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Evaluation of cadmium bioaccumulations in Pak Choi grown by hydroponics system

Piyachet Jinsart
Faculty of Science

Evaluation of Cadmium Bioaccumulations in Pak Choi Grown by Hydroponics system

Mr. Piyachet Jinsart

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การประเมินหาการสะสมทางชีวภาพของแคดเมียมในผักกวางตุ้งที่ปลูกโดยใช้ระบบ
ไฮโดรโปนิกส์

นายปิยเชษฐ์ จีนศาสตร์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Advisor	SUMETH WONGKIEW, Ph.D.

Accepted by the FACULTY OF SCIENCE, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

.... Dean of the FACULTY OF SCIENCE

(Professor POLKIT SANGVANICH, Ph.D.)

THESIS COMMITTEE

.... Chairman

(Associate Professor NAIYANAN ARIYAKANON, Ph.D.)

.... Thesis Advisor

(SUMETH WONGKIEW, Ph.D.)

.... Examiner

(Professor WANIDA JINSART, Ph.D.)

.... External Examiner

(Professor Thammarat Koottatep, D.Eng.)

ปิยเชษฐ์ จีนศาสตร์ : การประเมินหาการสะสมทางชีวภาพของแคดเมียมในผักกวางตุ้งที่ปลูกโดยใช้ระบบไฮโดรโปนิกส์. (Evaluation of Cadmium Bioaccumulations in Pak Choi Grown by Hydroponics system) อ.ที่ปรึกษาหลัก : ดร.สุเมธ วงศ์เขียว

แคดเมียมเป็นโลหะหนักที่อันตรายที่สุดชนิดหนึ่งที่มีรายงานว่าปนเปื้อนในห่วงโซ่อาหารและความเสี่ยงสูงต่อสุขภาพ รวมทั้งผลกระทบที่ไม่ก่อให้เกิดมะเร็งและสารก่อมะเร็งมีรายงานการปนเปื้อนแคดเมียมในระดับสูงเนื่องจากการปล่อยของเสียจากอุตสาหกรรมที่ส่งผลต่อการเจริญเติบโตและผลผลิตของพืช ซึ่งสามารถใช้เป็นตัวบ่งชี้ทางชีวภาพสำหรับการสะสมทางชีวภาพของแคดเมียมในพืช ซึ่งการใช้น้ำที่ปนเปื้อนแคดเมียมในการปลูกผัก เป็นที่ทราบกันดีว่าเพิ่มความเสี่ยงต่อสุขภาพสำหรับการะสมสมทางชีวภาพ ของแคดเมียมในพืช อย่างไรก็ตามก็ยังไม่ทราบระดับ พีเอช ที่เกี่ยวข้องกับความเข้มข้นของแคดเมียมสูงในแหล่งน้ำที่ใช้ในการเกษตร เช่น แม่น้ำ ทะเลสาบ น้ำทิ้ง เป็นต้นและส่งผลให้เกิดการสะสมทางชีวภาพและความเสี่ยงต่อสุขภาพของการบริโภคผักที่ปลูกโดยมีแคดเมียมที่ปนเปื้อนในน้ำในการศึกษานี้ ใช้ผักกวางตุ้งเป็นพืชต้นแบบในการปลูกพืชไร้ดินเพื่อเข้าถึงการสะสมทางชีวภาพของแคดเมียมและความเสี่ยงต่อสุขภาพ การทดลองดำเนินการโดยใช้ปริมาณแคดเมียมเริ่มต้นที่ 4 ขนาด (0,1,2,3 มก/ลิตร) มีระยะเวลาดำเนินการ 4 สัปดาห์ โดยมี pH เริ่มต้น 3 ระดับ (7.5, 6.5, 5.5) ใช้ในการทดลอง ครั้งที่ 1, 2, 3 ตามลำดับ ผลการวัดปริมาณแร่ธาตุที่พบในน้ำที่ใช้เลี้ยงผัก แอมโมเนีย = 0.91 ± 0.36 มก/ลิตร ไนเตรต = 0.50 ± 1.4 มก/ลิตร และ ฟอสเฟต = 0.23 ± 0.10 มก/ลิตร ผลการศึกษาแสดงว่า ความเข้มข้นของแคดเมียม 1 ถึง 3 มก/ลิตร ส่งผลถึงการยับยั้งการเจริญเติบโตของผักกวางตุ้ง อย่างมีนัยสำคัญ ($p<0.05$) โดยการสะสมทางชีวภาพของใบอยู่ที่ (0.035–2.963) และใบอยู่ที่ (0.434–1.038) ซึ่งบ่งชี้ว่า แคดเมียมไอออนถูกเคลื่อนย้ายจากน้ำสู่รากและสะสมในส่วนที่กินได้ คือ ส่วนใบและลำต้น โดยแคดเมียมสูงสุดที่มีอยู่ในน้ำอยู่ที่ (2.67 ± 0.1 มก/ลิตร) พบได้ที่ ระดับ พีเอช 5.5 เนื่องจากความสามารถ ในการละลายของแคดเมียมที่ระดับ พีเอชที่เป็นกรด จากการบริโภคเฉลี่ยต่อวันของ USEPA และการประเมินความเสี่ยงต่อสุขภาพ พบว่าการบริโภคผักกวางตุ้ง ที่ปลูกโดยแหล่งน้ำที่ปนเปื้อนแคดเมียมในช่วง 1-3 มก/ลิตร จะไม่ก่อให้เกิดมะเร็งเรื้อรัง (hazard quotient < 1) แต่สามารถก่อให้เกิดสารก่อมะเร็งได้ (cancer risk > 10^{-6}) หากบริโภคมากกว่า 30 ปี ดังนั้น จึงขอแนะนำให้มีการควบคุมดูแล ปริมาณแคดเมียมในน้ำเพื่อใช้ในการเกษตรอย่างเคร่งครัด

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Piyachet Jinsart : Evaluation of Cadmium Bioaccumulations in Pak Choi Grown by Hydroponics system. Advisor: SUMETH WONGKIEW, Ph.D.

