge regarding factors influencing Cd bioavailability, including both plant and soil factors. This chapter describes the potential for organic matter application to reduce Cd bioavailability and the impact of subsequent organic matter decomposition, potentially returning the Cd to soil solution before being taken up by plants.

Chapter 3 describes the influence of organic matter decomposition on cadmium availability in soil over time.

Chapter 4 describes the effect of compost application over time on biomass production and Cd uptake (concentration and content) by the commonly grown leafy green Bok Choy (*Brassica chinensis L).*

Chapter 5 summarises the key findings from the research chapters and discusses the knowledge developed in this study to establish frameworks for compost application rate and schedule that might limit Cd transfer through the food chain in Indonesia farming systems.

2. Literature review

2.1 Cadmium: Source and Toxicity

Cadmium is a trace element occurring in the Earth's crust at a low concentration between 0.1 to 0.2 mg/kg and is found in both sedimentary rocks and igneous rocks (Kabata-Pendias & Mukherjee, 2007). Cadmium has been industrially exploited for battery production alongside Zn and Ni (Kabata-Pendias & Mukherjee, 2007). Cadmium forms simple compounds in the environment as a result of weathering processes which are mobile affecting the environment and food quality. Therefore, it is a trace element that has adverse effects on plants, humans and animals (Kabata-Pendias & Mukherjee, 2007; Loganathan et al., 2012).

In soil, Cd accumulates from parent materials through pedogenic processes and from anthropogenic sources caused by human activities (Loganathan et al., 2012). Parent materials are the primary source of geogenic Cd content in soil, ranging from <0.03-2.6 mg/kg (Loganathan et al., 2003). According to Kabata-Pendias and Mukherjee (2007), the world range of Cd concentration in soils is between 0.2 and 1.1 mg/kg.

Cadmium has a harmful effect on the environment and impacts animals, plants, microorganisms and humans. Cadmium concentrations are known to be relatively high in topsoils due to phosphate fertilisers application, and in areas where industrial and household waste is disposed to soil (Kabata-Pendias & Mukherjee, 2007; Stafford et al., 2018). Cadmium contamination in agricultural topsoils leads to Cd toxicity to humans and animals through the food chain, as it is taken up by plants and then consumed by humans and animals (Qin et al., 2020).

Cadmium can accumulate in several human organs, such as bones and liver, but is mainly concentrated in kidneys resulting in serious disease such as renal tubular damage and kidney stones (Pooja & Jyotsna, 2018). Due to its similar charge, ionic radius, and chemical behaviour, Cd can replace calcium (Ca) in minerals (Kubier & Pichler, 2019). Therefore, as it enters the human body, Cd can be readily stored in several organs to a high level (Hajeb et al., 2014). The presence of Cd in human bodies can also decrease Ca uptake in the body (Lata et al., 2019).

Phosphate is a limiting nutrient for agricultural systems, particularly in acid tropical soils (Rochayati et al., 2011). Therefore, phosphate fertiliser application has been a necessity in some areas with low P concentrations. Raw phosphate rocks used for P fertilisers naturally contain Cd and therefore the use of phosphate fertilisers can increase Cd concentrations in the soil (Roberts, 2014). Consequently, in some cases, a high level of Cd in agricultural soils is linked to excessive use of P fertilisers, or the regular use of high-Cd P fertilisers (Loganathan et al., 2003; Roberts, 2014). The contribution of phosphate fertilisers to Cd accumulation in soil depends on the Cd content, application rate and phosphate fertiliser solubility of the phosphate fertilisers used (Loganathan et al., 1996).

Cadmium in agricultural areas can also come from urban and industrial wastes (Hardiyanto & De Guzman, 2008). Contamination of agricultural lands is becoming more common due to rapid urbanisation leading to direct contact between urban and agricultural areas (Mougeot, 2000; Nabulo et al., 2006). Hardiyanto and De Guzman (2008) studied Cd contamination in soil in three locations according to its distance from a main road (0, 25 and 50 m) in two cities (Las Piñas and Parañaque) in the Philipines. The study showed that Cd concentration in the soil at 0 m from the main road was the highest. The number declined by 40% at the distance of 25 m from the main road (Hardiyanto & De Guzman, 2008). In Indonesia, a recent study has shown that the Cd concentration in paddy field soil irrigated with sugarcane industry waste (Sragen Regency), Central Java was 0.164 mg Cd/kg, the highest compared to other land-use management options (Pradika et al., 2019).

2.2 Cadmium Accumulation in Crops

The Cd concentration in food crops is an important consideration as this controls the food chain transfer to humans. Rice, the second important cereal crop in the world after wheat, accumulates Cd to a relatively high concentration in grain (Table 2.1) (Bolan et al., 2013; Hu et al., 2013; Williams et al., 2007). The adverse effect of Cd on humans was first discovered in Japan in the 1950s (Roberts, 2014) after rice farmers were exposed to Cd poisoning following several years of consuming rice irrigated with water contaminated with Cd from mine wastes (Kobayashi, 1978). Cadmium contamination of rice is today a global environmental concern (Tsukahara et al., 2003; Williams et al., 2009).

Vegetable consumption is also an important pathway for Cd to enter human bodies as vegetables are an essential component in the human diet (Abdulla & Chmielnicka, 1989; Rafiq et al., 2014). Some research has demonstrated that leafy vegetables potentially accumulate a higher Cd concentration, particularly in edible parts, than other crops (Al Mamun et al., 2016; Alexander et al., 2006; Han-Song et al., 2010; He & Singh, 1993). Accumulation to high levels has been recorded for vegetables grown on soil with low Cd concentration (Han-Song et al., 2010).

According to research conducted by Yang et al. (2010), when growing on soil with a Cd concentration of 1 and 2 mg Cd/kg, the Cd concentration in spinach was 110-fold and 175-fold higher than in sweet pea, respectively. In this same study, through assessing 28 vegetables, the order for Cd accumulation was, leafy vegetables > solanaceous vegetables > kale vegetables > root vegetables > alliums > melon vegetables > legumes (Yang et al., 2010). A study conducted by Al Mamun et al. (2016) found that the leafy vegetables spinach and lettuce accumulated a higher Cd concentration when compared to onions. Meanwhile, according to study conducted by Alexander et al. (2006), the order for Cd accumulation in spiked soil with 4.2 mg kg^{-1} Cd was Spinach > Onion > Carrot > Pea. The literature summarised in Table 2.2 shows that spinach uptakes the highest level of Cd from soil.

Table 2. 1. Cadmium concentration in rice grain from the cultivars Japonica and Indica (Modified from Römkens et al. (2009)

Family	**Cultivar Name**	**Cd range in grain (mg/kg)**
Japonica	Tainung no. 70	0.02 – 4.6
Japonica	Taiken no. 8	0.11 – 6.0
Japonica	Tainung no. 72	0.02 – 3.0
Japonica	Kaohsiung no. 143	0.01 – 4.5
Japonica	Taitung no. 30	0.02 – 3.3
Japonica	Tainung Sen no. 20	0.02 – 12.6
Japonica	Tainung no. 71	0.10 – 3.7
Japonica	Tainung no. 67	0.09 – 3.4
Indica	Kaohsiung Sen Yu no. 1151	0.23 – 7.6
Indica	Taichung Sen Waxy no.1	0.25 – 25.3
Indica	Taichung Sen no. 10	0.19 – 29.1
Indica	Kaohsiung no. 144	0.08 – 3.7

Table 2. 2. Cd range concentrations in some crops

Food Crops	**Cd concentration range (mg kg^{-1} dry matter)**	**Cd Exposures (mg/kg)**	**Author**
Carrot	1.215 - 2.521	4.2	(Alexander et al., 2006)
Spinach	4.24 – 6.94	4.2	(Alexander et al., 2006)
Pea	0.1785 – 0.4282	4.2	(Alexander et al., 2006)
Onion	3.4464 – 4.0229	4.2	(Alexander et al., 2006)
Squash	0.163 0 0.253	2	(Yang et al., 2010)
Cucumber	0.118 – 0.205	2	(Yang et al., 2010)
Kidney bean	0.049 – 0.099	2	(Yang et al., 2010)
Bok Choy	1.05 – 1.79	4	(Rafiq et al., 2014)

Apart from crop types, tissue age can also have a role in determining the Cd concentration in plants. Older tissues potentially accumulate a higher concentration of trace elements, including Cd than younger tissues because cadmium accumulates over time in plant tissues (Bešter et al., 2013; Loganathan et al., 2003). Leaf age is therefore an important plant factor in terms of Cd uptake by plants. It can be assumed that younger leaves will accumulate a lower cadmium concentration compared to the older leaves (Thompson-Morrison, 2017). However, if the exchangeable cadmium concentrations in the soil are high throughout the growth period of a plant, consuming younger leaves might still pose risks to human health. Investigation of optimal harvest timing has been recommended to prevent risk of Cd accumulation as a function of time (Thompson-Morrison, 2017).

In order to reduce potential Cd risks linked to excessive Cd intake through the food chain, maximum permissible limits (MPL) for Cd in edible crops have been created by the Codex Alimentarius Commission of the Food and Agriculture Organization (FAO). Table 2.3 summarise the MPL for Cd in several edible crops.

Table 2. 3. Maximum Permissible limit of Cd in edible crops (CODEX, 2018)

Edible Crops	Cd level (mg/kg Fresh Weight (FW))
Brassica vegetables	0.05
Cereals, legume	0.1
Fruiting vegetables	0.05
Leafy vegetables	0.2
Root and tuber vegetables	0.1
Stalk vegetables	0.1
Bulb vegetables	0.05
Wheat	0.2
Rice	0.4

2.3 Cadmium in Indonesian Farming System

In Indonesia, Cd might potentially pollute agricultural lands via irrigation water and an abundance of reactive phosphate rock. A summary of studies that record the presence of Cd in Indonesian farming systems is displayed in Table 2.4. Trace elements might contaminate irrigation water via the direct discharge of sewage water and urban waste to water (Iskandar & Kirkham, 2001; Kirkham, 2006). According to Pradika et al. (2019), in Sragen Regency, Central Java, Indonesia, a high cadmium level in a paddy field was due to an abundance of P fertiliser application and therefore this remains a major pathway for Cd enrichment of agricultural land. The presence of Cd in Indonesian Farming Systems has become an environmental issue of concern to the Indonesian government due to its toxicity (Ferina et al., 2020). Therefore, the Indonesian government has regulated a Cd threshold value in the soil of 0.15 mg/kg (PP RI No. 101, 2014). Furthermore, in order to provide healthy food, the Agency for Drugs and Food Control of Indonesia (BPOM) has limited the Cd concentration in rice to a maximum safe level of 0.05 mg/kg dry weight (DW) (BPOM [The Agency Food and Drug Control of Indonesia], 2017).

Table 2. 4. Studies demonstrating the presence of Cd in the Indonesia Farming System

Author	**Plants Species**	**Primary Observations**
(Pradika et al., 2019)	Rice	Cadmium concentration in paddy soil was 0.28 mg/kg, higher than national threshold.
(Rochayati et al., 2011)	Maize	Pot trials using RPR high (RPRH) as a P source by 0.025% increased the Cd concentration in maize shoots planted in acid upland soils.
(Ali et al., 2021)	Forages	The mean cadmium concentration in forages from flooded pasture in South Sumatra, Indonesia was 0.0031 mg/kg, lower than the maximum permissible level of 1 mg/kg (FW) (Hejna et al., 2018).
(Suzuki et al., 1980)	Rice	Out of 116 rice samples from Java Island, two samples recorded high Cd concentrations of 0.27 and 0.34 mg/kg (DW). Assuming rice consumption of 300 g on a daily basis, this is 160 µg Cd intake per day. The Cd daily intake exceeded the tolerable limit, 57 to 71 µg per day proposed by FAO.

Ferina et al. (2020) reported the spatial distribution of Cd across different land-use management units in Sragen Regency. The study also investigated areas with the same land use management but with different soils (Table 2.5). Low levels of Cd where measured in forest soil (control) and organic paddy fields, which are classified as unpolluted (0.007 mg/kg) and lightly polluted (0.019 mg/kg) respectively (Ferina et al., 2020). Conventional paddy fields with different soils are classified as moderately polluted (ranging from 0.053-0.069 mg/kg). Paddy fields irrigated by wastewater showed a high Cd concentration in the soil. Paddy fields irrigated by the textile and batik industries had a moderate to heavy level of pollution with Cd concentrations of 0.112 and 0.115 mg/kg, respectively. Fields irrigated by the sugarcane industry contained the highest Cd concentration (0.164 mg/kg) and were classified has heavily

polluted. Table 2.5 shows that industrial wastes contribute a high Cd load to soil. Industrial wastes are one of the main Cd sources for agricultural lands alongside household waste, phosphate fertilisers and pesticides (Carabalí et al., 2020).

Table 2.5. Cadmium spatial distribution in different land use managements in Sragen Regency (adapted from Ferina et al. (2020)).

Land use	Cd in soil (mg/kg)	Total area (ha)	Pollution degree*
Forest	0.007 d	370	Unpolluted
Organic Paddy Field	0.019 d	388	Lightly polluted
Conventional Paddy Field (Alfisols)	0.054 c	13,461.8	Moderately polluted
Conventional Paddy Field (Entisol)	0.069 c	24,443,4	Moderately polluted
Conventional Paddy Field (Inceptisol)	0.053 c	12,577,7	Moderately polluted
Conventional Paddy Field (Vertisol)	0.057 c	10,668.5	Moderately polluted
Paddy Field Irrigated by Textile Industry	0.112 b	471.2	Moderate to heavily polluted
Paddy Field Irrigated by Batik Industry	0.115 b	1,196.5	Moderate to heavily polluted
Paddy Field Irrigated by Sugarcane Industry	0.164 a	246.7	Heavily polluted

* The pollution degree level is based on modified PP RI No 101, 2014.

Ferina et al. (2020) also analysed the Cd concentration in grain as a function of land use (Table 2.6). In contrast the ranking for the Cd concentration in the soil, the highest Cd concentration in grain was found from the conventional paddy field (Entisol) at 0.046 mg Cd/kg. This might be influenced by the excess use of overspray pesticides, common in conventional paddy field, leading to the pesticide remaining in the rice grain. The excessive utilisation of pesticides is another key anthropogenic source of Cd in rice grain (Ferina et al., 2020).

Table 2.6. Cadmium content in rice grain (adapted from Ferina et al. (2020))

Land use	Cd in rice grain (mg/kg)
Forest	0 g
Organic Paddy Field	0.0006 g
Conventional Paddy Field (Alfisols)	0.039 b
Conventional Paddy Field (Entisol)	0.046 a
Conventional Paddy Field (Inceptisol)	0.026 d
Conventional Paddy Field (Vertisol)	0.032 c
Paddy Field Irrigated by Textile Industry	0.011 f
Paddy Field Irrigated by Batik Industry	0.008 f
Paddy Field Irrigated by Sugarcane Industry	0.019 e

Reactive phosphate rock is commonly applied to acid upland soils across Indonesia because is a cheaper source of P fertilizer; highly water-soluble P fertilisers used in developed countries are often too expensive for some farmers (Rochayati et al., 2011). RPR use on acid soils has been shown to increase maize, soybean, groundnut and upland rice production leading to higher yields than triple superphosphate application (Partohardjono & Sri Adiningsih, 1991). However, superphosphate products can also contain Cd, as influenced by the phosphate rock used in manufacture. According to a pot experiment conducted by Rochayati et al. (2011), applying RPR high (RPRH) containing 69 mg Cd/kg significantly increased Cd concentration in maize shoots planted in acid upland soils from Indonesia.

2.4 Soil Factors Influencing Cadmium Availability

2.4.1 Total Cadmium and Cadmium Availability

Cadmium availability in soil is more a concern than the total soil concentration of this metal (Kirkham, 2006). As Cd accumulates in soil, only a small fraction is available for plant uptake, with a larger fraction complexed by soil colloids (Al Mamun et al., 2016). Therefore, a high Cd concentration in the soil does not always indicate pollution and represent an environmental risk (Chapman, 2007). Cadmium would pose a risk when it is available for plant uptake, leading to food chain transfer and exposure for animals and humans, potentially causing adverse health effects.

The form of Cd that is available for plant uptake is free Cd ions (Cd^{2+}) found in soil solution (Gray & Mclaren, 2006). Many studies have used regression models to demonstrate the relationship between the Cd level in the soil and Cd plant uptake. According to Gray et al.

(1999), while pH was the most important factor influencing Cd concentration in soil solution, total soil Cd was also important in New Zealand soils. Organic matter content and cation exchange capacity (CEC) and zinc (Zn) concentration are also key soil parameters that control Cd bioavailability.

2.4.2 Organic Matter Content

Organic matter (OM), according to Brady et al. (2008), is defined as animal and plant residues that undergo different stages of decomposition. Decomposed organic material consists of detritus (active OM), hummus (stable OM), plants residues, and microbial biomass. Soil organic matter affects the biological and physicochemical properties of the soil, and it therefore has vital roles in improving soil function. Organic matter will increase soil microbial diversity, water holding capacity, improve soil aeration, increase soil water infiltration, and cation exchange capacity (CEC) (Kaiser et al., 2008; Oades, 1984; Tate III, 1987).

Organic matter content is one of several critical soil factors that influence cadmium availability to plants (Benavides et al., 2005). High organic matter content is associated with a high cation exchange capacity (CEC) due to the presence of reactive cation adsorbing surfaces on colloidal organic matter particles (Kaiser et al., 2008; Tate III, 1987). CEC surfaces lead to an increase in Cd adsorption in the soil. Organic matter can also specifically bind Cd through the formation of organometallic compounds on soil particle surfaces (He & Singh, 1993; Simmler et al., 2013). Once adsorbed to the soil, Cd remains bound and insoluble, reducing the availability of the trace metal to plant uptake (Simmler et al., 2013).

Studies have demonstrated that the application of organic amendments to topsoil can reduce cadmium toxicity risks. According to research conducted by Al Mamun et al. (2016), applying a low-cost municipal compost at a rate of 2.5% decreased the Cd concentration in lettuce, spinach and onions by up to 60% in two New Zealand soils collected from Pukekohe and Levin. In another study, the application of compost of wheat straw biochar reduced the Cd ecological risk in Chinese cabbage (*Brassica rapa*. *L*) grown in contaminated soil (Awasthi et al., 2019). The combination of 18% compost with 300 kg/ha nitrogen treatment significantly decreased Cd bioavailability by 28 – 32% compared to other treatments.

Pinto et al. (2004) used an extract of Iris peat moss as organic matter to investigate the influence of this amendment on Cd uptake by sorghum plants. The Cd concentration in roots decreased as a function of reduced Cd bioavailability induced by the presence of organic matter. However, contrary to popular findings, the organic matter triggered the translocation of Cd from roots to shoots, resulting in greater mobility of Cd within the plant. This observation should be considered as an example of the risk of increased exposure to Cd that might be induced by organic matter leading to greater health risk (Pinto et al., 2004).