Cadmium is one of the most harmful heavy metals reported to contaminate the food chain and pose a high risk for health, including non-carcinogenic and carcinogenic effects. High levels of cadmium contamination due to industrial effluent discharge have been reported to affect plant growth and productivity, which could be used as a bio-indicator for cadmium bioaccumulation in plants. The utilization of cadmium-contaminated water for vegetable production has been known to increase the health risk for human consumption. Moreover, pH level was known as a factor affecting cadmium solubility and cadmium uptake by plants. However, there is still unknown about critical pH level that is associated with high cadmium concentrations in water resources used in agriculture (e.g., river, lake, effluent, etc.) and results in bioaccumulation and health risk of vegetable consumption grown by the cadmium contaminated water. In this study, pak choi as a model plant in a hydroponic setup was used to access cadmium bioaccumulation and the health risk of vegetable consumption. The experiment was conducted using four initial cadmium doses (control: 0 mg/L, 1 mg/L, 2 mg/L, and 3 mg/L) in a four-week operation. Three initial pH levels (7.5, 6.5, and 5.5) were used for experiments I, II, and III, respectively. The study was designed to use nitrogen and phosphorus levels at low concentrations (ammonium = 0.91 ± 0.36 mgN/L, nitrate = 0.50 ± 1.4 mgN/L, phosphate = 0.23 ± 0.10 mgP/L), which were in range of wastewater effluent and natural water for agricultural reuse. Results show that cadmium concentrations of 1-3 mg/L significantly inhibited pak choi growth ($p < 0.05$) compared with the control. Bioaccumulation in plant roots (0.035–2.963) and leaves (0.434–1.038) occurred with an increase in cadmium concentration ($p < 0.05$), suggesting that cadmium ions were translocated from water to roots and accumulated in the edible part of pak choi (leaves and stems). The highest cadmium available in water (2.67 ± 0.1 mg/L) was found at the initial pH of 5.5 due to the solubility of cadmium at the acidic pH level. Based on US EPA average daily intake and health risk assessment, it was found that consumption of pak choi grown by the cadmium contaminated water resource in the range of 1 to 3 mg/L will not cause chronic non-carcinogenic (hazard quotient <1) but can cause carcinogenic effect (cancer risk > 10-6) over 30-year consumption. Therefore, it is recommended that the monitoring of cadmium in water for agricultural use must be regulated strictly.

Field of Study:	Industrial Toxicology and Risk Assessment	Student's Signature ....
Academic Year:	2021	Advisor's Signature ....

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Piyachet Jinsart

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CHAPTER 1
INTRODUCTION

1.1 Introduction

Soil-based agriculture is now facing various challenges such as urbanization, natural disaster, climate change, and indiscriminate use of chemicals and pesticides, which deplete land fertility (Sharma et al., 2018). Hydroponics is an advanced technique for vegetable production. Although water resources such as rivers, groundwater, tap water, and other surface water are generally used in conventional agriculture, it was found that those water resources could be contaminated with heavy metals. Cadmium is one of the most harmful heavy metals found to contaminate both natural soil and water resources from commercial fertilizers, manure, sludge, and industrial effluent (López-Millán, Sagardoy, Solanas, Abadía, & Abadía, 2009). Moreover, it was found that cadmium in soil and water resources primarily resulted from industrial human activities, including mining, smelting, sludge, and wastewaters (Kabata-Pendias, 2004). Cadmium is the main contaminated pollutant that affects environments, agricultural lands, and human health, causing chronic adverse effects on kidneys, liver, bone, and blood from long-term exposure. Soils contaminated with cadmium and heavy metals have become a worldwide problem and serious disaster of the environment, leading to losses in agricultural yield and hazardous health effects as they enter the food chain(Anwar, Naz, Ashraf, & Malik, 2020)

Due to natural soil degradation, resource limitation, and urbanization, hydroponics has gained popularity in crop production as an emerging technology for resource recovery and reducing the effects of heavy metal contamination in soils.

Hydroponics is a method of growing crops without soil in which plants are grown in channels while recirculating water flowing through the root surface area.

Hydroponics is known as the innovation of growing plants without soil and has been developed for urban farming and smart agriculture. Hydroponic cultivation is also gaining popularity for the successful production of high-quality vegetables (e.g., lettuce, pak choi, spinach, tomatoes, etc.) due to efficient resources management and quality food production. Hydroponics also takes other advantages over soil-based systems such as pathogen-free, high plant yields, and predictable production rate(Arumugam, Sandeep, & Maheswari, 2021).Nutrients in hydroponics do not rely on soil fertility, moisture, and water availability; thus, hydroponics takes advantage to grow in different areas regardless of soil quality. In hydroponics, chemical solutions are supplied as external nutrient sources, and the nutrient concentrations (e.g., ammonium, nitrate, and phosphate) are controlled at a certain level. Moreover, natural water resources and wastewater containing nutrients were reported to be effective for growing plants in hydroponics (Prazeres, Albuquerque, Luz, Jerónimo, & Carvalho, 2017). With such advantages of NFT hydroponics, there is a high potential to integrate water resource reclamation with hydroponics for crop production.

Integration of hydroponics with water resource reuse has met limitations due to human perception and lack of information in health risk assessment from the resource recovery. Several studies reported that cadmium contamination in soils might cause an adverse effect on plant growth, and average daily intake was above the health risk level (Fang et al., 2019). Studies showed that cadmium contaminated water resources and resulted in bioaccumulation in plants at high levels (Woraharn, Meeinkuirt, Phusantisampan, & Chayapan, 2021).There is little information on an actual toxic level that causes adverse effects on plant growth and could result in health risks from vegetable consumption grown by hydroponics using cadmium contaminated water. Moreover, pH level is a key factor to control ions dissociation; thus, affecting nutrient and cadmium dissolution, availability, uptake, plant growth,

and toxic responses (Alexopoulos et al., 2021) .Thus, there is a need to evaluate the toxicity level of cadmium on plant growth and health risk assessment for human consumption.

The overarching goals of this study are to evaluate the toxic levels of cadmium on plant growth under nutrient concentrations for agricultural reuse. The objectives of this study are to (1) evaluate the bioaccumulation of cadmium and its effects on plant growth at different cadmium concentrations and pH levels and (2) to conduct health risk analyses of consuming pak choi grown by different levels of cadmium concentrations in hydroponics. The novelties of this study are (1) the low level of hydroponic nutrient concentrations that are in the range of reclaimed wastewater for agricultural use and (2) the effect of pH levels on the dissolution of high cadmium levels that could adversely affect plant growth.