2.4.3 Soil pH

There is a negative correlation between soil pH and Cd plant uptake (Kabata-Pendias & Mukherjee, 2007). At elevated pH Ca^{2+} competes with Cd^{2+} ions at uptake sites on the plant root surface, causing a decrease in Cd plant uptake from soils (Adriano, 1986). Furthermore, the presence of Cadmium Carbonate ($CdCO_3$) in neutral and alkaline soils limits Cd solubility (Santillan-Medrano & Jurinak, 1975). An increase in pH values within variable-charge soil causes an increase in surface negative charge, which increases cation adsorption, including Cd^{2+} (Naidu et al., 1994). In addition, in alkaline soil, the stable species of Cd^{2+}, $CdOH^{+}$ may form and this enhances soil surfaces sorption (Bolan et al., 2003). However, in low pH soil environments, surface negative sites available for adsorption of Cd^{2+} are reduced, and this increases the availability of Cd for plant uptake (Evans, 1989).

Xian and Shokohifard (1989) conducted an experiment where they altered soil pH values of 7.0, 6.0 and 4.50 and measured exchangeable trace elements in the soil, including Cd. They showed that the alteration of pH influenced the form of Cd in soil significantly. Reduction in pH from 7.0 to 4.5 raised the Cd exchangeable concentration from 5.9 to 7.4 mg Cd/kg, which would increase Cd bioavailability (Xian & Shokohifard, 1989). An increase in the pH of a tropical soil (oxisol) in Puerto Rico caused an increase in Cd sorption by 36% (Appel & Ma, 2002). Meng et al. (2018) studied the effect of alkaline amendments on Cd bioavailability in rice grown in clay loam sandy soil contaminated with Cd. Their results showed that increased soil pH can alter Cd to be more stable (i.e. reduced bioavailability) due to Cd immobilisation in soil.

2.4.4 Cation Exchange Capacity (CEC)

Cation exchange capacity (CEC) is a rating of the soil's ability to hold exchangeable nutrient cations such as Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and H^{+} (McLaren & Cameron, 1996). Cadmium is also a cation (Cd^{2+}) and binds to negative soil surfaces charges like other soil exchangeable cations. The higher the CEC value of a soil, the more Cd in the soil binds in the soil colloid (Kabata-Pendias & Mukherjee, 2007; Roberts, 2014).

The application of organic amendments to soil causes an increase in CEC as organic matter consists of humic substances which have a high concentration of negative surface charges (Al Mamun et al., 2016; McLaren & Cameron, 1996). The presence of humic substances in soil therefore improves cation exchange and decreases the concentration of Cd^{2+} in soil solution. A significant decrease in cadmium plant uptake due to an increase in soil pH has been associated with an increase in CEC values (Bolan et al., 2003; Loganathan et al., 2003).

2.4.5 Zinc

Due to chemical similarities between these two elements (both belong to group II in the periodic table), Zn and Cd ions compete for uptake by plant roots and subsequent translocation through a plant (He et al., 2020; Zare et al., 2018). According to research conducted by Gray and Wise (2020), applying 5-20 kg Zn/ha as zinc sulphate reduced the Cd concentration in spinach leaves by 19-28%. The effectiveness of Zn in reducing Cd plant uptake is influenced by varying factors such as Zn rate and form applied, plants species, and the procedure used to apply Zn to the soils (Fahad et al., 2015; Khoshgoftarmanesh et al., 2013; Zhao et al., 2005). Increasing the Zn level in the soil, at an appropriate rate, form and procedure, can be advantageous to decrease Cd uptake by plants.

2.5 Cadmium Uptake in Plants

Although Cd is non-essential element, it is taken up by plants because plants have a slight ability to regulate Cd uptake below phytotoxic concentration (Adriano, 1986). Some studies have shown a linear relationship between Cd availability in soil and Cd accumulation in plants (Adriano, 1986; Williams & David, 1977). Cadmium accumulation in plants significantly

depends on plant species and cultivar (Huang et al., 2019). Cadmium movement via root cells can be either passive through diffusion and convection via apoplast (apoplastic pathway) or active via selective transport from cell to cell (symplastic pathway) (Song et al., 2017).

In young wheat plants, Cd accumulates primarily in roots and tends to be transported slowly to shoots due to a phytochelatin-Cd transporter (Page & Feller, 2005). Phytochelatins have been involved in trace element detoxification by chelation and subsequent sequestration into vacuoles (Hart et al., 1998). Furthermore, phytochelatins influence a major long-distance Cd^{2+} transport mechanism in *Arabidopsis* (Gong et al., 2003). On the other hand, Xin et al. (2017) conducted an experiment to demonstrate Cd distribution in plant tissues in radish (*Raphanus sativus*) under different Cd concentrations (0.01, 0.05, 0.1, 0.5 and 1 mg Cd/L). They showed that there was a correlation between Cd accumulation in roots with cell walls (46-49%), soluble fraction (36-38%) and organelles (15%).

Plant absorbed Cd in root cells is diffused to the stem xylem through a process called xylem loading (Clemens et al., 2002). According to Mori et al. (2009), xylem loading has an important role in Cd accumulation in aerial plant parts. There are several important membrane transporters such as ZIP (ZRT, IRT-like protein), ATP Binding Cassette (ABC) superfamily, YSL (Yellow-Stripe-Like Transporter) and HMA (Heavy Metal ATPase) involved in Cd loading into the xylem (Hasanuzzaman et al., 2018; Song et al., 2017).

2.6 Cadmium Plant Uptake Mitigation

2.6.1 Organic Amendments

An understanding of the various soil and plant factors controlling Cd availability and uptake have enabled the adoption of appropriate management practices that can reduce the transfer of Cd into food crops. Amendment of soil with organic matter is one strategy that can influence cadmium availability in the soil. Cadmium plant uptake decreases as a result of increased organic matter content in the soil, so increasing organic matter content in the soil is an important factor associated with retaining Cd within the soil (Roberts, 2014). Increased organic matter content in the soil can be achieved by applying organic amendments such as peat, animal and plant waste, manure, compost, lignite, sawdust, and biochar.

Bolan et al. (2003) showed that biosolid compost applied to Egmont and Manawatu soils had a strong effect on Cd availability and immobilization. In pot trials, Indian mustard (*Brassica juncea* L.) plants were grown in contaminated soils. Compost application increased CEC and reduced the labile soil Cd concentration. There was also a production benefit for the plants as quantified by an increase in plant dry matter yield as the rate of biosolid compost application increased. In this work, amendment of soil with biosolid compost was demonstrated as a mitigation strategy to reduce Cd availability in agricultural soils (Bolan et al., 2003).

The work of Bolan et al. (2003) focused on temperate climatic conditions where organic matter turnover is relatively slow. Turnover in tropical regions is, by comparison, much more rapid (Coleman et al., 1989) and this may have a major influence on chemical and biochemical reaction rates in tropical soils. The rapid decomposition of organic matter could potentially decrease its ability to bind Cd in the soil with time, potentially leading to re-release of this Cd to soil solution, effectively increasing bioavailability of the metal (Coleman et al., 1989).

2.6.1.1 Compost

Compost is an abundant and low-cost soil amendment which can potentially reduce cadmium bioavailability in agricultural soils (Awasthi et al., 2019). Compost is readily available and could be a suitable and beneficial Cd management strategy, particularly for smallholder farmers in developing countries. A New Zealand trial reported by Al Mamun et al. (2016) utilised municipal compost, at a cost of \$12.50 t^{-1}, to reduce field uptake of Cd by spinach, lettuce and onions. The reduction in uptake was by up to 60% (Table 2.7) (Al Mamun et al., 2016).

Sato et al. (2010) studied the effect of animal waste compost (AWC) on Cd uptake reduction in spinach (*Spinacia oleracea* L) (Table 2.7). Spinach is known as a high Cd accumulator and is one of the most highly consumed vegetables in the world (Ramaiyan et al., 2020; Yang et al., 2010). The research was conducted in a horticultural field with five treatments: cattle compost, swine compost, poultry compost, chemical fertiliser (CF), and a no addition (NA) control (Sato et al., 2010). The AWC application increased spinach yield compared to fertiliser application and control. The yield for the treatment of cattle compost, swine compost, poultry compost, chemical fertiliser and the control were 21.7 t/ha, 20.9 t/ha, 21.0 t/ha, 18.5 t/ha

and 2.4 t/ha respectively. The trial also showed that there was a reduction in Cd concentration by 34-38% in harvested spinach through AWC application when compared to fertiliser application (Sato et al., 2010). Of the animal waste composts, cattle compost was the preferred amendment due to its high Cd affinity and low P content. Low P content can prevent excessive P accumulation in the soil and reduce P loss risk to waterways (McDowell et al., 2003).

Shan et al. (2016) investigated the effect of two composts (composted pig manure and wheat straw compost) on Cd bioavailability and uptake by cherry-red radish (*Raphanus sativus*) in two soil types (Table 2.7). Both wheat straw and pig compost application improved plant growth in the second harvest as quantified by dry matter yield. In the first harvest applied with wheat straw, the dry matter yields of leaves and roots of radish decreased significantly, except for the root at 4.8 g TOC kg^{-1} of soil on Calcaric Cambisols soil (Shan et al., 2016). At 11.9 g TOC kg^{-1} of soil, the dry matter yields of leaves and roots decreased by 72.5% and 49.4% on Calcaric Cambisols, respectively, and decreased by 37.2% and 25.1 on Acidic Ferralsols. For the second harvest, the root biomass increased regardless of soil types. On the other hand, pig manure application significantly increased radish leaves and roots yields on Acidic Ferralsols (Shan et al., 2016). At 11.9 g TOC kg^{-1} application, the dry matter yields of leaves and roots increased by 235.2 and 174.0 % at the first harvest, respectively, and increased by 157.1 and 509.1 % at the second harvest on Acidic Ferralsols.

With respect to Cd, Shan et al. (2016) showed that composted pig manure application was more effective in reducing Cd uptake by radish (Cd in leaves and roots) than wheat straw compost (table 2.7) The application of composted pig manure of 11.9 g kg^{-1} TOC amended into Acid Ferralsols decreased Cd levels in roots and leaves by 90.2% and 84.8% at first harvest and 95.7 % and 89.2% at the second harvest, respectively (Shan et al., 2016). On comparison, wheat straw application to both soils increased the Cd concentration in leaves and roots at first harvest except for leaves when 11.9 g kg^{-1} TOC was applied into Calcaric Cambisols. However, wheat straw at 11.9 g kg^{-1} TOC significantly decreased the Cd concentration in leaves and roots by 11.27% and 39.52% in radish grown on Calcaric Cambisols. There was no significant change for wheat straw application on Acid Ferralsols (Shan et al., 2016).

Shaheen et al. (2017) studied the effect of compost on Cd availability and biomass yield for two planted systems: 1) sorghum under dry-irrigated cycles (referred to as 'dry soil') and 2) barnyard grass saturated by water and irrigated everyday (referred to as 'wet soil') (Table 2.7). The experiment showed that application of 2.5% compost increased the biomass yield of sorghum (41%) and barnyard grass (17.4%) in fluvial soil spiked with 25 mg Cd/kg compared to the control treatment. Compost application reduced Cd availability by 11% to 13% under dry conditions. However the Cd availability increased by 28% to 62% under wet conditions due to the decomposition of soil organic matter which might release Cd in an available form (Shaheen et al., 2017). This study suggests that compost application to reduce Cd bioavailability would be more effective in a dry soil environment.

Table 2.7. Summary of the variable influence of compost application on Cd availability in contaminated soils

Author	**Compost Type**	**Primary Observations**
(Al Mamun et al., 2016)	Municipal Compost	Municipal compost reduced Cd concentration in spinach, lettuce and onions by up to 60%.
(Sato et al., 2010)	Animal Waste Compost (AWC)	Application of AWC reduced Cd concentration in harvested spinach by 34-38% compared to fertiliser application.
(Shan et al., 2016)	Composted Pig Manure	Application of composted pig manure (11.9 g TOC/kg) decreased Cd levels in roots and leaves by 95.7% and 89.2% respectively for second harvest of cherry red radish.
(Shaheen et al., 2017)	Combination of rice straw, cotton, and corn stalks (60%); farmyard manure (35%), and fertile soil (5%).	Compost increased biomass of sorghum (41%) and barnyard grass (17.4%). Compost reduced Cd availability in dry soil by 11% to 13%.

2.6.1.2 Biochar

Biochar is a soil amendment produced when biomass is heated (pyrolysed) with no or low air in a closed container (Lehmann & Joseph, 2009, 2015). Biochar can be made from organic waste such as agricultural residues, feedstock waste and municipal sewage sludge (Wang & Wang, 2019). Biochar has high stable carbon content and can be stored in soil, which could eventually reduce atmospheric CO^2 concentration (Rizwan et al., 2016; Woolf et al., 2010). Highly porous biochars which can act as carbon-based adsorbent materials potentially can sequester trace elements in soils, including Cd (Khan et al., 2020; Nguyen et al., 2007).

Cui et al. (2021) demonstrated the potential for biochar to act as a soil amendment for long-term cadmium sorption in contaminated paddy fields. In this work Cd bioavailability in soil was reduced as a function of the constituent organic functional groups of biochar and the precipitation of (hydr) oxide and carbonate phases induced through soil amendment (Cui et al., 2021). The physical disintegration of organic matter during biochar preparation results in greater surface area exposure and greater inner-particle exposure that would be expected for non-pyrolyzed organic residues and this increased surface areas is attributed to greater cadmium sorption (Cui et al., 2021).

The effect of biochar made from sugarcane bagasse on Cd availability in soils and the Cd concentration in corn roots growing in the amended soil was studied by Su et al. (2021). An incubation experiment was conducted for 60 days, and samples were taken at 0, 20, 40, and 60 days. The incubation soils where then used for pot trials in which corn seeds were planted in both biochar and no biochar amended treatments. Corn root biomass at day 42 collected from five different sampling points (S1, S2, S3, S4 and S5) increased by 24%, 113%, 74%, 31%, and 103% respectively compared to the control treatment, and the Cd concentrations decreased by 11.3%, 57.7%, 38.0%, 34.5%, and 44.8% (Su et al., 2021). With respect to available Cd, the values at Day 60 significantly reduced by 14.2%, 47.2%, 21.6%, 21.3% and 46.8% respectively. During subsequent corn cultivation on these soils, available Cd also decreased due to added biochar 14.2%–18.1%, 47.2%–56.4%, 21.6%–32.1%, 21.2%–26.8%, and 46.8%–57.0%, respectively, compared to control treatment in the same period (Su et al., 2021).

2.6.1.3 Liming

Liming is a practice in agriculture to increase soil pH (Sime & Hill, 2001). Lime increases the total number of base cations which enhance the base saturation, and reduces exchangeable acidity, Mn^{2+} and Al^{3+} (Cristancho et al., 2014). The most common lime form is $CaCO_3$ (aglime), but there are others forms of lime used in agricultural practices such as $Ca(OH)_2$ (slaked lime or hydrated lime), $CaMg(CO_3)_2$ (dolomite lime) and CaO (quicklime) (Sime & Hill, 2001).

Elevated pH values decreases Cd phytoavailability (Kabata-Pendias & Mukherjee, 2007). Lime also adds Ca^{2+} to soil solution which competes with Cd^{2+} at root surface exchange sites reducing Cd plant uptake (Simmler et al., 2013). According to Bolan et al. (2003), there are several mechanisms for lime induced Cd immobilisation; an increase in CEC in soils, metal precipitation, hydroxyl metal species formation, and sequestration due to augmented microbial activity.

Han and Lee (1996) investigated the effect of liming addition (0, 0.25, 0.5, 1 and 2%) on Cd uptake by Radish (*Raphanus sativa)*. The study showed that Cd uptake rate ranged from 8.1% to 148%. Furthermore, lime application decreased Cd uptake both in roots and shoots. Over the first 25 days, the recorded decrease in Cd uptake in both plant parts was not consistent. However, between 50 and 75 days, the Cd concentration in both roots and shoots decreased significantly as a function of lime application ($P < 0.0001$) (Han & Lee, 1996).

2.7 Organic Matter Decomposition over Time and Cd Availability

Organic matter turnover in tropical regions is more rapid than in temperate regions due to warmer weather (Coleman et al., 1989). This results in a several-fold increase in chemical and biochemical reaction rates. Precipitation and temperature changes in critical tropical areas could lead to organic matter decomposition and loss in the soil (Martius et al., 2001).

Soil organic matter (SOM) transformation involves biological and chemical processes such as hydrolysis and oxidation which causes the release of dissolved organic matter (DOM) and which eventually results in a potential increase in trace elements bioavailability (Martínez et al., 2003). The release of dissolved organic matter could increase metal solubility in polluted

soil due to formation of soluble OM-metal complexes (Zhou & Wong, 2001). Soluble organic compounds such as citrate, tartrate, histidione and deoxymuginetic acid have a significance effect on metal availability in polluted soils (Collins et al., 2003).

Su et al. (2021) reported that biochar application to soil for 60 days resulted in a decomposition which can be seen from pore water dissolved organic carbon (DOC) concentration. The samples were taken at 0, 20, 40, and 60 days. Biochar application at day 20 increase pore water dissolved organic carbon by 12.26%. Then, a significant increase occurred starting from Day 60 by 20.66%. The results showed that biochar application as soil amendment increased pore water DOC concentrations in the short term and then decrease eventually as the incubation time longer (Su et al., 2021).

Cavanagh et al. (2015) studied the effect of time and temperature on OM decomposition and Cd sorption in two NZ soils. Compost was added to two soils, and these were stored in incubators at either 20^{o}C or 30^{o}C. The study showed that C content started decreasing after week 9 and plateaued at week 13. Eventually, by week 49, soils at 20^{o}C contained slightly more C compared to 30^{o}C which experienced a high rate of decomposition (Cavanagh et al., 2015). Cadmium sorption showed a similar trend to C content, which increased after compost application and decreased past week 9.

As the organic matter content reduces with temperature, the light fraction of SOM which forms humus colloidal particles with negative charges also decreases (Al Mamun et al., 2016). A decrease in these negative soil surface charges reduces the concentration of Cd^{2+} bound to soil colloids leading to an increase in Cd availability in soil. Therefore, and rapid decomposition of organic matter in soil could potentially decrease its ability to bind soil Cd (Coleman et al., 1989), and potentially could release Cd back to the soil solution over time, particularly in tropical regions. Maximizing organic matter input onto soil and decreasing decomposition rate could be carried out to address the potential for organic matter reduction in the soil (Wambeke, 1992).

2.8 Problem Statement and Research Objective

Cadmium is an emerging and currently poorly understood environmental issue for smallholder horticultural production in Indonesia. Fertiliser- and wastewater-derived Cd are the dominant input sources for Cd to land. The science described in this literature review has highlighted that the application of organic matter to soil is an effective management strategy to reduce Cd bioavailability and uptake by plants. However, this review has also shown that there is potential for the re-release of Cd sorbed to organic matter as this organic matter turns over under tropical conditions. This risk has been poorly investigated in terms of food chain safety.

Based on this knowledge gap, the current study has been carried out to assess the effect of compost decomposition with time on Cd bioavailability. An incubation experiment was developed in which soil was sampled each month to quantify the influence of organic matter decomposition over time (quantified through pH and total organic matter content) on Cd availability under simulated tropical conditions. Overlapping with the incubation study, a pot trial was advanced to investigate the effect over time of compost on biomass production and the Cd uptake concentration of the commonly grown leafy green Bok Choy (*Brassica chinesis. L*). The overall objective of this work is to provide information that can support agriculture practitioners in Indonesia to advance compost application rates and schedule that will limit transfer of Cd through the food chain. Using such science, a cost-effective framework can be established to reduce Cd potential risk in Indonesian farming systems.

2.8.1 Research Aim

The specific aims of this research were to:

1. Investigate the influence of organic matter decomposition on Cd availability in two different soils over time by conducting an incubation experiment.
2. Determine the influence of Cd and organic matter applied at different levels on the biomass and Cd concentration in Bok Choy over time.
3. Quantify the effect of organic matter decomposition under tropical climatic conditions on the potential for Cd transfer to vegetables.

3. Incubation experiment to investigate the influence of compost decomposition on cadmium availability in soil

3.1 Introduction

Organic matter content is well-known to have a significant influence on trace element behaviour in the soil (Rieuwerts, 2007). Organic matter surfaces have large negative surface charges and are associated with high cation exchange capacity (Kaiser et al., 2008). Humic substances in organic matter increase Cd adsorption in the soil (Al Mamun et al., 2016; McLaren & Cameron, 1996) reducing the solubility of Cd^{2+} in soil solution and thereby reducing Cd availability for plant uptake (Simmler et al., 2013).

Organic matter can decompose with time and this is driven by high temperatures and precipitation (McCauley et al., 2009). The higher the temperature and precipitation, the more rapid organic decomposition can be, particularly in tropical regions (Rieuwerts, 2007). Rapid organic matter decomposition can reduce the abundance of humus colloidal particles, leading to a decrease in negative charges (Al Mamun et al., 2016) and this could potentially reduce the Cd^{2+} concentration bound to soil colloids, eventually causing an increase in Cd availability in the soil, increasing the risk that mobile Cd will be accumulated by plants.

Maintaining negative soil charges is therefore critical to maintaining the sorption of Cd to soil particles. To address this need in the field, decomposition of organic matter decomposition must be balanced by applying organic matter at specific rates and schedules (Wambeke, 1992).

In this context, organic matter decomposition in tropical regions and its influence on Cd availability should be studied further, particularly in the Indonesia farming system where compost could be a potential cost-effective soil amendment to improve crop production and reduce Cd availability. This chapter investigates the effect of organic matter decomposition with time on Cd availability under simulated tropical conditions. The incubation was carried out for three months, with soil sampling conducted each month.

3.2 Materials and Methods

3.2.1 Soil Sampling

Two soil orders were used for the incubation experiment: Recent Soil and Allophanic Soil (Figure 3.1). A bulk sample of Allophanic Soil was collected from Waiaruhe Road, Taihape, New Zealand. Recent Soil were collected from the Massey No 4 Dairy Farm, Palmerston North, New Zealand. Both soils were collected from the A horizon to depth of 15 cm.

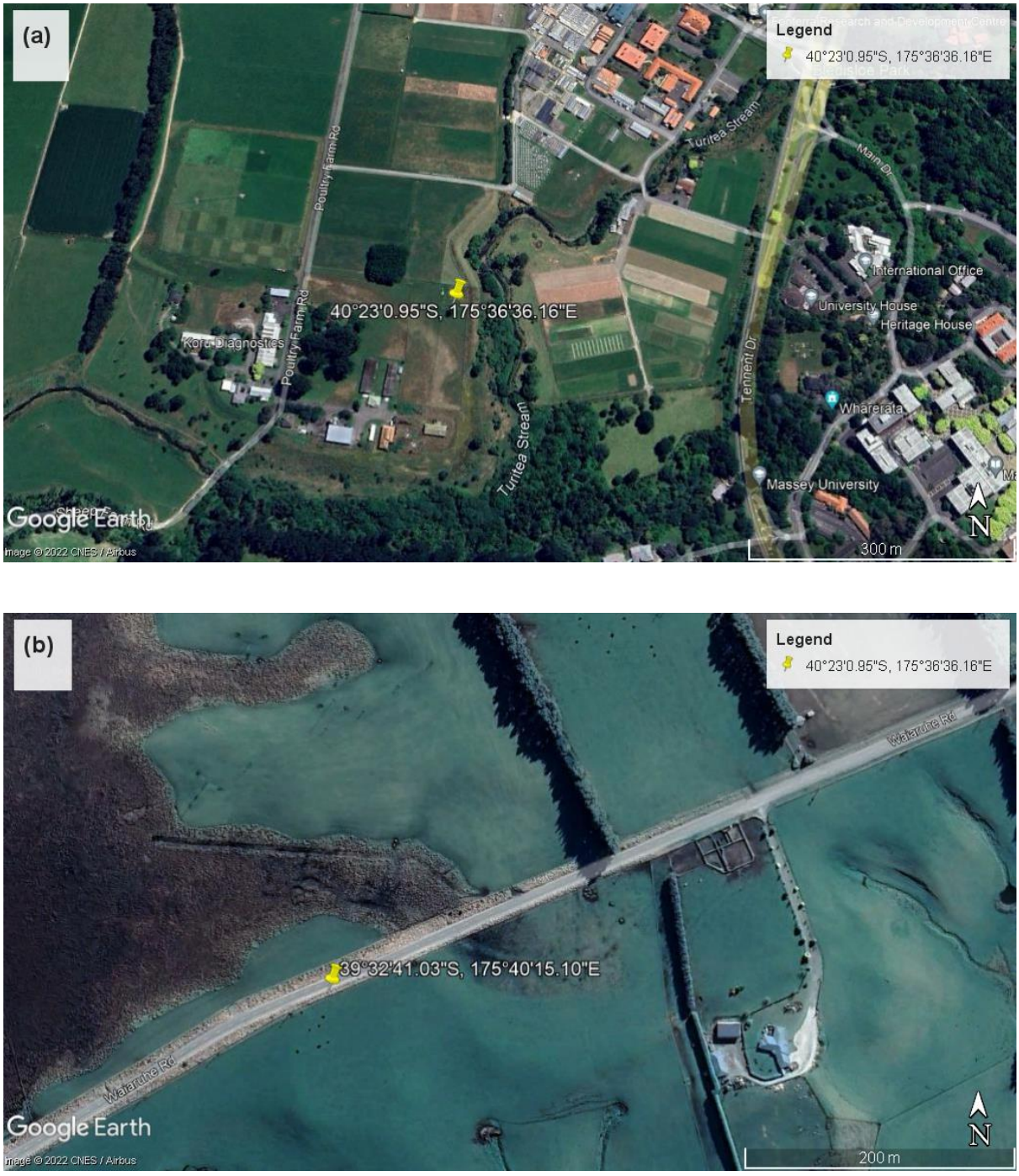


Figure 3. 1 Recent Soil sampling site (a), Allophanic Soil sampling site (b).

3.2.2 Soil Preparation Prior to Incubation

Soils were packed in the field into 20 plastic bags for each soil (21 x 30 cm)and the bags were sealed. Upon return to the laboratory, all soils were stored in a chilled room at -4^{0}C to maintain moisture and microbiological activity. All soil samples were subsequently sieved through a 4 mm sieve to remove gravel and plants roots and sieved soils were stored in a chilled room prior to incubation. The bulk sieved soil was subsampled with three replicate each soil, and these samples were sent to Hill Laboratories (Hamilton, New Zealand) for soil nutrient analysis (Table 3.1).

3.2.3 Soil Spiking

Soils were spiked with $CdCl_2$ to achieve target levels of cadmium: 0 (control), 1, 2, 5 and 10 mg/kg according to the method described by (Ubeynarayana et al., 2021). Soils were spread on to plastic trays and the spiking solution with a pre-determined amount of $CdCl_2$ was added before the soil and spiking solutions were mixed thoroughly. The soils were stored in the dark room for 30 days at a temperature of approximately 25^0 C to equilibrate Cd within the soil matrix.

3.2.4 Soil Incubation

A 5 x 4 x 2 x 3 complete randomised design with three replications per treatment was used (120 samples) to study the change of cadmium bioavailability as a function of time. Factors included were soil type (2), Cd level (5) and compost application (4 rates 0%, 5%, 10% and 15% on a weight basis) (Gadepalle et al., 2009). Control treatments with no Cd and no compost were included. The incubation was conducted for 90 days inside an incubation chamber under dark circumstance in 25^o C.

A separate plastic zip lock bag was used for each experimental unit. A subsample of fresh sieved soil (100 g) with relevant Cd and compost loading was contained within labelled bags. Distilled water was added to maintain soil moisture near 70% field capacity. Two holes were cut into each plastic bag to support aerobic conditions during incubation. To maintain moisture at about 70% field capacity throughout the incubation, water was added to a

constant weight once every 7 days. The soil in the plastic bags was mixed thoroughly by hand once every 14 days. Every 30 days a soil sample (20 g) was removed from each experimental unit for soil analysis.

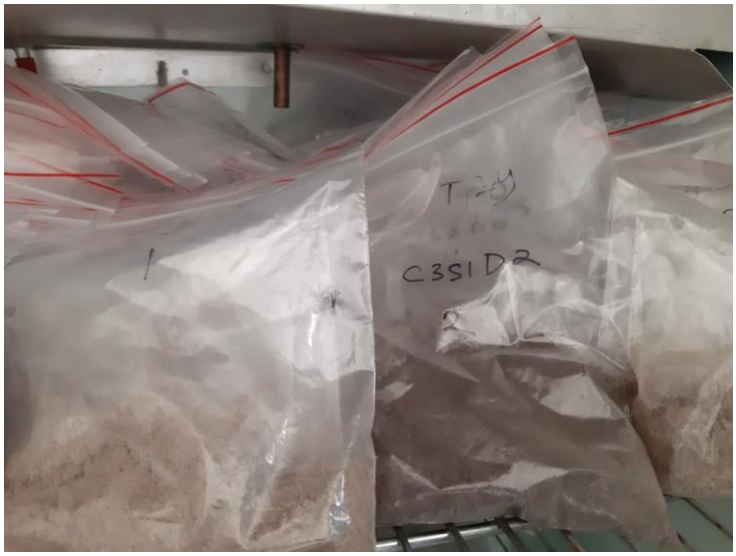


Figure 3. 2. Soil incubation process inside the incubation chamber.

Table 3.1. Chemical properties of soils and compost used in this experiment.

Soil Parameter	**Recent Soil**	**Allophanic Soil**	**Compost**
pH	5.47	5.8	6.3
TOC (g/100g dry wt)	1.22	6.67	22.97
OM (%)	2.4	12.77	58.9
Total Nitrogen (%)	0.15	0.64	0.81
CEC (me/100g)	8.3	22	67.3
Total Cd (mg Cd/kg)	0.04	0.21	0.12
Total Zinc (mg Zn/kg)	40.67	50.67	172.7
Total P (mg P/kg)	486.3	634.3	3,163
Olsen P (mg/L)	37	8.3	184

3.2.5 Laboratory Analysis

3.2.5.1 Cadmium Availability

Cadmium bioavailability in soil samples was quantified through extract with $CaCl_2$ (0.05 M). Five grams of soil was added to a 50 mL centrifuge tubes and then 30 mL of $CaCl_2$ was added. The tubes were shaken in an end-over-end shaker for two hours at room temperature. The solution was centrifuged at 5,000 g for 5 min and filtered through Whatman 42 filter paper. The filtered solution was then analysed using graphite furnace atomic absorption spectrometry (GFAAS) (Perkin Elmer 900z, Germany) (Figure 3.3).

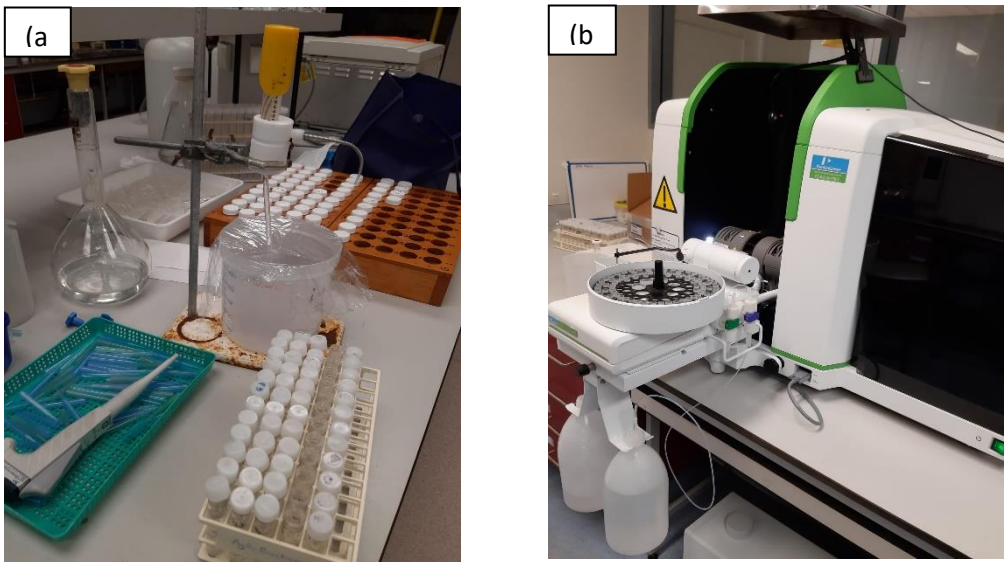


Figure 3. 3. Sample dilution prior to Cd availability analysis (a), graphite furnace atomic absorption spectrometry (GFAAS) (Perkin Elmer 900z, Germany) (b).

3.2.5.2 pH

Soil pH was measured using a pH electrode with 1:2.5 ratio of soil to water (Blakemore et al., 1987). Five grams of soil was weighed into pH beakers and then 12.5 mL of deionised water was added. The samples were stirred and left to sit for 16 hours. The next day, the pH of samples was measured.

3.2.5.3 Loss on Ignition

Loss on ignition was used as a proxy for organic matter due to the impact of Covid-19 restrictions on the progression of essential repairs to the laboratory's ELEMENTAR C, N, O analyser. Loss on Ignition analysis was performed on soils at treatment levels of 10 Mg Cd/kg and 15% compost application at day 0, Day 30 Day 60 and day 90. Sampled soil was air dried and a subsample accurately weighed into pre-weighed glass beakers. Glass beakers were placed in a muffle furnace and the temperature set at 500^{o}C for 6 hours (Figure 3.4). The samples were then removed from the muffle furnace and reweighed.

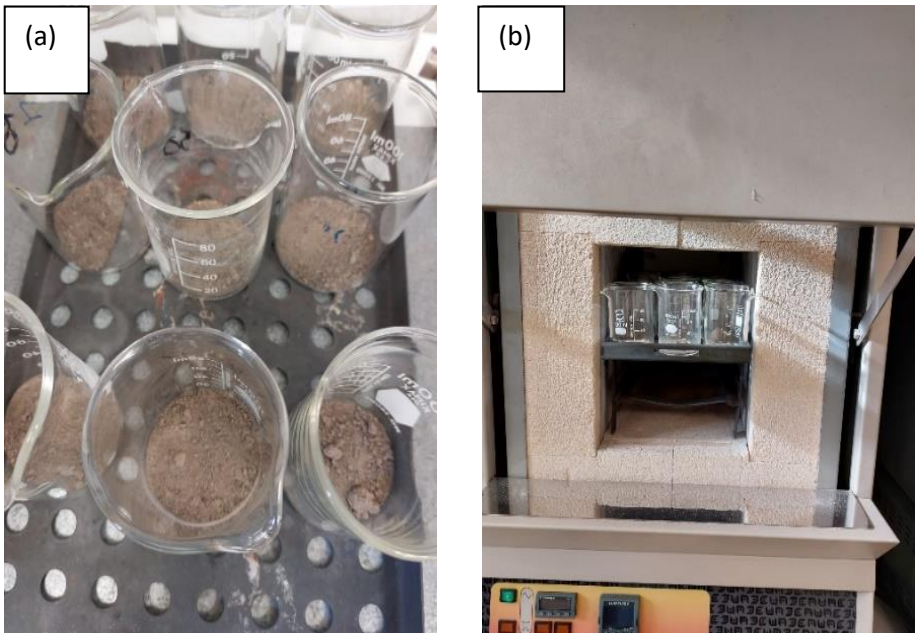


Figure 3.4. Soil subsamples on the tray (a), A muffle furnace used for loss on ignition process (b).

3.2.6 Quality Control Measures

Chemicals used in the experiments were of analytical grade. The limit of detection for Cd in this work was 0.002 mg Cd/L. The accuracy of the measurements was assessed by analysing certified reference materials in parallel with unknown samples. For total Cd concentration, CRM 051-050 clay 2 sample, a soil from western USA (total Cd 42.2 mg Cd/kg), was used as the certified reference material. For plant total tissue Cd analysis, NIST 1573a (National institute of standards and technology, tomato leaves-1.52 mg Cd/kg) was used.

3.2.7 Statistical analysis

Statistical analysis was carried out using Minitab 18 (OriginLab, USA) statistical software. The effect of Cd addition and organic matter application on different soil variables was analysed statistically using a one-way ANOVA test; if a significant ($P<0.05$) main effect was found, the difference among treatment means was analysed by a Tukey HSD posthoc test.

3.3 Results

3.3.1 pH result

The pH result from this experiment showed that overall, there was a trend for pH to increase as a function of compost application and the five different Cd levels (0.1,2,5,10 mg Cd/kg) and to decrease as a function of time (Figure 3.5, 3.6). The pH values tended to be similar at different Cd levels. Although the pH values experienced a similar trend for both soils, significant changes were observed as a function of compost application and incubation times in the Recent Soil ($P<0.05$) but not for the Allophanic Soil. The greatest pH values occurred for 15% of compost application on Day 0. The lowest pH values across all Cd levels were recorded at the 0% compost amendment rate on Day 90.

In the Recent Soil, the control compost treatment on Day 0 was significantly different to the other treatments, except for the application of 5% compost to the 0, 2 and 10 mg Cd/kg treatments. As the incubation period increased (Day 30, Day 60 and day 90), the control compost treatments showed significantly differences to the other compost treatments across all five Cd levels. From 2 mg Cd/kg onwards, there was no significant change in pH values between the 10% and 15% compost application.

In the Allophanic Soil, there was a trend towards pH increase as a function of compost application and decrease as a function of time although these changes in pH were not significant. The results from day 0 were the highest for all Cd levels, with an average of 5.09, 5.20, 5.30 and 5.38 for 0%, 5%, 10% and 15%, respectively. The results for days 30, 60 and 90 were similar for the 0%, 5%, 10% and 15%, with a range of 4.83-4.85, 4.95-5.0, 5.02-5.08 and 5.09-5.17 respectively. These results indicate that the magnitude of change in pH values on Day 0 was greater relative to subsequent days, where the difference was small from Day 30

onwards. The Allophanic have a higher pH buffering capacity, which explains the lower increase in pH compared to the Recent soil.