1.2 Objectives

(1) To evaluate effects of cadmium concentrations on bioaccumulation and growth of pak choi grown in hydroponics at different cadmium concentrations and pH levels

(2) To evaluate health risk of consuming pak choi grown under different levels of cadmium concentrations in hydroponics

1.3 Hypotheses

(1) Growing the hydroponic plants at different pH levels will relate to the concentrations of cadmium in water and cadmium bioaccumulation in plant biomass.

(2) After conducting a health risk assessment of consuming pak choi contaminated with cadmium, there will be a cadmium concentration in water that causes a hazard quotient above 1, suggesting risk for consumption.

1.4 Scope of Studying

- This study will emphasize on how Pak choi could grow in a hydroponic system at different Cd concentrations and pH levels.

This study will aim at determination of cadmium concentrations that accumulates in plants and then taken out for health risk assessment in the Pak choi using secondary data of vegetable consumption before performing statistical analysis.

CHAPTER 2

THEORIES AND LITERATURE REVIEW

2.1 Problems and significance of study

2.1.1 Cadmium pollution in water resources

Cadmium concentrations in unpolluted natural waters are usually below 1 µg/l. Median concentrations of dissolved cadmium measured at 110 stations around the world were <1 µg/l. The maximum value recorded was found at 100 µg/l in the Rio Rimao in Peru Average levels in the Rhine and Danube in 1988 were 0.1 µg/l (range 0.02–0.3 µg/l) and 0.025 µg/l, respectively (WHO/UNEP, 1988-2004). Contamination of drinking-water may occur because of the presence of cadmium as an impurity in the zinc of galvanized pipes or cadmium-containing solders in fittings, water heaters, water coolers and taps. Drinking-water from shallow wells of areas in Sweden where the soil had been acidified contained concentrations of cadmium approaching 5 µg/l. (Organization, 2004; STRAŽANAC et al.)

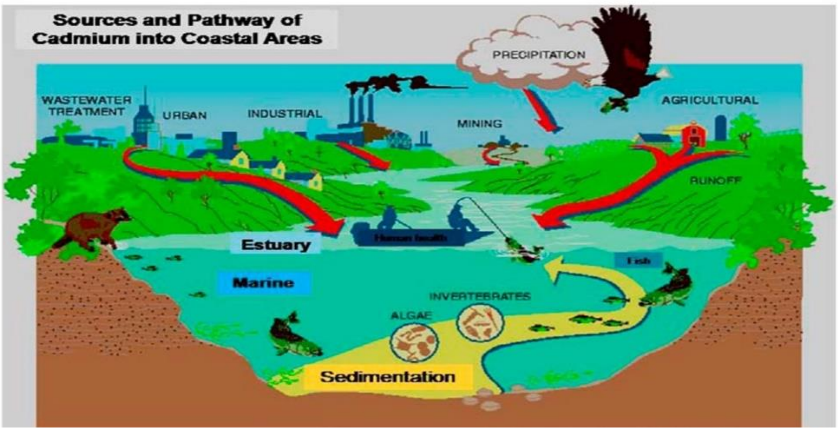
Figures 1 Soil Contamination in Water

(Source: https://en.wikipedia.org/wiki/Soilcontamination)

2.1.2 Sources and toxicity of cadmium

Components of automobiles include mechanical components, electronic and electrical devices, polymeric and sundry components that may contain toxic substances. Examples include vehicular crankshaft, engine block and connecting rod, which contains steel, chromium, nickel, titanium, copper while switches, batteries, headlamp bulbs, break light, data tapes, floppy disk, power supply boxes, and car stereo equipment contain cadmium, chromium and nickel etc.

These metals cause array of symptoms and chronic diseases. Studies have suggested that cadmium at low environmental exposures as currently found in industrialized countries, may result to subtle renal effects leading to noticeable level in urinary excretion of micro-proteins. Cadmium is hazardous both by inhalation and ingestion and can cause acute and chronic intoxications in humans with exceptional long half-life and accumulation in kidney, lungs, and liver, very toxic. Cd can disrupt biological systems, even at very low concentrations than most toxic metals.



Figures 2 Source of Pathway of Cadmium

(Source: http://what-when-how.com/mechanisms-of-cadmium-toxicity-to-various-trophic-saltwater organisms/sources-and-pathways-of-cadmium-in-the-environment-part-1/

2.2 Use of hydroponics in agriculture

Hydroponics is a method to grow plant without soil. Hydroponics required low amount of water for plant productions. Hydroponics can be used in serval urbanized areas where land and soil quality are limited. Water used in hydroponics can be taken from surface water and ground water. However, due to a rapid industrialization, water resources have been contaminated with heavy metals such as cadmium, resulting to the risk of reusing water resources, especially nearby industrial sectors which has been found to have cadmium contaminations. Although hydroponics can use water from several resources, it is still doubt that if the water resources are contaminated with cadmium, the safe level of cadmium contamination for growing plants using hydroponics from water resources is still unknown.

Hydroponics divide in 2 types, first is in non-circulating water and another one is in circulating water. It is a system in which the plants are grown where the roots are immersed in a solution. The plants are planted on foam pads or floating material to hold the stems. This system is commonly grown and can be used for planting troughs from various materials such as water pipes, foam boxes, buckets, and plastic bottles. Another one is in Circulating water called Nutrient Film Technique. The NFT system is the one we see often see in hydroponic growing. Both in Thailand and abroad look like a flat rail. When nutrients will flow through the pipes, it passes through the roots of plant in the planting trough. Plants in the system will get nutrients from water that

flows through it. There is also a gap in the planting trough for aerial roots to grow. There are 2 disadvantages; The price is quite high compared to other systems. And another disadvantage is that if the power goes out for a long time, this will cause the plant to die as the chute and will dry out of water.

Figures 3 Example of Hydroponics system in farm

(Source: https://www.conserve-energy-future.com/advantages-disadvantages-hydroponics.php)

2.3 Significance of this study

The main ideas of this research are to use hydroponic method to solve the agricultural productions although water is contaminated with some values of heavy metal contamination in water resources, which is commonly found in industrialized areas. Performing health risk assessment can suggest strategies to reduce the health risk for people consuming agricultural product from contaminated water resources.