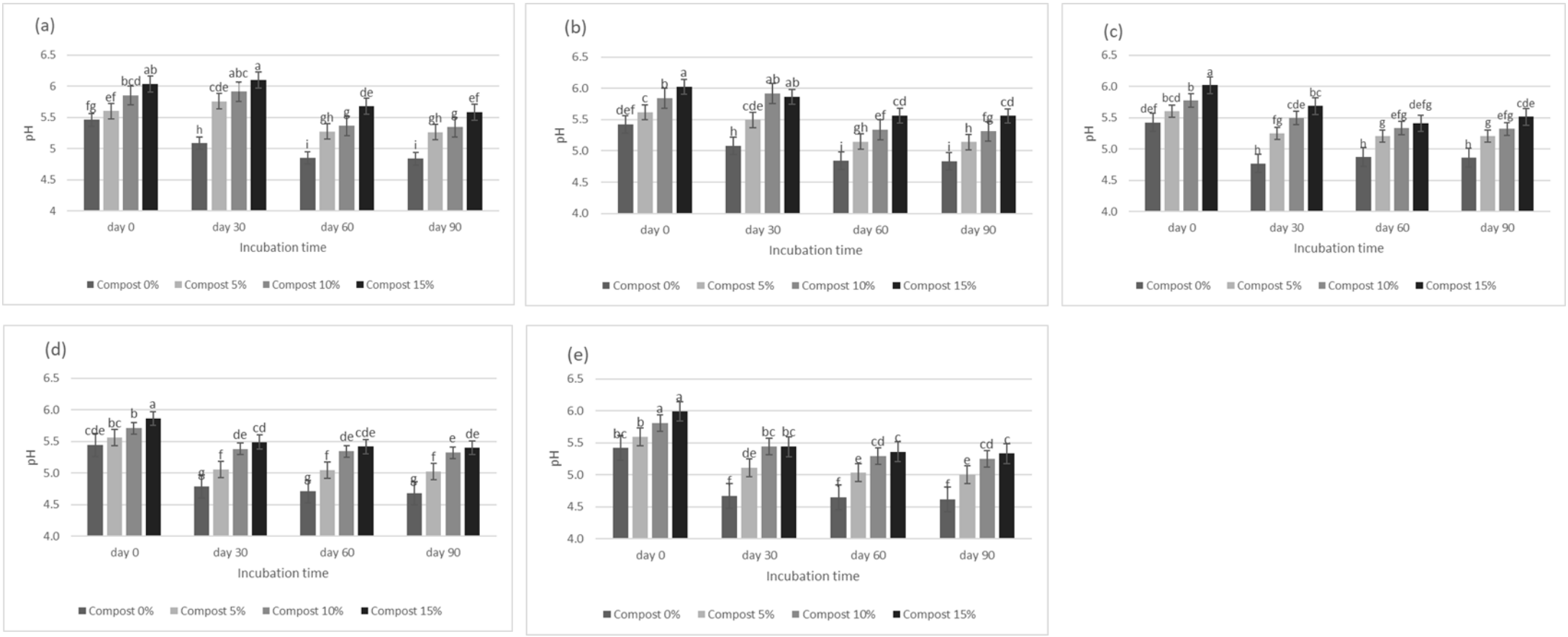


Figure 3. 5. The effect of variable rates of compost application and 4 different incubation times on pH for Recent Soil amended with 5 Cd application rates: 0 mg Cd/kg (a), 1 mg Cd/kg (b), 2 mg Cd/kg (c), 5 mg Cd/kg (d), and 10 mg Cd/kg (e). Means with the same letter are not significantly different at $P<0.05$ (n=3).

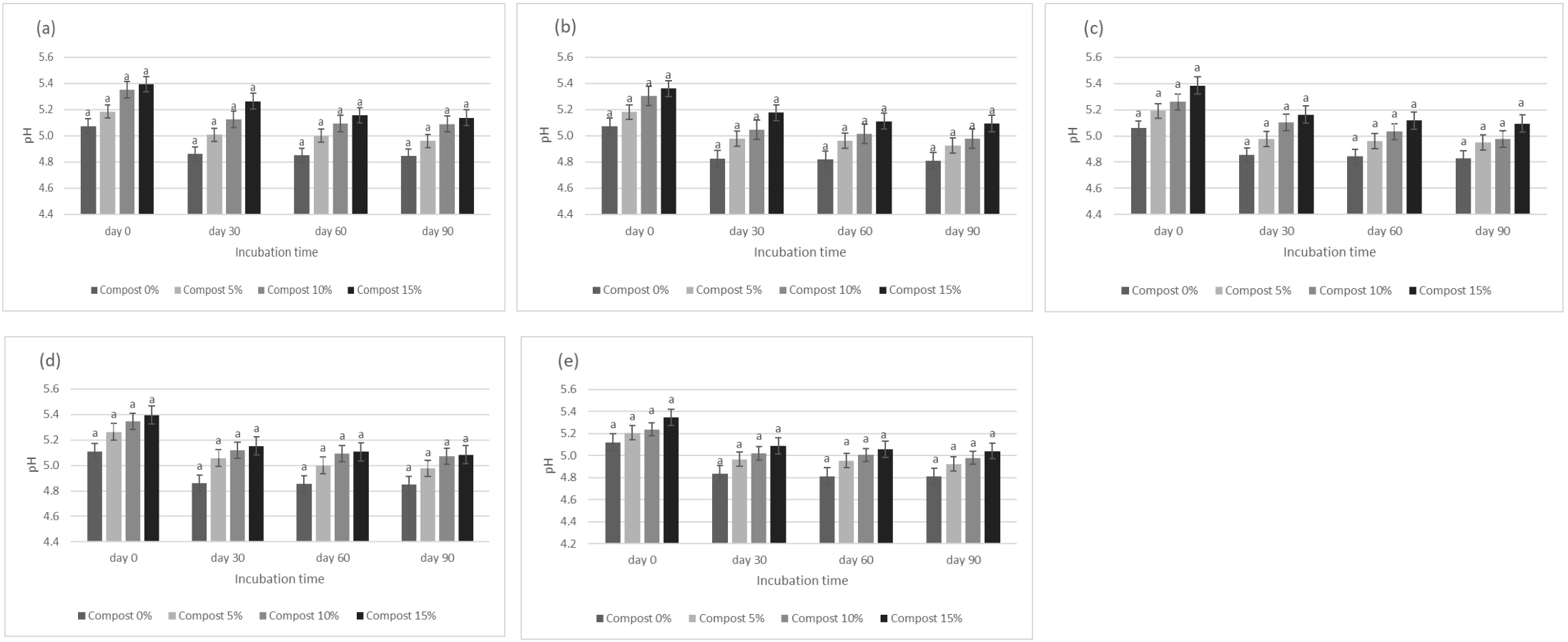


Figure 3.6. The effect of variable rates of compost and 4 different incubation times on pH for Allophanic Soil amended with 5 Cd application rates: 0 mg Cd/kg (a), 1 mg Cd/kg (b), 2 mg Cd/kg (c), 5 mg Cd/kg (d), and 10 mg Cd/kg (e). Means with the same letter are not significantly different at $P<0.05$ (n=3).

3.3.2 Cadmium Availability

Cadmium availability (quantified through extraction) increased as a function of increasing Cd concentration in the soil. Generally, in both soils, Cd availability decreased as a function of compost application, for all treatments and at all sampling times (Figure 3.7, 3.8). . There was a significant difference between the combination of compost application and incubation time on Cd availability.

In the Recent Soil, the observed increase in Cd availability was not significant from Day 0 to Day 30 (Figure 3.7). However, a significant increase in Cd availability was recorded from Day 30 to Day 60. This indicates that there was potentially a compost effect in reducing Cd availability decreases over time, but that this effect was time dependent, with Cd availability reaching the highest value on Day 60. Different levels of compost application had a significant effect on Cd availability for all soil Cd amendments at all sampling times, except for 0 mg Cd/kg on all days. There was a general trend that from Day 60 to day 90, the values tended to decrease, although these were mostly nominal, with limited significant differences between mean values.

For the Allophanic Soil, there was no significant change over time recorded for Cd availability except for a significant decrease recorded for the control (0 mg/kg) Cd treatment between Day 0 and Day 60. For the 1,2, and 5 mg Cd/kg treatments, there was a similar trend: on day 0, the Cd availability was low and experienced an increase on Day 30, then the available concentration stablisied. For the 10 mg Cd/kg application, from day 0 to day 90, the values tended to be the same. No trend was observed for the 0 mg Cd/kg on Day 60, which experiencing a decrease in Cd availability relative to Day 30.

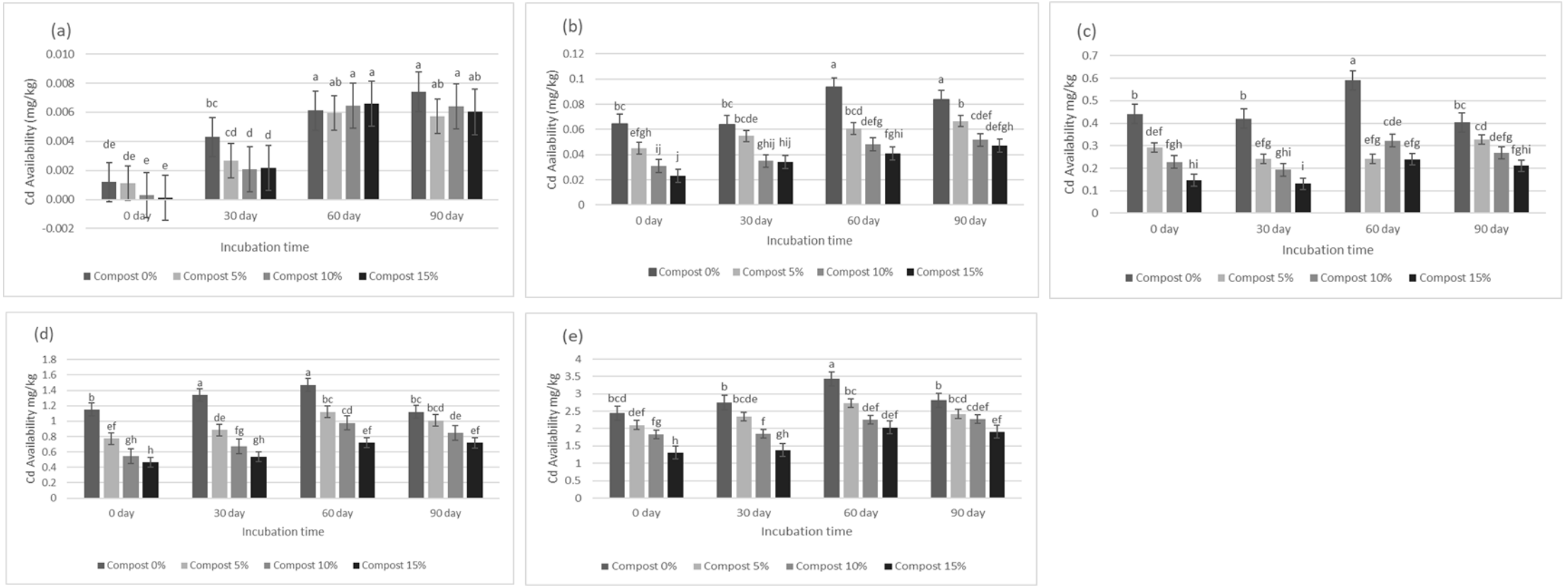


Figure 3.7. The effect of variable rates of compost application and 4 different incubation times on Cd availability (mg Cd/kg) for Recent Soil amended with 5 Cd application rates: 0 mg Cd/kg (a), 1 mg Cd/kg (b), 2 mg Cd/kg (c), 5 mg Cd/kg (d), and 10 mg Cd/kg (e). Means with the same letter are not significantly different at $P<0.05$ (n=3).

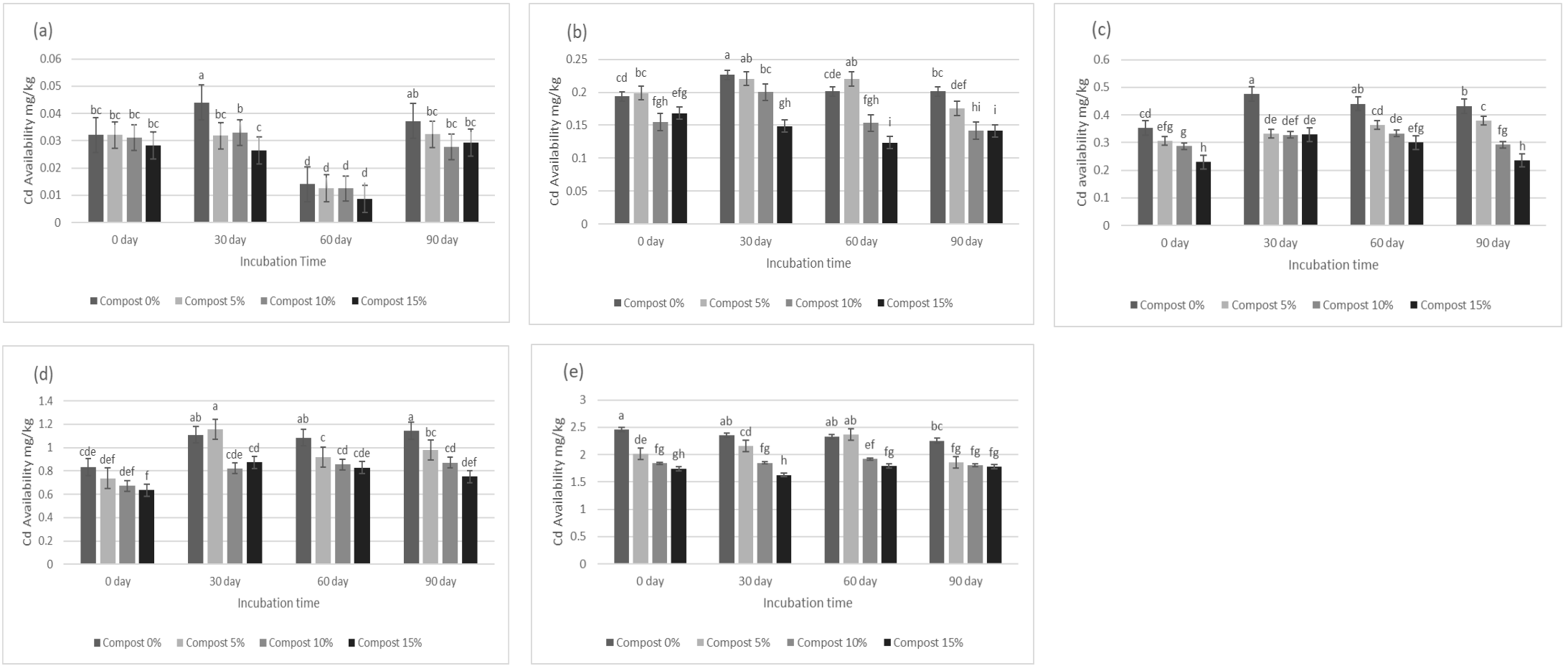


Figure 3.8. The effect of variable rates of compost application and 4 different incubation times on Cd availability (mg Cd/kg) for Allophanic Soil amended with 5 Cd application rates, 0 mg Cd/kg (a), 1 mg Cd/kg (b), 2 mg Cd/kg (c), 5 mg Cd/kg (d), and 10 mg Cd/kg (e). Means with the same letter are not significantly different at $P<0.05$ (n=3)

3.3.3 Loss on Ignition

Loss on ignition was used to observe organic matter (%) changes over time during the incubation experiment. Due to limited time and instrument malfunctions that could not be resolved due to Covid travel constraints, loss on ignition was used to quantify organic matter for the 10 mg Cd/kg and 15% compost applications from both soils. The results showed that in both soils, generally there was no significant influence of incubation time on organic matter (%) although there was a trend to a slight decrease in both soils (Table 3.2) and (Table 3.3).

Table 3.2. Organic matter (%) in the Recent Soil used for the Incubation Experiment.

Cd Application (mg/kg)	Compost Application (%)	Incubation Times (days)	Organic matter (%)
10	15	0	10.11±0.87 (a)
10	15	30	10.03±0.53 (a)
10	15	60	10.05±0.64 (a)
10	15	90	10.02±0.26 (a)

Data are mean±standard error of three replicates. Means with the same letter are not significantly different at P<0.05 (n=3).

Table 3.3. Organic matter (%) in the Allophanic Soil used for Incubation Experiment.

Cd Application (mg/kg)	Compost Application (%)	Incubation Times (days)	Organic matter (%)
10	15	0	24.56±0.65 (a)
10	15	30	24.56±0.57 (a)
10	15	60	24.34±0.23 (a)
10	15	90	24.29±0.42 (a)

Data are mean±standard error of three replicates. Means with the same letter are not significantly different at P<0.05 (n=3).

3.4 Discussion

3.4.1 pH

The increasing trend of pH as a function of compost application, for sampling at the same time, could be explained through organic matter input on soil improving buffering capacity allowing the soil to maintain a stable pH despite the presence of external influences such as added trace elements (in this case, Cd) (Curtin & Trolove, 2013; Dvořáčková et al., 2022).

Buffering capacity is primarily influenced by cation exchange capacity (CEC) due to the presence of organic matter (Jansen van Rensburg et al., 2009). Humic substances in organic matter contain a high concentration of negative surfaces leading to an increase in CEC and improving buffering capacity (McLaren & Cameron, 1996; Naramabuye & Haynes, 2006). A high CEC value also causes competition of Cd and Ca in the soil solution due to both Cd-sorption and Ca-desorption by soil, as Ca is one of the major cations of base saturations (Temminghoff et al., 1995).

From a temporal perspective, pH values tended to decrease over time. Organic matter in this experiment might have experienced decomposition due constantly elevated temperature of the incubation (Zech et al., 1997). Decomposition would lead to a reduction in organic matter percentage which in turn decreased Cation Exchange Capacity (CEC) causing a gradual reduction in soil in pH (Naramabuye & Haynes, 2006). A decline in soil pH might also be associated with a a reduction in soil buffering capacity. Therefore, the highest pH values which occurred on soil amended with 15% compost on day 0 may be due to the high soil buffering capacity as a function of the greatest rate of compost input, where the time was insufficient for decomposition to have begun. In comparison, the lowest pH values occurred for all Cd levels at a treatment rate of 0% compost application on day 90. This indicates that where no organic matter was applied to the soil, pH was due to the low soil buffering capacity and the decomposition of any native organic matter (i.e. that in the soil already) occurring by day 90.

3.4.2 Cd Availability

The results from both soils showed that a decrease in Cd availability occurred as a function of compost application. This observation is in agreement with the study conducted by Al Mamun et al. (2016) who showed that application of compost can reduce Cd availability in soil. Awasthi et al. (2019) describe an experiment where wheat-straw compost application reduced Cd availability in the soil up to 28.45 – 32.04%. Organic matter in soils has an important role in Cd immobilisation and Cd availability via a complex interaction between the negative charges in SOM (carboxylic and phenolic hydroxyl groups) and Cd (Zhao et al., 2014).

This experiment also highlighted that Cd availability increased as a function of the increasing level of applied Cd in both soils. Cadmium availability is influenced by Cd concentration,

although in some cases, a high Cd concentration does not directly correlate with a high Cd availability (Chapman, 2007; Gray et al., 1999). A high Cd concentration in the soil with a high Cd concentration history should be a concern for agricultural systems and trigger more focused environmental monitored. This is because such land can pose a potential risk for Cd transfer to edible plants (Wang et al., 2015).

The available cadmium concentrations over time were different between the two soils, which might be influenced by the chemical characteristics of each soil (Table 3.1). Organic matter decomposition could possibly reduce its ability to complex Cd in the soil due to a decrease of negative soil charges (Wambeke, 1992). A pot experiment by Antoniadis and Alloway (2001) with ryegrass (*Lolium perenne* L.) showed that at 25^{o}C, organic matter percentage decreased by 16% due to a high decomposition rate and this resulted in an increased Cd concentration in the soil. Decomposition is mainly driven by a high temperature and high precipitation (McCauley et al., 2009).

The recorded increase in Cd availability from day 0 to Day 30 was not significant in the Recent Soil, but this increase become significant from Day 30 to Day 60 (reaching the peak at Day 60). This significant increase of Cd availability in the Recent Soil might be influenced by its low organic matter percentage (2.4%) (Table 3.1). In contrast, there was no significant change in Cd availability over time for the Allophanic Soil. The Allophanic Soil used in this experiment had a greater organic matter content than the Recent Soil (12.77%) which improves baseline Cd retention (Table 3.1). The presence of highly reactive allophane in the Allophanic Soil may also decrease the rate of decomposition of organic matter attaching to it (Miltner & Zech, 1998). Allophanic Soil has an abundance of microaggregates which have a significant ability to preserve organic matter in the soil (Chevallier et al., 2010). Therefore, in this experiment, organic matter in Allophanic Soil experienced slower decomposition and better baseline Cd retention relative to Recent Soil.

The results of Cd availability on Day 90 decreased relative to Day 60 for the Recent Soil. This anomalous result might be influenced by the Covid-19 lockdown period in 2021. This occurred over the time period between Day 60 and Day 90 and during this time 70% field capacity could not be maintained. One of the possibilities of this result could be the lack of moisture at Day 90 in the Recent Soil. Lack of moisture due to soil drying would decrease Cd availability in the soil solution as a function of increased metal sorption onto soil surfaces (Li et al., 2015). Such

results did not occur on Allophanic Soil because the Allophanic Soil had a higher moisture content (25.26%) compared to Recent Soil (4.3%). A decrease in moisture may not have affected the Allophanic Soil due to its natural soil properties.

3.4.3 Loss on Ignition

Considering the loss on ignition data, a trend of decreasing organic matter (%) was observed as a function of incubation time. This result agrees with a study conducted by Martius et al. (2001) who found that organic matter decomposition increased over time, particularly at high temperature. Han-Song et al. (2010) also mentioned that there was a possibility of organic matter decomposition months after compost application to soils. In the decomposition process, there are chemical and biochemical process which cause a release of dissolved organic matter (DOM). The important factors causing decomposition are high temperature and high precipitation (McCauley et al., 2009). Decomposition can be more rapid if it occurs in a high temperature for longer period.

3.5 Conclusion

Cadmium availability in the current study was influenced by several factors, including total Cd in the soil, compost application also soil pH. A higher level of compost input increased soil pH when considering samples at the same time point. This is because organic matter from the compost improved soil buffering capacity, and this eventually caused a decrease in Cd availability. High Cd input resulted in a high Cd availability in the soil. An increase of Cd availability is therefore associated with the organic matter percentage in both soils which experienced a decrease over time. However, the degree of Cd availability change between the two soils was different. In the Recent Soil, a significant increase was observed from Day 30 to Day 60. For the Allophanic Soil a slight decrease was observed, but this change was not significant as a function of time.

The data presented in this Chapter show that the native organic matter content of each soil played an important role in explaining Cd availability results over time. The Allophanic Soil had a higher organic matter percentage than the Recent Soil.

The Covid-19 lockdown of 2021 potentially had an impact on the results presented in this Chapter. To an inability to access the lab, incubation soils were not watered from Day 60 to day 90, meaning that a soil moisture near 70% field capacity could not be maintained. As the soil dried over this period, the moisture content would have decreased, and this may have induced a lower concentration of available Cd on day 90 compared to Day 60. Further research on the influence of applied organic matter on Cd availability in the soil over a longer incubation period and different incubation temperature is recommended to confirm these results and to provide data to support more in-depth explanation for the effect of organic matter and time on Cd availability in soil.

4. Pot trial experiment to investigate the effect over time of compost on biomass production and the Cd uptake concentration by Bok Choy (Brassica chinensis. L).

4.1. Introduction

Bok Choy (*Brassica chinensis.* L) is a leafy vegetable which is grown and consumed worldwide due to its high nutritional value (Rafiq et al., 2014; Sun et al., 2010). In some countries such as China and the South East Asian countries, Bok Choy is highly cultivated because of its rapid growth and low cultivation cost (Capistrano & Marten, 1986; Sun et al., 2010) . However, As a leafy vegetable, Bok Choy (*Brassica chinensis.* L) uptakes relatively high amounts of trace elements, including Cd (Han-Song et al., 2010). Furthermore, due to globalisation and rapid construction, there are more urban settlements and agricultural lands side by side resulting in a higher release of Cd contamination to agricultural soils from manufacturing, sewage sludge and wastewater irrigation (Coudon et al., 2018; Ma et al., 2018). Therefore, it is important to limit Cd transfer from soil to the edible parts of Bok Choy (Rafiq et al., 2014) in order to reduce the potential for Cd accumulation and the potential for risk to food safety and human health (Wang et al., 2004).

Applying soil amendments such as manure compost, zeolite and organic materials is an affordable option to reduce plant uptake of Cd in intensive agricultural systems (Kiran et al., 2017). Han-Song et al. (2010) showed that compost application to Cd spiked soils reduced Cd uptake by 56.2%–62.5% compared to control soils without compost input. Furthermore, an increase of the Bok Choy root biomass of 4.6- 10.1% was recorded when 120 g of compost was applied , with an associated increase of 4.5-5.0% for the shoot biomass (Han-Song et al., 2010). However, the application of such amendments should be renewed periodically otherwise Cd could be re-released in the soil solution (Antoniadis & Alloway, 2001).

The effect of compost application over time on Cd uptake and dry weight plant biomass in leafy vegetables is limited in the literature. Although compost application potentially could decrease Cd uptake by plants and increase plant biomass in Cd contaminated soils, the decomposition of organic matter over time and the potential of Cd releasing back to soil solution should be considered. This chapter investigates the effect of compost application on

the total Cd concentration in Bok Choy (*Brassica chinensis.* L), root and shoot biomass. Supporting results such as pH, Cd availability and organic matter percentage in the soil over time are used to explain the plant observations. The pot trial was carried out in greenhouse, and plant harvest and soil sampling were conducted at both Day 30 and Day 60 after planting to observe any change in Cd uptake over time.

4.2 Materials and Method

4.1.1 Outline of the Experiment

Bok Choy (*Brassica chinensis* L) was grown in soil (Recent Soil and Allophanic Soil) spiked with Cd to a concentration of 10 mg kg^{-1} Cd and four levels of compost application. Three replicates per treatment were used in this study which conducted under greenhouse conditions. Total plant Cd concentration, dry weight biomass and Cd availability, pH were analysed at Day 30 and Day 60 of plant growth.

4.1.2 Pot experiment

The pot trial was conducted in a greenhouse of the Massey University Plant Growth Unit with day and night temperature of 25^0C and 22^0C. Two bulk samples of Recent Soil and Allophanic Soil collected from the locations described in Chapter 3 were used in this experiment. The Cd concentration of these soils was 0.04 and 0.21 mg Cd/kg, respectively (Table 3.1). The two soil types were air dried for approximately 5 days at 30^0C and passed through 4 mm sieve.

Soils were spiked with a calculated amount of $CdCl_2$ at 10 mg Cd/kg and combined with compost at rates 0%, 5%, 10% and 15% separately to form four treatments for each soil with three replicates in a 2 x 1 x 4 x 3 complete randomised design. Two identical experimental sets were maintained, the first set was harvested on Day 30, and the second was harvested on Day 60. Therefore, the total number of pots was 48, with each pot containing one kilogram soil, and kept for two weeks at 70% pot field capacity to equilibrate Cd with the soil matrix. After two weeks, three seeds of Bok Choy (*Brassica chinensis* L) were planted in each pot, and

one week after plant germination, two plants were removed, maintaining just the healthiest one.

Figure 4.1. Pot trial experiment in the greenhouse.

4.1.3 Plant Analysis

4.1.4.1 Plant Biomass

At harvest, the plants were removed from the pot and the shoot and root parts were separated. Both shoot and root parts were gently washed to remove the remaining soil and dried at 60^{o}C to reach a constant weight. The total dry weight of shoot and root then was recorded. Dry shoot and root were finely ground using a mortar and pestle for further chemical analysis.

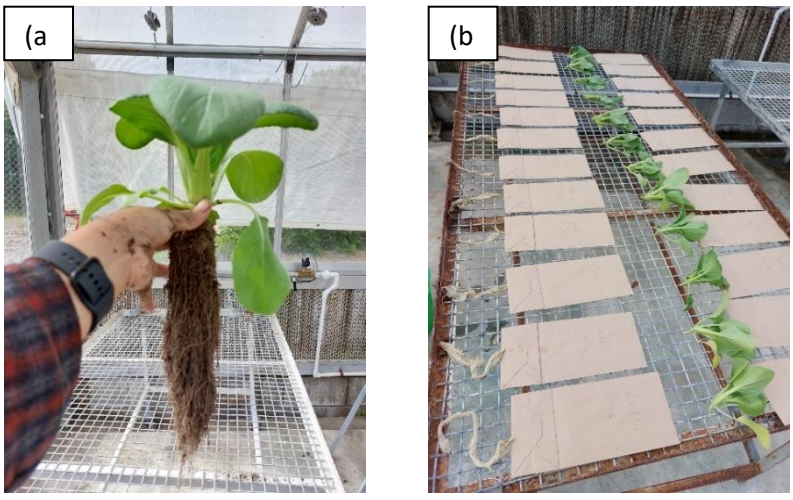


Figure 4.2. Removing plant from pot (a), Separating roots and shoots (b).

4.1.4.2 Cadmium Concentration and Cadmium Content

A subsample (0.1g) of dried and ground shoot and root biomass sample was digested with 10 mL of 70% HNO_3 at 120^oC for three hours. The final digest solution was diluted with de-ionised water and filtered through Whatman 42 filter paper. The Cd concentration in digest solution then was analysed using Microwave Plasma Atomic Emission Spectroscopy (MP-AES). For Cd content, the result of Cd concentration from MP-AES was multiplied by plant biomass.

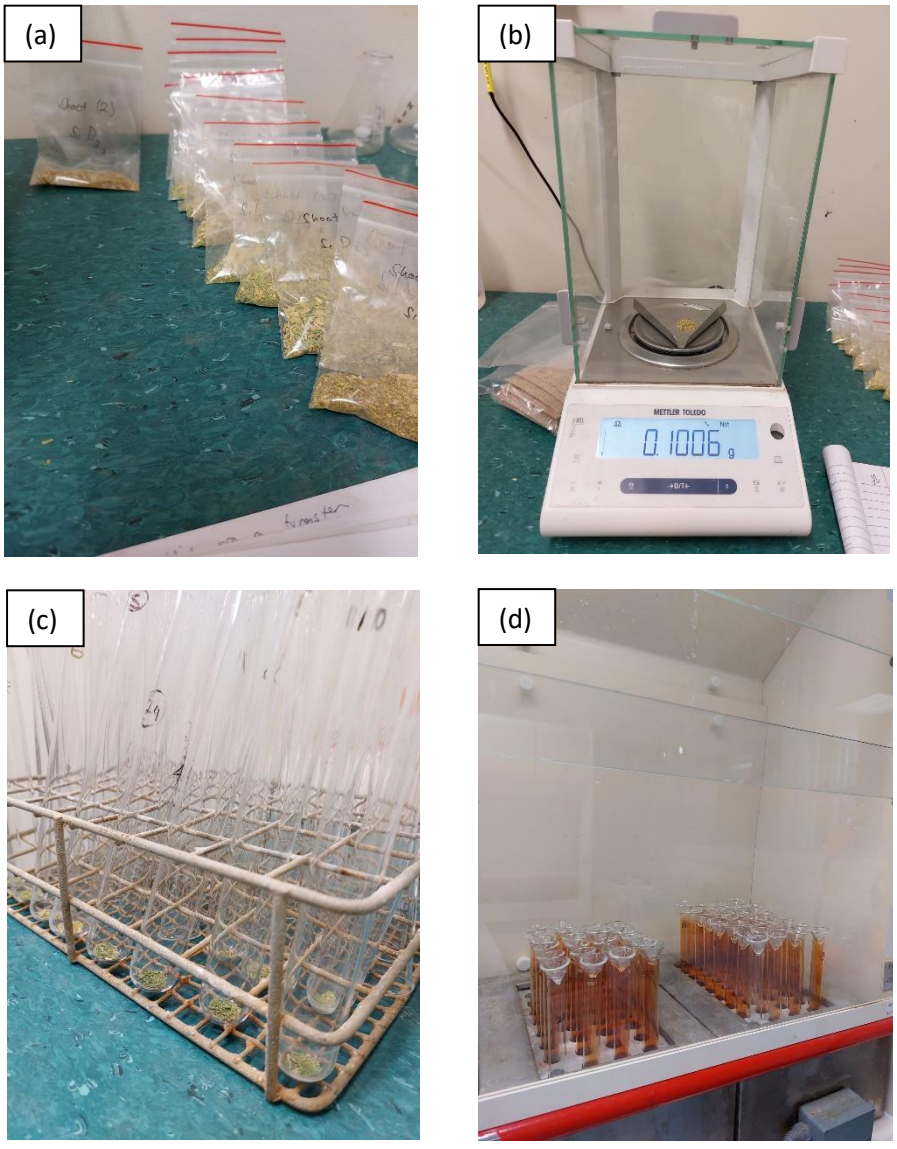


Figure 4. 3 Crushed plant subsamples (a), Weighing plant subsamples for digestion (b), Plant subsamples inside digestion tubes (c), A digestion process (d).

4.1.5 Soil Analysis

4.1.5.1 pH

The pH of soils was measured (1:2.5 w/w soil:water ratio) using a pH electrode (Blakemore et al., 1987). Five grams of soil was weighed into a pH cup and 12.5 mL deionised water was added. The samples were stirred and left for overnight. The next day, the pH was measured.

4.1.5.2 Cadmium Availability

Five grams of soil and 30 mL of $CaCl_2$ (0.05 M) were added to centrifuge tubes and extracted with an end-over-end shaker for two hours at room temperature. The extracted solution was centrifuged at 5,000 g for 5 min and filtered through Whatman 42 filter paper. The filtered solution was then analysed for Cd concentration using Microwave Plasma Atomic Emission Spectroscopy (MP-AES).

4.1.5.3 Loss on Ignition

Loss on ignition was used as a proxy for organic matter due to Covid-19 technical issues with the ELEMENTAR C, N, O analyser. Loss on Ignition analysis was performed on soils at treatment levels of 10 mg Cd/kg and 15% compost application at day 0, Day 30 Day 60 and Day 90. Sampled soil was air dried and a subsample accurately weighed into pre-weighed glass beakers. Glass beakers were placed in a muffle furnace and the temperature set at 500^{o}C for 6 hours. The samples were then removed from the muffle furnace and reweighed.

4.1.5.3 Quality Control Measures

All chemicals used in the experiments were of analytical grade. The limit of detection for Cd in this work was 0.002 mg Cd/L. The accuracy of the measurements was assessed by analysing certified reference materials in parallel with unknown samples. For total Cd concentration, CRM 051-050 clay 2 sample, a soil from western USA (total Cd 42.2 mg Cd/kg), was used as the certified reference material. For plant total tissue Cd analysis, NIST 1573a (National institute of standards and technology, tomato leaves-1.52 mg Cd/kg) was used.

4.3. Results

4.3.1 Dry Weight Biomass

Generally, the application of compost increased dry weight biomass compared to treatment with 0% compost in both soils (Figure 4.4). The results also showed that dry weight biomass at Day 60 was higher compared to Day 30. There was a significant difference in dry weight biomass of both roots and shoots of Bok Choy grown in both soils as a function of the combination of compost application and 10 mg Cd/kg, except for shoots in the Recent Soil.

In the Recent Soil, the highest root biomass occurred for the 10% compost application on Day 60 (2.14 g), and the lowest was for the 0% compost on Day 30 (0.13 g). There was no significant difference in root biomass that could be attributed to compost application on Day 30 when the root biomass across the four levels of compost application ranged between 0.13 – 0.35 g. On Day 60 there was a significant difference in root biomass between the 0% compost application (1.53 g) and the 5% (1.95 g), 10% (2.14 g) and 15% (1.89 g) applications. For shoot biomass, there was no significant difference in biomass as a function of compost application. On Day 30, the shoot biomass ranged between 1.38 – 1.67 g, and on Day 90 ranged between 2.74 – 3.50 g.

In the Allophanic Soil, the growth of Bok Choy without compost application was limited on both Day 30 and Day 60. The root and shoot biomass ranged from 0.02 – 0.15 g and 0.39 – 1 g respectively. On Day 30, both the root and shoot dry weight biomass was significantly increased by the 5% compost application and remained constant for further increases of compost application. However, on Day 30, root biomass (DW) increased up to the 10% compost application rate. The increase in root dry weight biomass as a function of the 5% compost application rate was by a factor of 25 on Day 30, and 12 on Day 60 relative to the 0% compost treatment. These increases for the shoot biomass were by factors of 8 and 5 for Day 30 and Day 60, respectively.