2.4 Theoretical Backgrounds

2.4.1 Hydroponics and nutrient in agricultural treated and raw domestic wastewater for agricultural reuse

Hydroponics means growing plants in water, and it can be defined as growing plants without using soil. Although the benefits of hydroponics have sometimes been questioned, there seem to be many advantages in growing without soil. Some hydroponic growers have found to get yields many times greater than conventional methods. Because hydroponically grown plants dip their roots directly into nutrient rich solutions, plants get nutrients much more easily than plants growing in soil. Plants need much smaller root systems and can divert more energy into leaf and stem growth. With smaller roots, more plants can grow in the same area and get more yield from the same amount of ground.

There are various ways of growing plants hydroponically. In one popular method, plants can grow in a plastic trough and let a nutrient solution trickle past their roots (with the help of gravity and a pump). This is called the nutrient-film technique (NFT) systems. The nutrient is like a kind of liquid conveyor belt, it is constantly sliding past the roots delivering to them the goodness they need. Alternatively, plants can grow with their roots supported by a nutrient-enriched medium such as rockwool, sand, or vermiculite, which acts as a sterile substitute for soil.

Table 1 Nutrient in a variant of waste water and plant species

Wastewater	Plant Species	Phosphate	Ammonia	Nitrate	References
Municipal domestic water (25%)	Tomato (L.esculentum)	0.094 ± 0.011	0.72 ± 0.084	0.842 ± 0.091	Rana et a. (2011)
Municipal domestic water (50%)	Tomato (L.esculentum)	0.147 ± 0.042	1.81 ± 0.201	7.14 ± 0.084	Rana et a. (2011)
Municipal domestic water (75%)	Tomato (L.esculentum)	0.182 ± 0.027	4.0 ± 0.53	8.13 ± 0.761	Rana et a. (2011)
Municipal domestic water (100%)	Tomato (L.esculentum)	0.209 ± 0.018	5.91 ± 0.641	8.83 ± 0.875	Rana et a. (2011)

2.4.2 The optimum range of growing pak choi in hydroponics

In this study, pak choi (*Brassica rapa* subsp. *chinensis*) will be used. Pak choi can be grown on a wide range of soils, but the crop is sensitive to soil acidity. The

optimum pH is 5.6 to 6.2. The pak choi is ready to harvest as soon as it has usable leaves. The small varieties are mature at 6 inches tall, and the larger types grow 2 feet tall. The baby varieties are ready in about 30 days and the larger ones are ready four to six weeks after sowing. Electro-conductivity (EC) is generally controlled at 1.8 – 2.4(Mehta, 2018). Ammonia, nitrate, and phosphate in water are nutrients resource for pak choi in a good condition, which can be measured by spectroscopic methods or using EC as the representative of nutrient levels.

2.4.3 Intoxication of cadmium

Cadmium poisoning has been reported from many parts of the world. It is one of the global health problems that affect many organs, and in some cases, it can cause deaths annually. Long-term exposure to cadmium through air, water, soil, and food leads to cancer and organ system toxicity such as skeletal, urinary, reproductive, cardiovascular, central, and peripheral nervous, and respiratory systems. Cadmium levels can be measured in the blood, urine, hair, nail, and saliva samples. Patients with cadmium toxicity need gastrointestinal tract irrigation, supportive care, and chemical decontamination traditional-based chelation therapy with appropriate new chelating agents and nanoparticle-based antidotes.

2.5. Literature reviews

2.5.1 Contaminations and bioaccumulations of heavy metals in vegetables

(Singh, Singh, Kumar, Bhargava, & Barman, 2010) studied the accumulation and translocation of heavy metals in soil and plants from fly ash contaminated area. They investigated and deals with the accumulation of heavy metals in fields contaminated with fly ash from a thermal power plant and subsequent uptake in different parts of naturally grown plants. Results revealed that in the contaminated site, the mean level of all the metals (Cd, Zn, Cr, Pb, Cu, Ni, Mn and Fe) in soil and different parts (root and shoots) of plant species were found to be significantly

($p<0.01$) higher than the uncontaminated site. The enrichment factor (EF) of these metals in contaminated soil was found to be in the sequence of Cd (2.33) > Fe (1.88) > Ni (1.58) > Pb (1.42) > Zn (1.31) > Mn (1.27) > Cr (1.11) > Cu (1.10).

(Bawa, Sharif, & Hashim, 2015) found that Artemisia plants and foliaceous lichens are known to be capable of accumulating heavy metals (HM) from soil and air. These plant species are widespread on polluted sites of Azerbaijan. However, so far, their capacity to accumulate HM in their shoots and roots has not been tested. In this study, three Artemisia and two lichen species were collected from different contaminated sites of Azerbaijan. Plant and surface soil samples were measured for Cd, Cu, Pb, Ni and Zn concentrations. The results indicated that among the Artemisia species, *A. scoparia* showed the best HM accumulation properties. Lichen species were also distinguished by very high amounts of HM in their biomass, while in surrounding soil samples HM concentrations had higher contents than the soils occupied only with Artemisia species. The results indicated that on contaminated sites, Artemisia, lichens accumulated metals in their biomass without toxicity symptoms. Heavy metals may enter the food chain because of their uptake by edible plants, thus, the determination of heavy metals in environmental samples is very important.

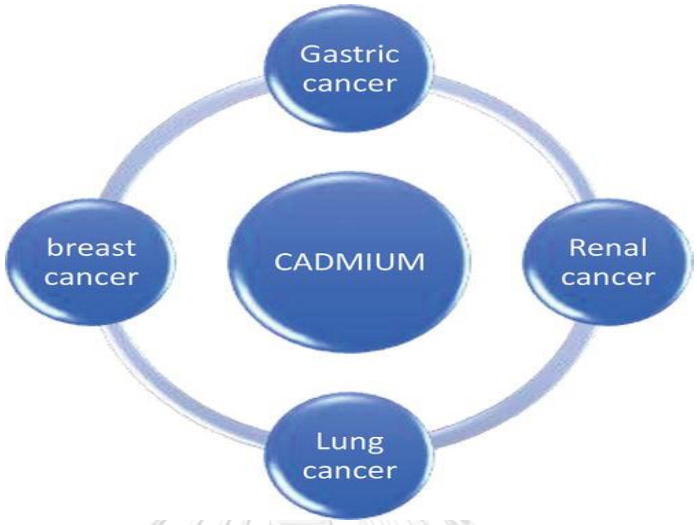
(Debnath et al., 2014) analyzed HMs in *Lycopersicon esculentum.* The study was carried out in agricultural field of Sanganer town. In the study area (Amanishah nallah, Sanganer, Jaipur) vegetables were grown in the field receiving sewage and textile wastewater. Water, soil, and crop plant samples were collected from the agricultural field of Sanganer for analysis. Soil from agricultural field contained 28.860 mg/g of Zn, 23.410 mg/g of Cu, 6.690 mg/g of Cr and 8.613 mg/g of Pb.