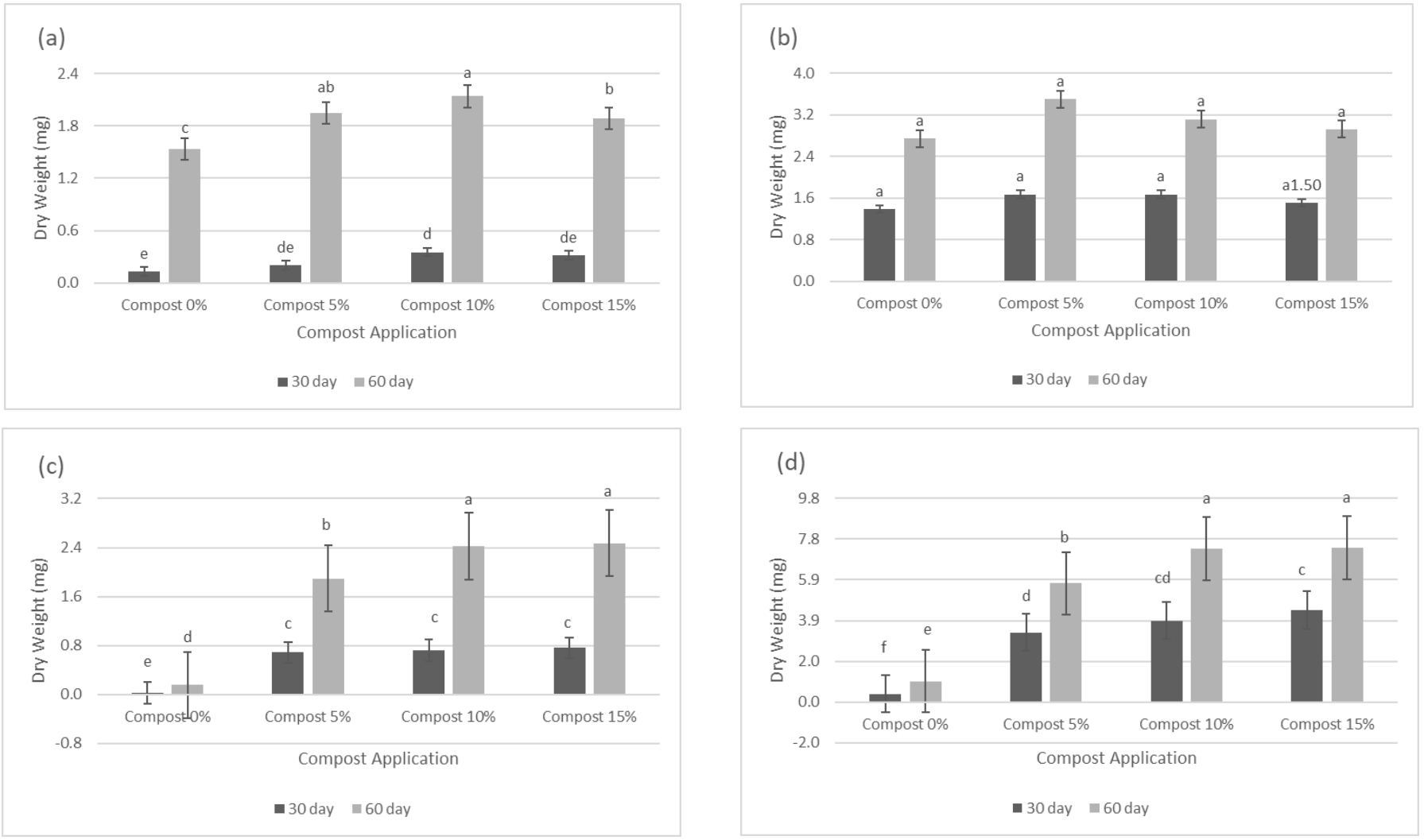


Figure 4.4. The effect of variable rates of compost application to soil with 10 mg Cd/kg on Dry Weight Biomass of Bok Choy, Recent Soil Root (a), Recent Soil Shoot (b), Allophanic Soil Root (c), Allophanic Soil Shoot (d). Means with the same letter are not significantly different at $P<0.05$ (n=3).

4.3.2 Cadmium Concentration

In general, the majority of plant tissue Cd concentrations on Day 60 were lower than the corresponding concentration on Day 30 (Figure 4.5). For roots in the Recent Soil, no significant difference was observed between these two harvests, but there was a trend towards decreasing Cd concentration as the compost application rate increased. However, the Cd concentration in shoots was significantly lower on Day 60 compared to Day 30 at each level of compost application, and the magnitude of the difference increasing as the compost application increased. For the Allophanic Soil, there was no trend in Cd concentration in roots across the levels of compost application. The shoot Cd concentration in the Allophanic Soil also showed no trend as a function of compost application for the first harvest (Day 30). However, there was a decrease in shoot Cd concentration observed on Day 60 and this reduction was significant up to the 5% compost application.

There was a trend towards a reduction in the Cd concentration of Bok Choy roots growing in the Recent Soil as a function of rate of compost application for both harvesting times. On Day 30, there was no significant difference in Cd concentration between the 0% and 5% compost application rates with mean values of 223 mg Cd/kg and 231 mg Cd/kg recorded respectively. The mean Cd concentration reduced for the 10% (193 mg Cd/kg) and 15% compost application rates (182 mg Cd/kg). On Day 60, there was a significant decrease in the Cd concentration of roots in the Recent Soil amended with 5% compost (60 mg Cd/kg). Relative to the 0% compost application rate (166.67 mg Cd/kg) the mean Cd concentration for the 5% and 10% compost application were similar but was significantly reduced at the 15% compost application rate (34.14 g). Shoots harvested on Day 30 from the 0% compost application rate had a mean Cd concentration of 180 mg Cd/kg, which reduced for all compost application rates (5%,10% and 15%) with a range of concentration values for these compost applications from 148-155 mg Cd/kg. On Day 60, the mean Cd concentration for the 0% compost rate was 122 mg Cd/kg, which significantly reduced for all compost applications. The minimum Cd shoot concentration was 26.7 mg Cd/kg, recorded for the 10% compost application rate although this was not statically different to the Cd concentration for the 15% treatment (33.33 mg Cd/kg).

In the Allophanic Soil, two trends were observed. There was initially an increase then a decrease in the Cd concentration of roots on Day 30, with a general trend towards decreasing Cd concentration in roots at Day 60 (this difference relative to the control was only significant for the 5% compost application rate). For shoots, there was a decrease in Cd concentration as a function of compost application at both sampling times. For root biomass collected from the 0% compost application, the Cd concentration was greater on Day 60 (174 mg Cd/kg) when compared to Day 30 (107 mg Cd/kg). This increase with time was not observed for any other compost application rate for shoots or roots for either soil. For roots, there was no significant change in Cd concentration in plants as a function of any applied compost rate (5-15%) on either day. For shoots, the Cd concentration at both Day 30 and Day 60 was significantly lower for the 15% compost application relative to the other treatments.

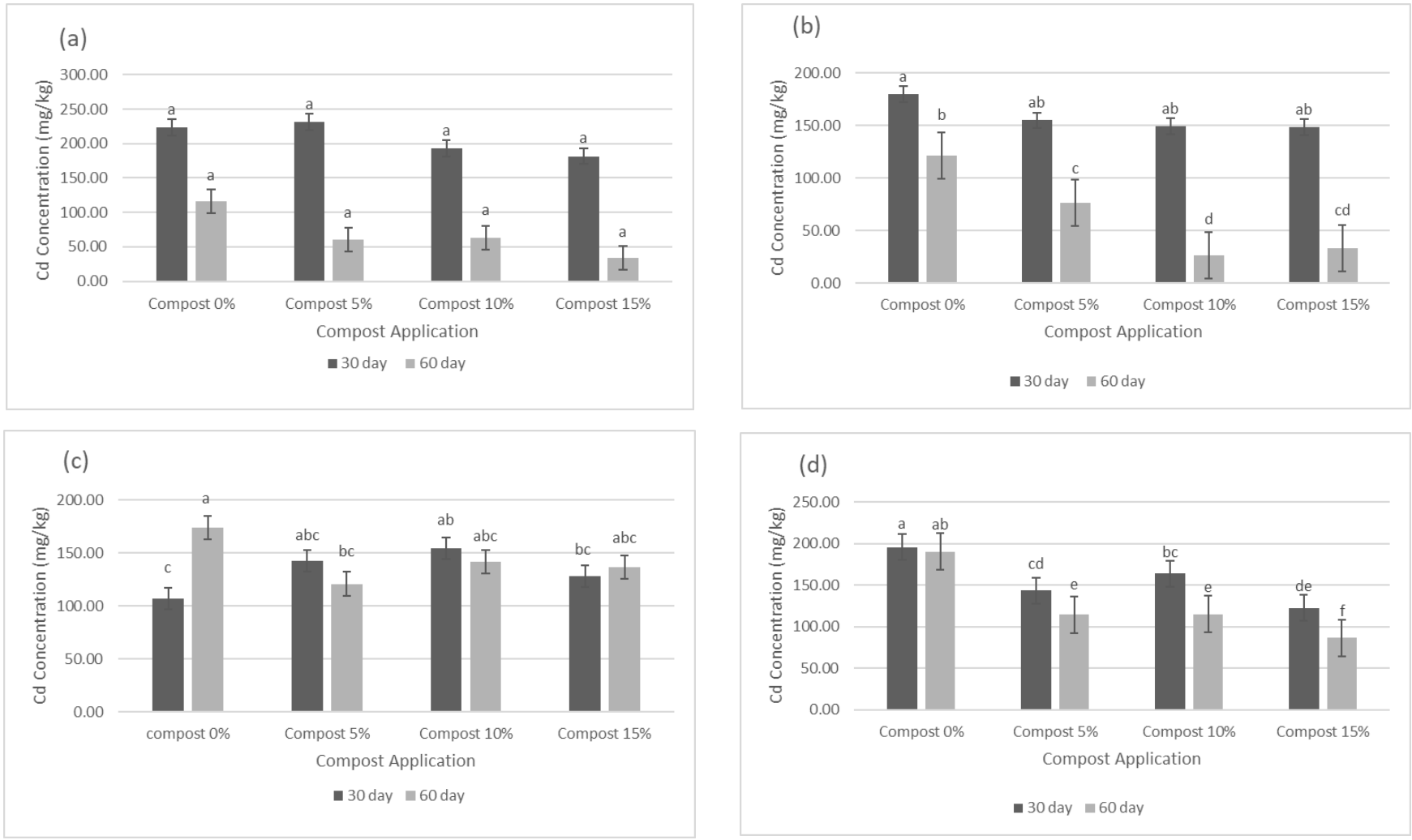


Figure 4.5. The effect of variable rates of compost application to soil with 10 mg Cd/kg on Cd Concentration (mg/kg) of Dry Bok Choy: Recent Soil Root (a), Recent Soil Shoot (b), Allophanic Soil Root (c), Allophanic Soil Shoot (d). Means with the same letter are not significantly different at $P<0.05$ (n=3).

4.3.3 Cadmium Content

Cadmium content (mg) is the total mass of accumulated Cd in the plants and is calculated as the product of concentration times biomass. This number is important in any investigation of the potential toxicity of the plants under the experimental conditions of the study (Rodda et al., 2011). Total Cd content in plants is presented in Figure 4.6 and the results showed that for the majority of harvested plants, the Cd content in both shoots and roots is higher on Day 60 relative to Day 30 (Figure 4.6). This is because the plants grew bigger leading to an increase in dry weight biomass (Figure 4.4). Therefore, the plants accumulated a greater Cd content.

In the Recent Soil, the difference in plant Cd content between the harvest times as a function of compost application decreased for roots. The root Cd content on Day 60 for the 0% compost treatment was the highest recorded (0.18 mg). There was no significant difference observed between the 5% and 10% compost application, with an average content of 0.12 mg which was the same as the control. A significant decrease in root Cd content was recorded for the 15% compost application rate, with a Cd content of 0.06 mg. At Day 30 there was no significant difference in Cd content between the 4 compost treatments, with content ranging from 0.03-0.06 mg. In shoots, there was again no significant difference in Cd content between the compost levels at Day 30 (range of 0.2-0.5 mg). On Day 60, there was no significant difference between the 0% and 5% compost application rates, however Cd content dropped significantly for the 10% and 15% compost applications (to a minimum value of approximately 0.1 mg).

In the Allophanic Soil, the Cd content on Day 60 was higher than on Day 30 for both roots and shoots and Cd content increased as a function of compost application, with the exception of the 15% compost application rate. In roots, the Cd content at the 0% compost application rate was extremely low on both days (<0.03 mg). On Day 30, there was no significant difference in Cd content between the 5%, 10% and 15% compost applications, with an average value of 0.1 mg. On Day 60, root Cd content for the 5%, 10% and 15% compost applications increased by 133%, 205% and 248% respectively, although the difference between 10% and 15% was not significant. In shoots harvested from Allophanic Soil, there was no significant change in Cd content as a function of compost level at either day.

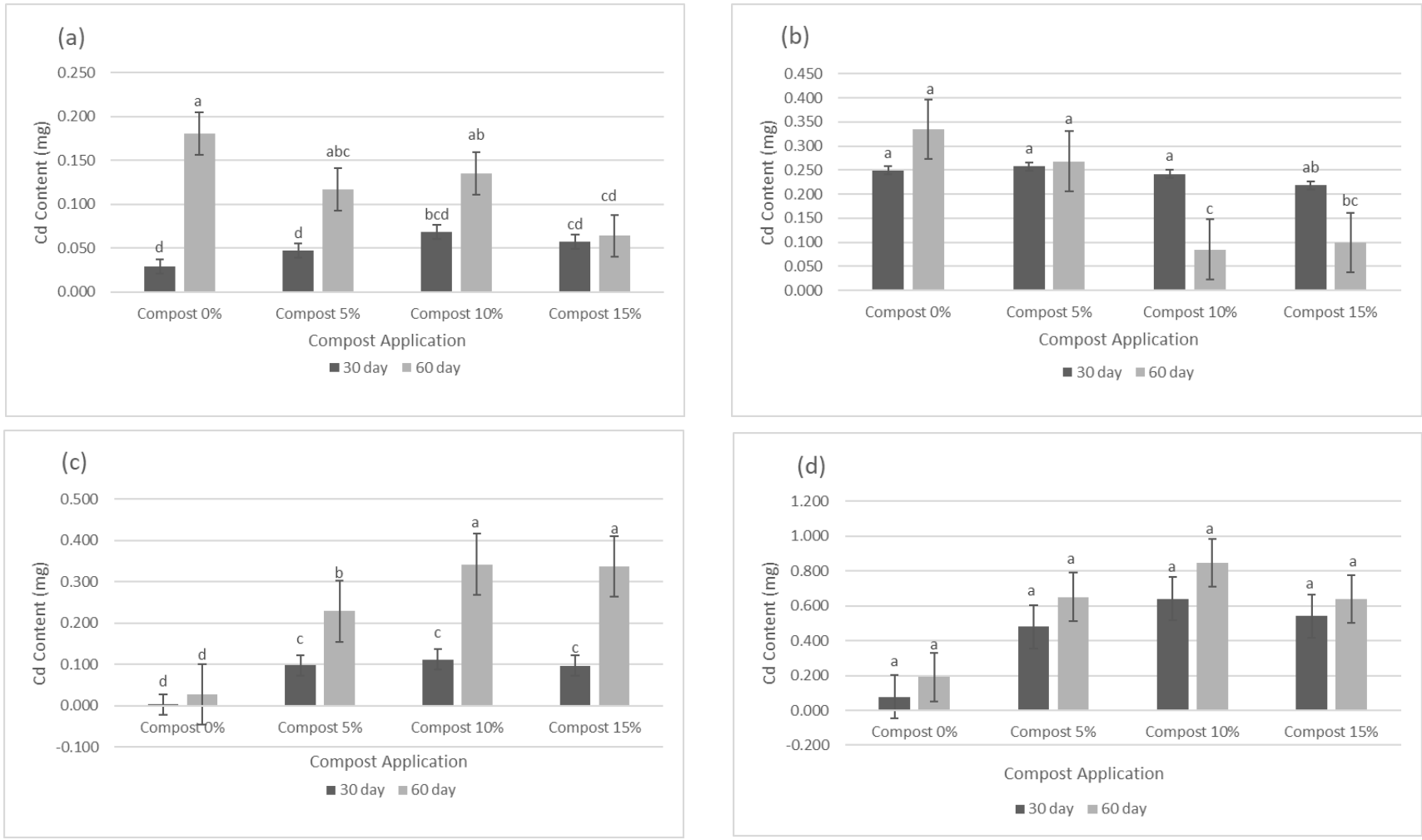


Figure 4.6. The effect of variable rates of compost application to soil with 10 mg Cd/kg on Cadmium Content (mg) of Bok Choy: Recent Soil Root (a), Recent Soil Shoot (b), Allophanic Soil Root (c), Allophanic Soil Shoot (d). Means with the same letter are not significantly different $P<0.05$ (n=3).

4.3.4 Cadmium Availability

Overall, there was a trend to a decrease in Cd availability in soil as a function of increasing compost amendment (Figure 4.7). For the Recent Soil this reduction in availability with compost was significant for both Day 30 and 60, but for the Allophanic Soil, the reduction was only significant on Day 30.

When comparing Cd availability between the two sampling times, there was a nominal decrease for the 0% compost application rate for the Recent Soil and a significant decrease for the Allophanic Soil (by 31.4%). However, for all other treatments, for both soils, Cd availability was the same or increased on Day 60 relative to Day 30. The magnitude of the change in availability with time was greater for the Recent Soil.

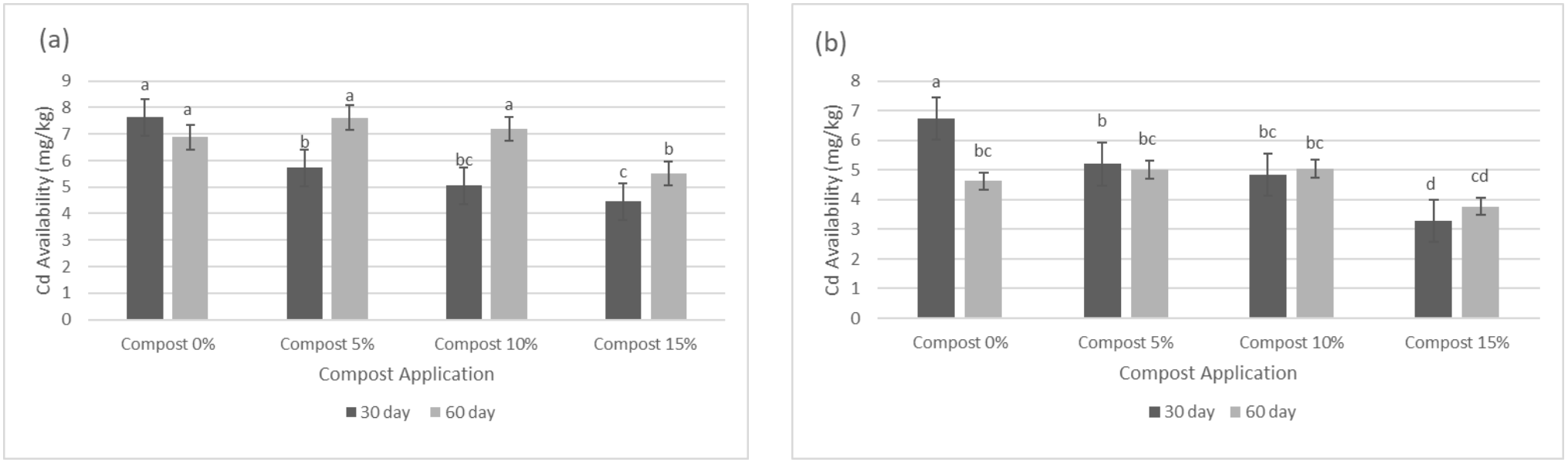


Figure 4. 7. The effect of variable rates of compost application to soil with 10 mg Cd/kg on Cadmium Availability (mg/kg) in the soil: Recent Soil (a), Allophanic Soil (b). Alphabetic letters are significantly different at $P<0.05$ (n=3).