(Akün, 2020) studies heavy metal contamination and remediation of water and soil with case studies from Cyprus. Some of heavy metals tended to endanger to health, found above critical limits in soil and water and becoming carcinogenic. This research studied heavy metals arsenic, cadmium, chromium, and nickel. Actual case studies from North Cyprus are provided with real contamination levels observed. Different areas and soil water plant species were assessed in detail, displaying concentrations, critical limits transfer and recommendations. Selected locations in Cyprus were investigated by the Cancer Research Fund and Frederick Institute of Technology. To achieve an analytical distribution, 260 composite soil samples were investigated at nine different locations for heavy metals using absorption spectrophotometer. The findings displayed average concentration level mg/kg as follows; Cu 208.4, PB 119.4, Cr 18.38, Cd 6.19, and Zn 144.2.

2.5.2 Health risk assessment of heavy metals in different areas

Workers in the battery, pigment, and electroplating sectors are among those who are exposed to cadmium on the job. Because of its long-term accumulation in the human body, even modest amounts are toxic and carcinogenic. Another key source of cadmium in the soil is sewage sludge, which may produce about the same amount of cadmium as fertilizer usage. Figure 4 depicts the many forms of carcinogenic consequences of cadmium poisoning. Cadmium has harmful effects on the stomach system, causing gastric cancer, breast cancer, and lung cancer, as well as on the excretory system, causing renal cancer. Symptoms of acute poisoning from rating are nausea, vomiting, diarrhea, headache, muscle pain, salivation, abdominal pain, shock,

kidney, and liver damage. From breathing causes to chest pain, shortness of breath, metallic odor in the mouth, weakness, pain in the legs, less urine output, fever, and symptoms of pneumonia.



Figures 4 Carcinogenic effect of cadmium

(Achakzai & Bazai, 2006) found phytoaccumulation of heavy metals in Spinach (*Spinacea oleraceac L.*) irrigated with wastewater of Quetta city. Their study was conducted to determine the concentration of heavy metals in spinach when irrigated with 5 different concentrations (treatments) of wastewater collected from three different localities of Quetta city. The spinach was used as a test plant and was grown in pots. Results revealed that localities, treatment, and their interactions were generally exhibited highly significant ($P < 0.01$) effect an accumulation of heavy metals in spinach. The maximum values of 16.09, 602.60, 36.82, 161.28, 11.35, 5.61 and 8.25 mg/kg for Cu, Fe, Mn, Zn, Pb, Ni and Cd, respectively, were usually obtained in highest treatment of effluents. Results further demonstrated that Fe, Zn and Cd of spinach lie in toxic range and Ni was found near to toxicity, whereas Cu,

Mn and Pb concentration lie below toxicity level. Based on findings it can be assumed that wastewater irrigation produces excess of heavy metals in vegetables (spinach).

(Athar & Ahmad, 2002) found the toxic effects of certain heavy metals on the plant growth and grain yield of wheat (*Triticum aestivum* L.). For this study, a pot experiment was conducted, and the results revealed that heavy metals brought about significant reductions in both parameters. Cd was the most toxic metal followed by Cu, Ni, Zn, Pb and Cr. Moreover, the presence of Cd in the soil resulted in the maximum inhibition (84.9%) in the number of free livings *Azotobacter chroococcum* cells over the control. The phytotoxicity was apparently due to the susceptibility of the free living *Azotobacter chroococcum* cells to the toxic doses of heavy metals. Protein content decreased from 19.0–71.4% in metal exposed plants at metal concentrations equivalent to those found in polluted soil. Metal uptake by grains was directly related to the applied heavy metal with greater concentrations of metals found in cases where metals were added separately rather than in combinations.

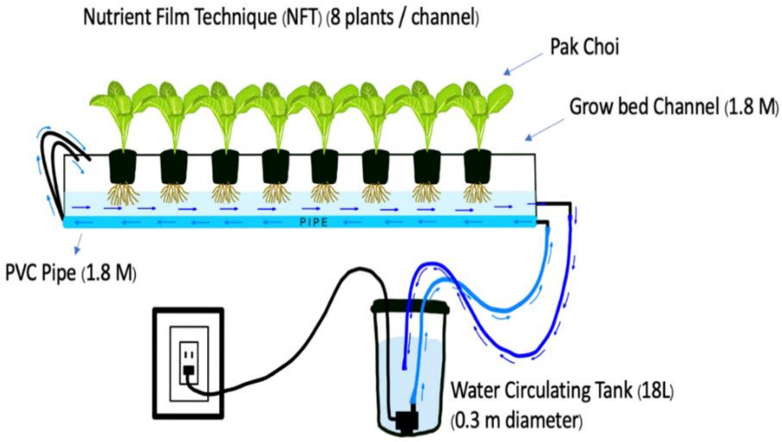
(Chove, Ballegu, & Chove, 2006) have determined copper and lead levels in two popular leafy vegetables grown around Morogoro Municipally Tanzania. They collected vegetables samples of Pumpkin leaves (*Cucurbita moschata*) and Chinese pak choi (*Brassica chinensis*) from three sites and analyzed for their concentration of two metals using an Atomic Absorption Spectrophotometer. The results indicated that the levels (mg/100gm dry weight) ranged from 0.885 to 1.39 for copper and 0.05 to 0.315 for lead.