4.3.5 Loss on Ignition

Loss on ignition was used to quantify organic matter (%) changes in soil over time from Day 30 to Day 60 in this pot trial experiment. Due to laboratory equipment constraints, loss on ignition testing was limited to the 10 mg Cd/kg Cd treatment for the 15% compost application rate for both soils. The data shows that although OM% values decreased from Day 30 to Day 60, there was no significant difference in loss on ignition quantified soil organic matter. Loss on ignition data from pot trial agreed with the results of the incubation experiment. The organic matter percentage in the Allophanic Soil was higher compared to the Recent Soil. Furthermore, the Recent Soil greater drastic decrease in organic matter compared to Allophanic Soil, where the reduction was by 9.25% and 2.92% respectively.

Table 4. 1. Organic matter (%) of Recent Soil used for the Pot Trials Experiment.

Cd Application (mg/kg)	Compost Application (%)	Harvesting Time (days)	Organic matter (%)
10	15	30	10.62±1.63 (a)
10	15	60	9.63±1.88 (a)

Data are mean±standard error of three replicates. Means with the same letter are significantly different $P<0.05$ (n=3).

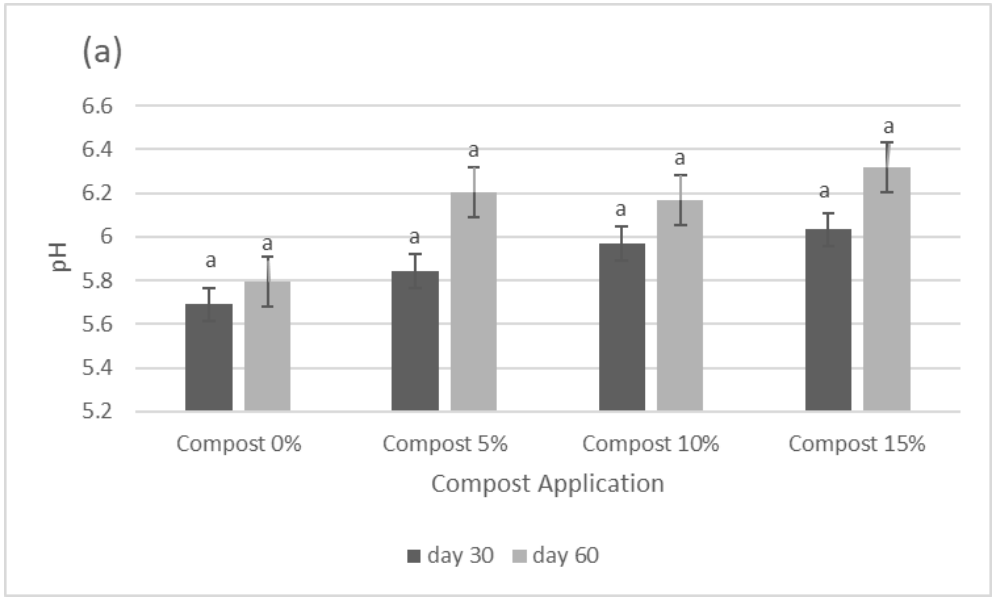
Table 4. 2 Organic matter (%) of Allophanic Soil used for the Pot Trials Experiment.

Cd Application (mg/kg)	Compost Application (%)	Harvesting Time (days)	Organic matter (%)
10	15	30	26.9±0.70 (a)
10	15	60	26.1±0.40 (a)

Data are mean±standard error of three replicates. Means with the same letter are significantly different $P<0.05$ (n=3).

4.3.6 pH

There was a slight increase in soil pH was associated with compost application to both soils although this increase was not significant (Figure 4.8). This trend agreed with the pH results from the incubation experiment in Chapter 3. The increase of pH from Day 30 to Day 60 was similar between the Recent Soil and Allophanic Soil, on average by 4% and 4.4% respectively. In both soils, the treatment with 0% compost showed a lower pH on both Day 30 and Day 60 of sampling compared to those with compost application.



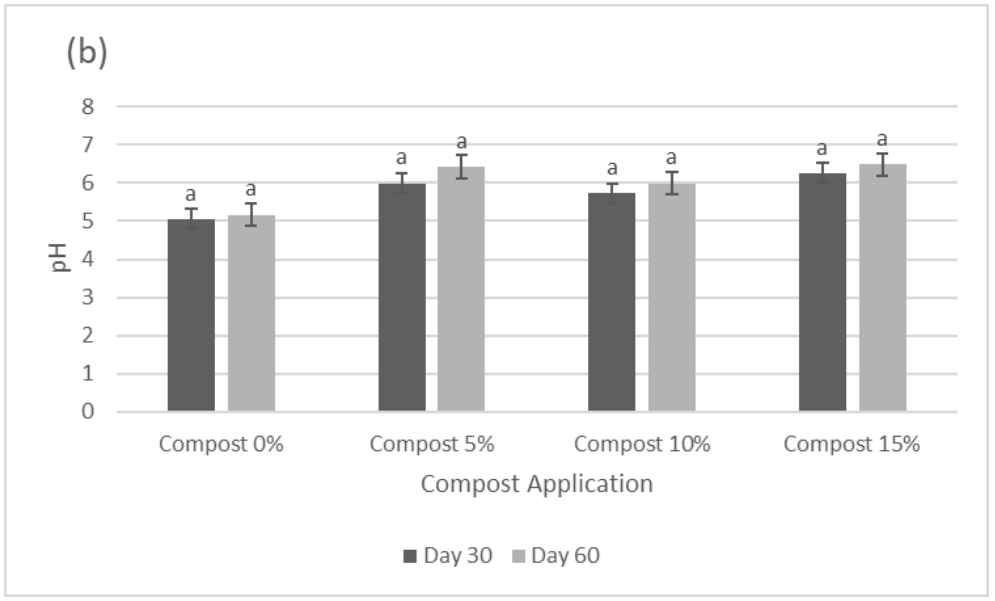


Figure 4. 8. The effect of variable rates of compost application to soil with 10 mg Cd/kg on pH in the soil: Recent Soil (a), Allophanic Soil (b). Alphabetic letters are significantly different at $P<0.05$ (n=3).

4.4 Discussion

4.4.1 Dry weight biomass

Overall, compost application increased the dry weight of harvested Bok Choy biomass compared to the 0% compost treatment in both soils (figure 4.1). As compost application increased, the root and shoot biomass increased. This observation is in agreement with a study conducted by Bashir et al. (2018) who showed that soil amendments (rice straw biochar and zeolite) applied to Cd contaminated soils can increase dry weight biomass of water spinach (*Ipomea aquatica*). Han-Song et al. (2010) showed that compost application increased the dry weight biomass of Bok Choy even when grown in soil with high Cd concentration (0, 2,10 and 50 mg Cd/kg). Compost application will increase total organic carbon in the soil which can increase porosity and hydraulic conductivity (Wong et al., 1999). Compost also can promote soil fertility because it contains nutrients for plants (Na, Mg, Ca, K and P) and increased nutrient supply will increase the dry weight biomass of plants (Fritz et al., 2012; Wong et al., 1999).

In this experiment, the growth of plants on soil with 0% compost application was reduced relative to the compost treatments, as observed through the low biomass of both roots and shoots, particularly for the Allophanic Soil. This result could be influenced by the elevated native Cd concentration in the Allophanic Soil (0.21 mg Cd/kg) relative to the Recent Soil (0.04 mg Cd/kg), and the absence of organic matter to bind soil Cd. A high Cd concentration in the soil can inhibit plant growth and reduce plant biomass (Xu et al., 2012). Han-Song et al. (2010) described a pot trial experiment where no plants survived at high Cd input (50 mg Cd/kg) without compost application.

4.4.2 Cd Concentration and Cd Content

Overall, the Cd concentration in plants decreased as a function of compost application. There was generally a significant change in Cd concentration associated with the application of 5% compost to soil, however the change associated with additional compost loading was of lower magnitude. This suggests that even a relatively small addition of organic matter to soil can

have a prominent impact on Cd concentration in Bok Choy. As the organic matter input increased, the magnitude of the effect on Cd concentration reduced.

The Cd concentration (mg Cd/kg) in plants at Day 60 decreased relative to Day 30 except for the Allophanic Soil at the 0% compost application for both roots. In the case of Allophanic Soil, total soil Cd prior to the experiment was higher (0.21 mg Cd/kg) than the Recent Soil (0.04 mg Cd/kg). A relatively higher total Cd in the soil, and the absence of organic amendment, caused a major increase in the Cd concentration of Bok Choy growing on the Allophanic Soil from Day 30 to Day 60 (0% compost application). Total Cd in the soil is one of important factors influencing Cd availability (Chapman, 2007).

To better identify total cadmium uptake in the plants, Cd content (mg) was quantified. Cadmium content was highly influenced by plant biomass. Plant roots experienced a greater change in Cd content from Day 30 to Day 60 compared to shoots. In the Allophanic Soil, Cd content increased as a function of compost application due to an increase in plant biomass. In the Recent Soil, at lower compost application rates (0% and 5%), Cd content increased significantly in root parts over time however this was not associated with a significant change in biomass. This increase could have been influenced by a release of soil-bound Cd during decomposition (McBride, 1995). In shoots, no significant increase in Cd content was found from Day 30 to Day 60 and a decrease in content was observed for the 10% and 15% compost application rates from Day 30 to 60. He et al. (2020) showed that Cd tends to accumulate more in roots compared to shoots. Therefore, in this study, the effect of time on Cd content increase was more prominent in roots. The observed decrease in content may be due to leaf fall during the pot trials. Some first leaves may have taken up a higher Cd concentration compared to newer leaves. Fallen leaves were not collected and analysed in this work.

4.4.3 Cd availability

Cadmium soil availability recorded in the pot trials showed a similar trend with the results from the incubation experiment. There was a decrease of Cd availability as a function of compost application to the soil. Compost application significantly reduced Cd availability in the Recent Soil, while for the Allophanic Soil, a significant difference occurred only for the 0% compost application on Day 30. Organic matter input in soil decreases Cd availability due to

the presence of colloidal organic matter particles that increase Cd sorption potential (Benavides et al., 2005; Kaiser et al., 2008). In the Allophanic Soil there was no significant effect of increasing percentage of compost applications (5%,10% and 15%) on Cd availability. This might be due to the dominant influence the high native organic matter content of the Allophanic Soil

In terms of changes over time from Day 30 to Day 60, Cd availability in the Recent Soil experienced a more prominent increase relative to the Allophanic Soil. Antoniadis and Alloway (2001) reported that Cd availability increased over time simultaneously with organic matter decomposition. The breaking of humic substances during decomposition potentially re-releases Cd-bond in organic matter into more labile forms, thus increasing Cd availability for uptake by plants (Alloway, 1995). However, the results for the two soils of this might differ due to soil characteristics. In this study, Allophanic Soil contains a high content of reactive Allophane and an abundance of microaggregates that preserve organic matter in the soil reducing the potential for organic matter decomposition (Chevallier et al., 2010; Miltner & Zech, 1998). This could account of the lack of any significant increase in Cd availability from Day 30 to Day 60 observed for the Allophanic Soil.

4.4.4 Loss on Ignition

The loss on ignition results from the pot trial experiment showed the same trend with results from the incubation experiment. A decrease in organic matter percentage as a function of incubation time was observed. The day and night temperature range of 25^0C and 22^0C in the greenhouse potentially created an environment suitable for organic matter decomposition. Temperature is the major influence on chemical and biochemical processes that lead to a release of dissolved organic matter (DOM) and organic matter mineralisation (Martius et al., 2001; McCauley et al., 2009). Antoniadis and Alloway (2001) reported that organic matter percentage during a pot trial with ryegrass (*Lolium perenne* L.) declined significantly in the first four months due to a decomposition under 25^0C.

4.4.5 pH

Organic matter application caused an increase in pH although there was no significant difference found. Contrary to the incubation results, pH values for the pot trials slightly increased as a function of time. Organic matter presence in the soil increases buffering capacity, increasing the ability of soil to maintain stable pH (Curtin & Trolove, 2013). Cation exchange capacity is one of important factor in soil buffering capacity because of an abundance of organic matter in the soil (Jansen van Rensburg et al., 2009). While CEC was not measured in the experimental units of this trial, it is possible that nighttime temperatures (22^0C) slowed down the rate of organic matter decomposition relative to that for the constant incubation temperature of 25^0C. A high temperature is one of the major factors that influence organic matter decomposition and will impact pH as well as total organic matter content (Zech et al., 1997).

4.5 Conclusion

Organic matter application increased plant biomass and reduced Cd concentration in Bok Choy plants when compared to the control treatment. A reduction in Cd concentration of the plants was a function of the reduction in soil Cd availability due to higher rates of compost application. However, despite this reduction in concentration, the Cd content of plants increased as a function of compost application due to the growth-promoting effect of compost application to soil.

In terms of changes over time, the increase in Cd concentration was more prominent in plant roots compared to shoots from Day 30 to Day 60. Some insignificant increases were found in shoot parts. These observations indicate that roots are more prone to a high Cd availability in the soil over time. The magnitude of the increase in Cd content in plants for the 0% compost application over time was greater than that observed for higher compost applications. This suggests that a high organic matter application to soil could reduce the overall rate of Cd availability increase over time. The data in this Chapter suggest that Cd availability increased on Day 60 due to organic matter decomposition. The increased Cd content in plants on Day 60 was a function of the longer growth period of plants on Day 60 relative to Day 30.

5. General Discussion and Conclusion

5.1 Cadmium in the Indonesian Farming System

Cadmium in the soil can pose a serious risk to the agriculture sector (Loganathan et al., 2012). Cadmium contamination leads to human and animal toxicity through the food chain because Cd taken up by plants can be consumed by animals and humans (Qin et al., 2020). In the Indonesian agriculture sector, Cd in the soil mainly comes from the excessive application of phosphate fertilisers and the land-based disposal of waste from urban areas which exist alongside agricultural lands due to extensive urbanisation (Ferina et al., 2020; Pradika et al., 2019). Bok Choy (*Brassica chinensis.* L) is widely consumed vegetable in Indonesia and has been noted as a high Cd accumulator (Capistrano & Marten, 1986; Han-Song et al., 2010). This plant represents a potential risk for Cd transfer into the food chain.

The risk describes here requires serious attention, however awareness of the issue is lacking. Pradika et al. (2019) found that approximately 60% farmers in Sragen Regency, Central Java, Indonesia did not acknowledge that a high Cd levels in the soil might enter the food chain and cause a toxicity to humans. To address this issue of limited Cd awareness, manure compost application has been implemented by farmers in developing countries as a low cost way to reduce Cd transfer to the food chain (Agegnehu et al., 2017; Welikala et al., 2018). However, the effect of organic matter decomposition over time on Cd availability is not well studied and requires a further investigation to establish proper agricultural framework in Cd enriched soils.

Based on this background information, two experiments were designed in this study. In Chapter 3, an incubation experiment was conducted to investigate the influence of compost decomposition on Cd availability in soil where the temperature was set at 25^0C (imitating the ambient environmental conditions in Indonesia). Secondly, in Chapter 4, a pot trial experiment was conducted to investigate the effect over time of compost on biomass production and the Cd uptake concentration of Bok Choy (*Brassica chinensis*. L). Understanding the effect of organic matter decomposition over time on Cd availability in the

soil and Cd uptake by plants could underpin more effective Cd management in Indonesian agriculture systems.

5.2 Summary of the Key Findings

The experiments conducted in this study investigated the effect of low-cost compost application to reduce Cd risk transfer to plants and its efficiency over time. The key points of this research outcome are summarised below.

5.2.1 Organic matter in the soil experienced a slight decrease over time

The organic matter percentage in incubation soils and pot trial soils was assessed using the loss on ignition method (Chapters 3 and 4). Although the two soils used in the study have different soil characteristics, results showed that organic matter experienced a slow decomposition in both soils as quantified by a decrease in soil organic matter percentage over time. The organic matter percentage in soil prior to experiments had a significant role on the decomposition process; Allophanic Soils contained a higher organic matter content as compared to the Recent Soil. In the Recent Soil, organic matter percentage from both experiments was around 10%, while in the Allophanic Soil the percentage was around 25%.

5.2.2 Cd availability in both soils varies as a function of compost application and time in recent and Allophanic Soils

Cd availability in the Recent and Allophanic Soils increased over time and this was explained as a function of the decrease in organic matter that was observed in both soils. While Cd availability increased in both soils, the rate of this increase was different between the Recent Soil and the Allophanic Soil.

From experiments in Chapters 3 and 4, the increase in Cd availability as a fucntion of time was more prominent in the Recent Soil. A significant increase occurred between Day 30 and Day 60 in the Recent Soil indicating that a peak in organic matter decomposition occurred during this period (Chapter 3). In contrast, the increase in Cd availability with time for the Allophanic Soil was insignificant. The abundance of microaggregates and secondary clay minerals in the

Allophanic Soil creates an ability for this soil to preserve organic matter, explaining the insignificant increase in Cd availability. The organic matter percentage during the experiments remained higher in the Allophanic Soil compared to the Recent Soil and this afforded greater protection of soil organic matter.

5.3.3 Cd content in plants increased as plant biomass rose

There was an increase in the Cd content in roots and shoots on Day 60 relative to Day 30. The increase of Cd content was in line with the dry weight biomass: as plant dry weight biomass increased, the plants accumulated more Cd. A greater rate of compost application promoted plant growth due to the increase in soil essential nutrients such as Mg, Ca, K and P associated with compost - this is the point of adding compost. However, compost use is also associated with the uptake of mobile nonessential trace elements that exist in soil solution, such as Cd. If added organic matter does not adsorb Cd in the soil system, the increased growth of plants risks increased Cd uptake.

5.3.4 A higher compost application decreased a gap increase in Cd content over time

Although, in general, Cd content increased as a function of compost application, a higher compost application decreased the rate of Cd content increase over time. The increase of plant Cd content for the 0% compost application rate was greater than for the 5%, 10% and 15% compost applications. This result was most obvious for the Recent Soil where the increase of root Cd content for the 0% compost application from Day 30 to Day 60 was 515%. However, this increase in Cd content was only 12.1% for the 15% compost application. The similar trend was observed for the Cd content of plant roots grown in the Allophanic Soil. At the 0% compost application rate, the increase was 850% and reduced to 248% for the 15% compost application rate. A high loading of organic matter to soil can therefore potentially reduce the rate of increase in Cd availability and plant uptake over time. However, for all treatments, Cd availability experienced an increase on Day 60 due to organic matter decomposition. The results in this thesis suggest that at a higher compost application loading, an increase of Cd availability (and the risk of uptake) from Day 30 to Day 60 could be reduced

5.2.5 The increase of Cd concentration over time in roots is higher than in shoots

Over time, Bok Choy roots experienced a higher Cd concentration increase compared to the shoots and some insignificant differences were found in shoots between treatments. This observation suggests that root parts are more sensitive to Cd toxicity in the soil compared to shoots. For plant roots at the 0% compost application, Cd content increased by 515% and 850% for the Recent Soil and Allophanic Soil respectively. For shoots at the 0% compost application, the increase was 34.31% for the Recent Soil and 149% for the Allophanic Soil. These results indicate that bok Choy roots take up a greater content of Cd compared to shoots, particularly under conditions of low organic matter that can be promoted by decomposition processes over time.