CHAPTER 3

METHODOLOGY

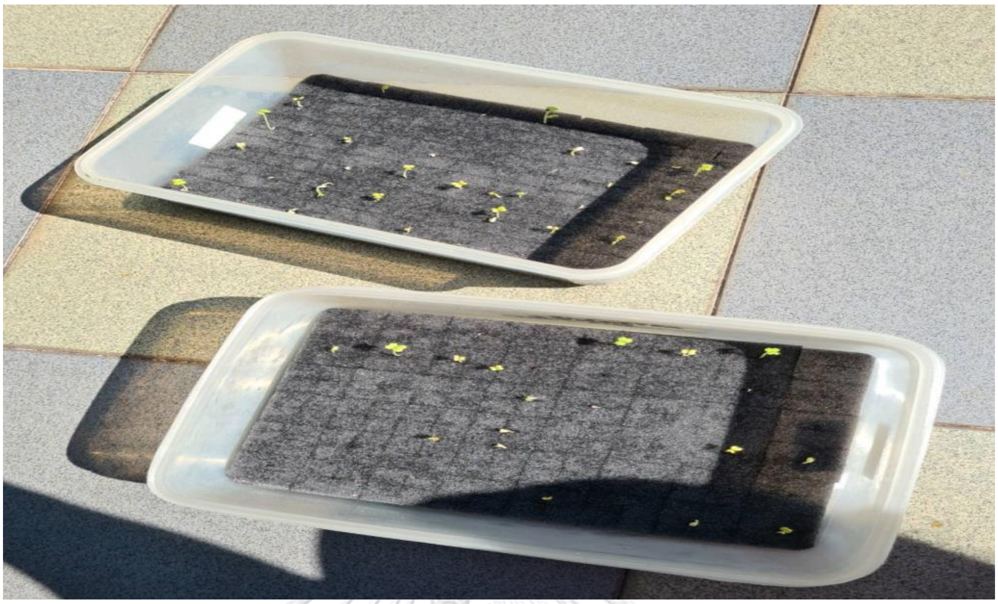
3.1 Hydroponic set up and operation

Hydroponics Nutrient film technique (NFT) systems were constructed and operated at the Department of Environmental Sciences, Chulalongkorn University. The NFT consisted of a channel allowing a thin layer of recirculating water to flow through the root zone in grow bed and a recirculating tank with a water pump for water circulation (Fig. 1). Pak choi (*Brassica rapa* sub. *Chinesis*) was used as a model plant for the bioaccumulation test of cadmium. Seeding was taken within 14 days and sprayed water twice a day. After the seeding, plants were transferred to the hydroponic growing bed for 28 days in the NFT systems. A growing bed containing 8 plants was designed as a grow bed, and a recirculating system (a 5-gallon water tank was designed using a recirculating pump) (Fig. 1). Before starting each experiment, commercial hydroponic nutrient solutions were added to the hydroponic systems to adjust ammonium and phosphorus concentration in the range of wastewater for agricultural reuse (~1 ± 0.5 mgN/L, ~0.3 ± 0.1 mgP/L).



Figures 5 Schematic diagram of a NFT hydroponic system used in this study

Figures 6 NFT system at the Department of Environmental Sciences, Chulalongkorn University.



Figures 7 First weeks of seedings Pak Choi

Figures 8 Pak Choi after seedings for 2 weeks

3.2 Experimental design

The hydroponic experiments were conducted in duplicate. Three experiments were designed using pH levels of 7.5, 6.5, and 5.5, respectively, as shown in Table 2. In each experiment, four conditions were conducted using four initial cadmium doses (0 mg/L, 1 mg/L, 2 mg/L, and 3 mg/L) in a 28-day operation. A stock solution of cadmium chloride was spiked to the hydroponic systems with an amount according to the four initial cadmium doses. Three initial pH levels (7.5, 6.5, and 5.5) were used for experiments I, II, and III, respectively. Different pH was adjusted by sulfuric acid and potassium hydroxide. The pH level was varied from experiments I to III because it was hypothesized that pH could affect the cadmium and ion dissolutions in water including the plant growth and nutrient/heavy metal uptake. pH levels were adjected every week. Water samples were taken every week for ammonium, nitrate, phosphate, and pH levels. Pak choi were harvested at the end of each experiment for wet weight and dry weight (70^{o}C for 48 hours). The dried plants were mixed, ground, and analyzed for cadmium content in plant tissues. Cadmium in the edible part of pak choi (whole edible part) and root was measured separately. The bioaccumulation of hydroponically grown pak choi at various cadmium concentrations was compared with control (no cadmium addition). The plant yield in each experiment was evaluated, and the pH level that resulted in the highest plant yield and cadmium accumulation was identified. The level of Cd maximum concentration giving no Chronic non-carcinogenic and

No table of figures entries found.carcinogenic health risks from consuming pak choi grown by cadmium contaminated water in hydroponics were analyzed using the standard USEPA risk assessment equations.

Table 2 Experimental design one experiment consisted of 4 conditions of Cadmium concentrations and each condition growing 8 plants in duplicate set

Experiment No.	Condition No.	Cadmium dose (mg/L)	pH
1	1	control (no Cd added)	7.5
	2	1	
	3	2	
	4	3	
2	1	control (no Cd added)	6.5
	2	1	
	3	2	
	4	3	
3	1	control (no Cd added)	5.5
	2	1	
	3	2	
	4	3	

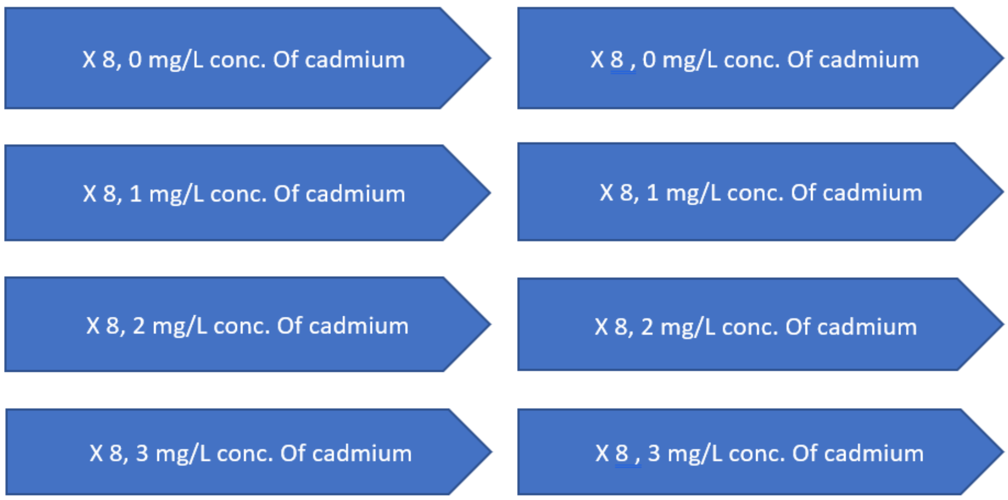
3.3 Preparing for cadmium concentration in container

To adjust cadmium concentration in water, this study will add $CdCl_2$ to each hydroponic system. Solution of $CdCl_2$ (10000 ug Cd/L = 16350 ug $CdCl_2$/L) will be prepared and added to the systems using different concentrations as follow:

- Condition 1 = 0 mg/L Cd
- Condition 2 = 1 mg/L Cd
- Condition 3 = 2 mg/L Cd

Condition 4 = 3 mg/L Cd

- Therefore, the addition of cadmium will be redone in 4 different experiments (Table 3.1).
- The calculation used to add cadmium solution is based on mass balance.