5.3 Importance of the Findings for Indonesian Farming Systems

Bok Choy (*Brassica chinensis* L) is one of most widely grown vegetables in Indonesia due to its high nutritional value, rapid growth and low cultivation cost (Capistrano & Marten, 1986; Rafiq et al., 2014). Unfortunately, Bok Choy is widely known as a high Cd accumulator and could pose a serious risk to health if it is grown in Cd contaminated soils and consumed by humans (Han-Song et al., 2010; He et al., 2020). Although trace element toxicity in soil and crops (including Bok Choy) has been studied by some Indonesian scholars, there have been no robust regulations or initiatives to address this risk. Some agricultural areas in Indonesia are situated in rural areas and these are difficult to reach. This limits the potential to implement education and innovations in contaminant management. Unfamiliarity regarding Cd's potential toxicity in food crops among small-holder farmers makes this a challenging for the Indonesian government (Pradika et al., 2019).

A ban in vegetable cultivation in Cd-enriched soils would not be a wise decision. Any restriction on agriculture as a source of income for smallholder farmers in Indonesia would affect livelihoods. This would also cause vegetable scarcity due to a decrease in vegetable yield and food supply in certain areas. Therefore, comprehensive management guidelines should be implemented to address the issue. Compost application is an affordable soil treatment for small holder farmers to manage the risk of Cd transfer within the food chain in developing countries, including Indonesia (Agegnehu et al., 2017; Ferina et al., 2020).

However, as this research has shown, organic matter in the soil can decompose with time, reducing its ability to bind Cd in the soil and potentially releasing large amounts of Cd to growing plants (Wambeke, 1992). It is known that a high temperature in the tropics can accelerate decomposition process (Coleman et al., 1989).

The Indonesian government could develop regulations to reduce the risk of increased Cd transfer through organic matter decomposition. This could start with regulations to educate small holder farmers regarding trace elements and their potential risks in food crops. Education would be via consultative programs because some farmers have baseline low understanding of this issue, particularly in remote areas. As the farmers become better informed, the adoption of agronomic practices and practical frameworks would be easier to develop and apply. Secondly, the government could publish detailed frameworks that regulate the cultivation of crops in Cd-enriched soils to reduce the risk. With the case of Bok Choy growing in Cd-enriched areas, according to this study, a monthly re-application is necessary to avoid the risk of decomposition causing a rise in Cd availability. Soils have a limit to the amount of organic matter that they can hold, and rate guidelines would need to be developed to ensure that soils remain at this maximum (or optimal) organic matter level. It is also advocated that Bok Choy and other high Cd accumulator leafy vegetables are harvested as soon as they are ready. Delaying the harvesting time potentially increases Cd uptake even under scenarios where compost has been applied continuously.

In terms of farmer responsibilities, farmers are advised to attend training events and adopt practical frameworks proposed by the government. The government and scientific community are encouraged to follow progress and to provide continuous training to farmers. This engagement with farmers could be achieved through the service responsibility of Indonesian universities. Ongoing research should be conducted to keep up to date with the current issues, particularly field trials where Cd-contaminated land is identified. The initiatives described here will require teamwork from several parties, such as the government, farmers, and scientists.

5.4 Recommendation of ongoing work

There are several knowledge gaps that could be targeted in ongoing research. Specifically,

- The incubation experiment could be conducted over a longer period to observe further the effect of organic matter decomposition on Cd availability.
- A range of temperatures could be added as a parameter to observe the decomposition difference at each temperature and to assess how this affects Cd availability.
- In a subsequent pot trial experiment, individual leaves could be analysed to identify the difference of Cd content in new and old leaves. This work would verify the mobility status of Cd in Bok Choy under the experimental conditions of the current work

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Appendix

Appendix 1: Moisture Percentage and Water Holding Capacity

- Recent Soil

Table A1.1. Moisture Percentage of Recent Soil

Sample No:	Beaker Weight (g)	Initial Beaker +Sample W (g)	After Beaker + Sample W(g)	Moisture Percentage (%) on dry weight	Dry soil weight
1	31.38	65.26	63.84	4.37	32.46
2	20.98	58.99	57.38	4.42	36.40
3	20.45	56.35	54.89	4.24	34.44
		Average		4.35	34.43

Moisture percentage: 4.5%

Table A1.2. Water Holding Capacity of Recent Soil

Sample No:	Beaker Weight (g)	Initial Beaker +Sample W (g)	After Beaker + Sample W(g)	Moisture Percentage (%) on dry weight	Dry soil weight
1	20.44	32.32	30.5	18.09	10.06
2	20.98	35.1	32.94	18.06	11.96
3	30.1	43.53	41.45	18.33	11.35
		Average		18.16	11.12

Water Holding Capacity: 18.16%

- Allophanic Soil

Table A1.3 Moisture Percentage of Allophanic Soil

Sample No:	Beaker Weight (g)	Initial Beaker +Sample W (g)	After Beaker + Sample W(g)	Moisture Percentage (%) on dry weight	Dry soil weight
1	31.4	52.76	48.48	25.06	17.08
2	23.73	50.76	45.27	25.49	21.54
3	30.1	56.9	51.5	25.23	21.40
				25.26	20.01

Moisture percentage: 25.26%

Table A1.4 Water Holding Capacity

Sample No:	Beaker Weight (g)	Initial Beaker +Sample W (g)	After Beaker + Sample W(g)	Moisture Percentage (%) on dry weight	Dry soil weight
1	22.53	34.53	29.94	61.94	7.41
2	23.73	33.74	29.93	61.45	6.20
3	23.08	31.22	28.11	61.83	5.03
				61.74	6.21

Water Holding Capacity: 61.74%

Appendix 2: Brief Research Details

- Brief Incubation Experiment Detail

- Incubation temperature: 25^{o}C (imitating the average temperature in West Java)
- Incubation time: 90 days and sampling for analysis every 30 days

Parameters:

- Cd concentration: 0, 1, 2, 5, 10 ppm

  C_0 = 0 ppm

  C_1 = 1 ppm

  C_2 = 2 ppm

  C_3 = 5 ppm

  C_4 = 10 ppm

- Compost rate: 0%, 5%, 10% and 15% of the total soil weight
  D_0 = 0%

  D_1 = 5%

  D_2 = 10%

  D_3 = 15%

  - Soil type: 2 (Recent Soil and Allophanic Soil)

  S_1 = Alophanic

  S_2 = Recent Soil

- Treatment combinations will be replicated three times and arranged in a 5 x 4 x 2 x 3 Complete Randomised Design = 120 pots
- Soil moisture during experimental period: pot-field capacity of 70%
- Sampling procedure: Soils are collected from A horizon (10-15 depth) from two sites and then will be air-dried at 30^{o}C for 5 days.

Table A2.1 Incubation Design

Cadmium C	S_1				S_2			
	D_0	D_1	D_2	D_3	D_0	D_1	D_2	D_3
C_0	C_0S_1 D_0	$C_0S_1D_1$	$C_0S_1D_2$	$C_0S_1D_3$	C_0 S_2 D_0	C_0 S_2 D_1	C_0 S_2 D_2	C_0 S_2 D_3
C_1	C_1S_1 D_0	$C_1S_1D_1$	$C_1S_1D_2$	$C_1S_1D_3$	C_1S_2 D_0	C_1S_2 D_1	C_1S_2 D_2	C_1S_2 D_3
C_2	C_2S_1 D_0	$C_2S_1D_1$	$C_2S_1D_2$	$C_2S_1D_3$	C_2S_2 D_0	C_2S_2 D_1	C_2S_2 D_2	C_2S_2 D_3
C_3	C_3S_1 D_0	$C_3S_1D_1$	$C_3S_1D_2$	$C_3S_1D_3$	C_3S_2 D_0	C_3S_2 D_1	C_3S_2 D_2	C_3S_2 D_3
C_4	C_4S_1 D_0	$C_4S_1D_1$	$C_4S_1D_2$	$C_4S_1D_3$	C_4S_2 D_0	C_4S_2 D_1	C_4S_2 D_2	C_4S_2 D_3

- Brief Pot Trial Experiment Detail

Parameters

- Cd concentration:
  C= 10 Cd mg/kg

- Compost rate: 0%, 5%, 10% and 15% of the total soil weight
  D_0 = 0%

  D_1 = 5%

  D_2 = 10%

  D_3 = 15%

- Soil type: 2 (Recent Soil and Allophanic Soil)

  S_1 = Alophanic

  S_2 = Recent Soil

A2.2 Pot Trial Design

Cadmium	s_1				S_2			
	D_0	D_1	D_2	D_3	D_0	D_1	D_2	D_3
C	CS_1D_0	CS_1D_1	CS_1D_2	CS_1D_3	CS_2D_0	CS_2D_1	CS_2D_2	CS_2D_3

- The experiment will be replicated 3 times.
- There will be 2 experiments, will be harvested each month for 2 months.
- Total pots needed: 1x2x4x3x2= 48 (1 Cadmium application, 2 soils, 4 compost application, 3 replicates, 2 harvest time).

Appendix 3: Results on mean Cd availability values

Representative of the mean Cd availability values data of different compost application (%) and incubation time (day) with standard deviation (sd) and standard error (se).

3.1. Recent Soil

Table A3.1 Mean of Cd Availability at 0 mg/kg Cd application in Recent Soil over time

Compost (%)	Incubation time (day)	Mean (mg/kg)	sd	se
0	0	0.001	0.004	0.002
0	30	0.004	0.007	0.004
0	60	0.006	0.000	0.000
0	90	0.007	0.000	0.000
5	0	0.001	0.080	0.046
5	30	0.003	0.042	0.024
5	60	0.006	0.014	0.008
5	90	0.006	0.002	0.001
10	0	0.000	0.004	0.003
10	30	0.002	0.002	0.001
10	60	0.006	0.001	0.000
10	90	0.006	0.001	0.000
15	0	0.000	0.003	0.017
15	30	0.002	0.002	0.000
15	60	0.007	0.004	0.000
15	90	0.006	0.009	0.001

Table A3.2 Mean of Cd Availability at 1 mg/kg Cd application in Recent Soil over time

Compost (%)	Incubation time (day)	Mean (mg/kg)	sd	se
0	0	0.065	0.002	0.001
0	30	0.064	0.002	0.001
0	60	0.094	0.001	0.001
0	90	0.084	0.001	0.001
5	0	0.045	0.002	0.001
5	30	0.055	0.008	0.005
5	60	0.061	0.004	0.002
5	90	0.067	0.007	0.004
10	0	0.031	0.004	0.002
10	30	0.035	0.008	0.004
10	60	0.048	0.005	0.003
10	90	0.052	0.006	0.003
15	0	0.023	0.000	0.000
15	30	0.034	0.003	0.002
15	60	0.041	0.005	0.003
15	90	0.047	0.005	0.003

Table A3.3 Mean of Cd Availability at 2 mg/kg Cd application in Recent Soil over time

Compost (%)	Incubation time (day)	Mean (mg/kg)	sd	se
0	0	0.441	0.011	0.006
0	30	0.421	0.009	0.005
0	60	0.591	0.044	0.025
0	90	0.404	0.022	0.013
5	0	0.292	0.078	0.045
5	30	0.242	0.010	0.006
5	60	0.242	0.002	0.001
5	90	0.328	0.015	0.009
10	0	0.227	0.014	0.008
10	30	0.193	0.010	0.006
10	60	0.322	0.014	0.008
10	90	0.268	0.034	0.020
15	0	0.147	0.021	0.012
15	30	0.131	0.014	0.008
15	60	0.240	0.026	0.015
15	90	0.211	0.020	0.011

Table A3.4 Mean of Cd Availability at 5 mg/kg Cd application in Recent Soil over time

Compost (%)	Incubation time (day)	Mean (mg/kg)	sd	se
0	0	1.151	0.110	0.063
0	30	1.340	0.031	0.018
0	60	1.470	0.046	0.026
0	90	1.121	0.040	0.023
5	0	0.774	0.083	0.048
5	30	0.885	0.041	0.024
5	60	1.121	0.040	0.023
5	90	1.009	0.072	0.042
10	0	0.547	0.008	0.005
10	30	0.670	0.037	0.021
10	60	0.979	0.022	0.013
10	90	0.846	0.044	0.025
15	0	0.465	0.104	0.060
15	30	0.536	0.047	0.027
15	60	0.721	0.020	0.012
15	90	0.717	0.035	0.020

Table A3.5 Mean of Cd Availability at 10 mg/kg Cd application in Recent Soil over time

Compost (%)	Incubation time (day)	Mean (mg/kg)	sd	se
0	0	2.441	0.769	0.444
0	30	2.745	0.422	0.244
0	60	3.340	0.210	0.121
0	90	2.810	0.061	0.035
5	0	2.104	0.109	0.063
5	30	2.347	0.102	0.059
5	60	2.729	0.149	0.086
5	90	2.420	0.214	0.124
10	0	1.840	0.034	0.020
10	30	1.848	0.087	0.050
10	60	2.257	0.146	0.084
10	90	2.274	0.183	0.106
15	0	1.309	0.050	0.029
15	30	1.381	0.008	0.005
15	60	2.033	0.332	0.192
15	90	1.907	0.155	0.089

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Table A3.6 Mean of Cd Availability at 0 mg/kg Cd application in Allophanic Soil over time

Compost (%)	Incubation time (day)	Mean (mg/kg)	sd	se
0	0	0.032	0.004	0.002
0	30	0.044	0.002	0.001
0	60	0.014	0.005	0.003
0	90	0.037	0.002	0.001
5	0	0.032	0.005	0.003
5	30	0.032	0.003	0.002
5	60	0.013	0.006	0.004
5	90	0.032	0.006	0.003
10	0	0.031	0.005	0.003
10	30	0.033	0.002	0.001
10	60	0.012	0.006	0.003
10	90	0.028	0.002	0.001
15	0	0.028	0.010	0.006
15	30	0.026	0.000	0.000
15	60	0.009	0.002	0.001
15	90	0.029	0.002	0.001

Table A3.7 Mean of Cd Availability at 1 mg/kg Cd application in Allophanic Soil over time

Compost (%)	Incubation time (day)	Mean (mg/kg)	sd	se
0	0	0.194	0.017	0.010
0	30	0.226	0.022	0.013
0	60	0.201	0.012	0.007
0	90	0.201	0.004	0.002
5	0	0.199	0.006	0.004
5	30	0.221	0.015	0.009
5	60	0.220	0.016	0.009
5	90	0.175	0.022	0.013
10	0	0.155	0.002	0.001
10	30	0.200	0.039	0.022
10	60	0.153	0.016	0.009
10	90	0.142	0.009	0.005
15	0	0.168	0.016	0.009
15	30	0.148	0.005	0.003
15	60	0.124	0.003	0.002
15	90	0.142	0.007	0.004

Table A3.8 Mean of Cd Availability at 2 mg/kg Cd application in Allophanic Soil over time

Compost (%)	Incubation time (day)	Mean (mg/kg)	sd	se
0	0	0.353	0.041	0.024
0	30	0.474	0.030	0.017
0	60	0.441	0.011	0.007
0	90	0.433	0.019	0.011
5	0	0.307	0.029	0.017
5	30	0.333	0.062	0.036
5	60	0.364	0.009	0.005
5	90	0.378	0.183	0.106
10	0	0.288	0.021	0.012
10	30	0.327	0.027	0.015
10	60	0.333	0.015	0.008
10	90	0.292	0.010	0.006
15	0	0.229	0.038	0.022
15	30	0.329	0.013	0.007
15	60	0.300	0.019	0.011
15	90	0.235	0.013	0.008

Table A3.9 Mean of Cd Availability at 5 mg/kg Cd application in Allophanic Soil over time

Compost (%)	Incubation time (day)	Mean (mg/kg)	sd	se
0	0	0.832	0.115	0.067
0	30	1.110	0.046	0.026
0	60	1.085	0.025	0.015
0	90	1.143	0.114	0.066
5	0	0.738	0.161	0.093
5	30	1.155	0.119	0.069
5	60	0.916	0.095	0.055
5	90	0.980	0.023	0.013
10	0	0.672	0.095	0.055
10	30	0.821	0.012	0.007
10	60	0.857	0.029	0.017
10	90	0.871	0.031	0.018
15	0	0.636	0.004	0.002
15	30	0.874	0.050	0.029
15	60	0.829	0.038	0.022
15	90	0.751	0.008	0.005

Table A3.10 Mean of Cd Availability at 10 mg/kg Cd application in Allophanic Soil over time

Compost (%)	Incubation time (day)	Mean (mg/kg)	sd	se
0	0	2.456	0.082	0.047
0	30	2.351	0.143	0.083
0	60	2.333	0.104	0.060
0	90	2.256	0.053	0.031
5	0	2.014	0.119	0.069
5	30	2.156	0.109	0.063
5	60	2.368	0.016	0.009
5	90	1.860	0.021	0.012
10	0	1.838	0.116	0.067
10	30	1.850	0.177	0.102
10	60	1.918	0.050	0.029
10	90	1.803	0.094	0.054
15	0	1.744	0.180	0.104
15	30	1.628	0.032	0.018
15	60	1.794	0.013	0.008
15	90	1.778	0.010	0.006

Table A3.11 Mean of Cd Content in Recent Soil (root) over time

Compost (%)	Harvest time (day)	Mean (mg)	sd	se
0	30	0.029	0.007	0.004
0	60	0.181	0.055	0.032
5	30	0.047	0.011	0.007
5	60	0.117	0.019	0.011
10	30	0.068	0.015	0.009
10	60	0.136	0.028	0.016
15	30	0.057	0.008	0.005
15	60	0.064	0.011	0.006

Table A3.12 Mean of Cd Content in Recent Soil (shoot) over time

Compost (%)	Harvest time (day)	Mean (mg)	sd	se
0	30	0.249	0.048	0.028
0	60	0.335	0.052	0.030
5	30	0.257	0.029	0.017
5	60	0.268	0.014	0.008
10	30	0.242	0.049	0.028
10	60	0.085	0.032	0.018
15	30	0.218	0.009	0.005
15	60	0.100	0.072	0.042

Table A3.13 Mean of Cd Content in Allophanic Soil (root) over time

Compost (%)	Harvest time (day)	Mean (mg)	sd	se
0	30	0.003	0.001	0.001
0	60	0.027	0.003	0.002
5	30	0.098	0.005	0.003
5	60	0.228	0.027	0.016
10	30	0.112	0.005	0.003
10	60	0.342	0.051	0.029
15	30	0.097	0.009	0.005
15	60	0.337	0.015	0.009

Table A3.14 Mean of Cd Content in Allophanic Soil (shoot) over time

Compost (%)	Harvest time (day)	Mean (mg)	sd	se
0	30	0.077	0.013	0.008
0	60	0.191	0.020	0.011
5	30	0.480	0.062	0.036
5	60	0.651	0.061	0.035
10	30	0.640	0.112	0.064
10	60	0.847	0.030	0.017
15	30	0.541	0.026	0.015
15	60	0.639	0.040	0.023