Figures 9 Experimental design in 2 duplicates

3.4 Parameters and sampling for analyses

- pH using pH meter to control 3 different values of 7.5, 6.5 and 5.5 in the systems (once a week)
- pH will be adjusted by sulfuric acid and potassium hydroxide

- Cadmium concentration in water will be measured once a week. Cd in pak choi (edible part and root) will be measured at end of each experiment. Cd measurement will be performed using Inductively Couple Plasma Optical Emission Spectrometer (ICP-OES).
- Ammonia, nitrate, phosphate in water using chemical analyses (once a week)
- Measure weight net and length of pak choi root (end of each experiment)
- Planting time: It takes about 4 weeks after transplanting to get the plant ready for harvest

3.5 Bioaccumulation Factor

Bioconcentration factors (BCF) were used to evaluate the levels of cadmium concentrations in plant tissues relative to concentration in water. In this study, BCF was used to compare the ability of the plant to concentrate cadmium in their tissues. BCF of pak choi and roots were calculated using the following equations.

$$BCF_{plant\text{-}water} = C_{plant}\ (\mu g/g)/C_{water}\ (ug/L) \qquad (1)$$

$$BCF_{root\text{-}water} = C_{root}\ (\mu g/g)/C_{water}\ (ug/L) \qquad (2)$$

Where, $BCF_{plant\text{-}water}$ and $BCF_{root\text{-}water}$ are bioconcentration factors in pak choi (edible part) and root, respectively; C_{plant} and C_{root} are concentrations of cadmium in pak choi (edible part) and root, respectively (mg/kg); C_{water} is cadmium concentration in hydroponic recirculating water (mg/L). BCF was determined using the slope of cadmium concentrations in the plant tissues by average cadmium concentrations in water.

BCF is described as the ability of plants for elemental accumulation from the substrate (Mishra & Pandey, 2019).A key assumption for the calculation or measurement of BCF is that of equilibrium between the contaminant and organism.

Equilibrium can be easily achieved for small organisms with fast growth rates; however, equilibrium is very difficult to reach for large organisms such as fish, in which equilibrium may not be reached during their entire life history.

3.6 Health risk assessment

3.6.1 Exposure assessment

The average daily dose (ADD) was used to evaluate cadmium intake per day from vegetables during the lifetime of consumption. In this study, the consumption of plant roots was neglected, and only edible part was accounted for ADD on pak choi grown by the hydroponic systems. The dermal and inhalation routes were negligible because the intake was considered based on the oral route of the vegetable consumption.

$$ADD = C_{medium} \times IngR \times EF \times ED / BW \times AT \quad (3)$$

Where ADD is an average daily dose (mg/kg-day); C_{medium} is cadmium concentration in pak choi (mg/g wet wt.); IngR is ingestion rate of pak choi (2.86 g wet/day of the whole population) (Manjón et al., 2020); EF is exposure frequency (365 days/years); ED is exposure duration (30 years for non-carcinogenic and carcinogenic risk assessment) . AT is averaging time (days, AT = ED × 365). AT for non-carcinogenic risk is 10,950 days (30 years) and, AT for carcinogenic risk is 25,550 days (70 years). Cadmium content in wet weight basis was calculated from cadmium content in dry weight basis using a multiplication factor as follows.

$$C_{medium} = C_{dry\ wt.} \times (100 - \%m)/100 \quad (4)$$

Where, $C_{dry\ wt.}$ is cadmium concentration on a dry weight basis (mg/g wet wt.); %m is the average moisture content in pak choi (%).

3.6.2 Risk characterization

Hazard quotient (HQ) and cancer risk (R_c) were used as indices to evaluate non-carcinogenic risk and carcinogenic cadmium uptake from pak choi consumption. HQ and R_c were calculated using the following equations.

$$HQ = ADD/RfD \quad (5)$$

$$R_c = ADD \times CSF \quad (6)$$

Where, RfD is the reference dose of the non-carcinogenic risk of cadmium via ingestion route (0.001 mg/kg-day) and CSF is the cancer slope factor for carcinogenic risk of cadmium via ingestion route (15 kg-day/mg). Cadmium intake in a person was considered not to cause a human health risk when HQ was below 1.0 for non-carcinogenic risk and R_c below 10^{-6} for carcinogenic risk (Quispe et al., 2021)

RC = Cancer risk

ADD = Average daily dose (mg/ kg-day)

SF = Slope factor (mg/ kg-day)

3.7 Statistical analyses

The one-way analysis of variance (ANOVA) will be used to determine whether there are any statistically significant differences between the means of two or more independent (unrelated) groups.

Pearson's Correlation will be used to investigate the relationship between two quantitative, continuous variables. Pearson's correlation coefficient will be used to see strength of the association between the two variables. The independent (or explanatory) variable is plotted on the x-axis and the dependent (or response) variable is plotted on the y-axis.

CHAPTER 4

RESULTS AND DISSCUSSION

4.1. Nutrient availability and cadmium solubility at varying pH levels

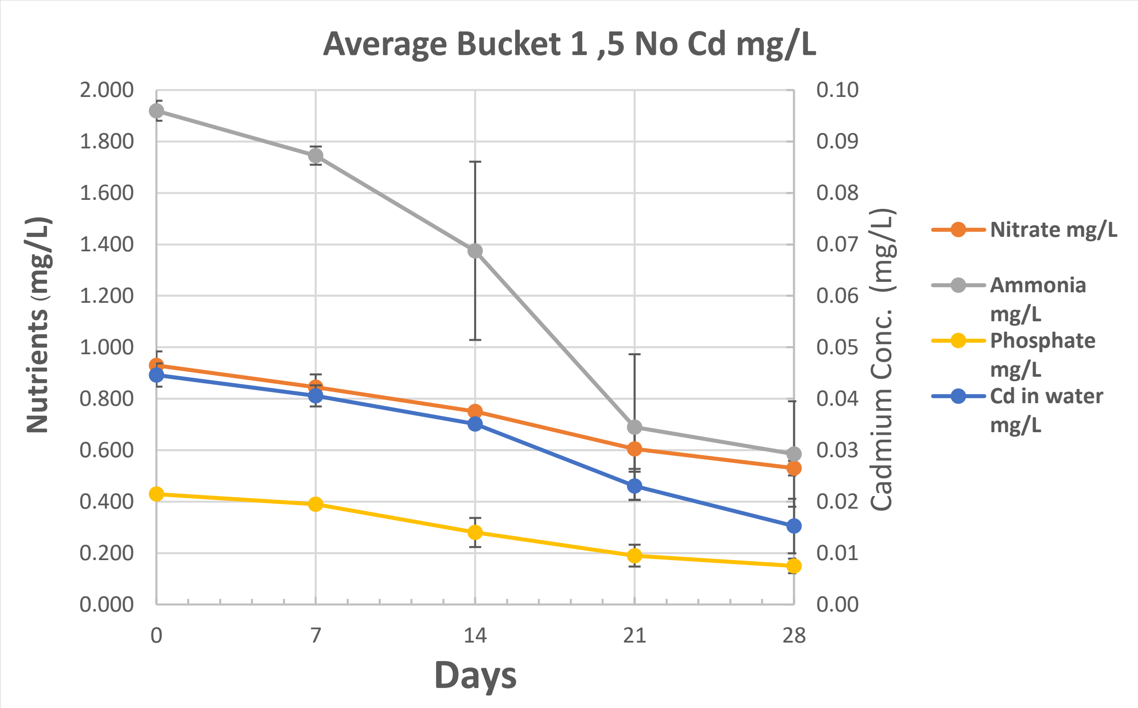
Ammonium, nitrate, and phosphate concentrations were in ranges of 3.9–12.6 mgN/L, 3.2–7.5 mN/L, and 0.22–0.43 mgP/L, respectively (Table 2), which were in ranges of domestic waste/wastewater used in agricultural systems (Prazeres et al., 2017; Rana et al., 2011). For example, municipal wastewater with ammonium, nitrate, and phosphate concentrations of 0.72 mgN/L, 0.842 mgN/L, and 0.209 mgP/L was found to be successful for growing tomatoes from domestic wastewater (Rana et al., 2011)

The pH levels were within the designed levels, suggesting pH levels were well maintained during the whole experiment. Results showed that cadmium doses supplemented into hydroponic systems did not affect ammonium, nitrate, and phosphate concentrations ($p > 0.05$). Although ions concentrations (e.g., cadmium concentration) can affect inorganic nutrient solubility in water, pH level could be the main factor to control nutrient availability and dissociation within each experiment (da Silva Cerozi & Fitzsimmons, 2016). Thus, there was no effect of cadmium doses that make a significant difference in nutrient concentrations. The results suggested that by controlling a constant pH level, hydroponic systems can maintain a constant dissociation of available nutrients for plant uptake.

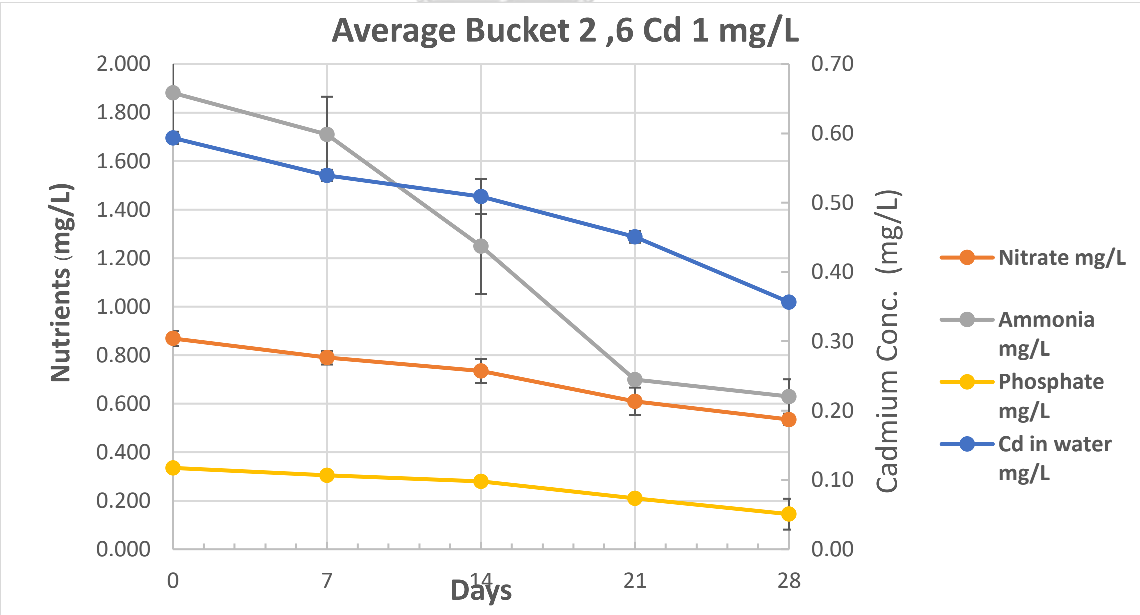
Table 3 Ammonium, Nitrate and Phosphate concentrations at varying Cadmium and pH levels

Exp no. /pH	Cadmium dose (mg/L)	pH level	Ammonium (mgN/L)	Nitrate (mgN/L)	Phosphate (mgP/L)
1/7.5	control	$7.49 \pm 0.03^{A, *}$	1.26 ± 0.59^{A}	0.73 ± 0.16^{A}	0.29 ± 0.12^{A}
	1.0	7.46 ± 0.04^{A}	1.23 ± 0.55^{A}	0.71 ± 0.13^{A}	$0.26 \pm 0.08^{A, B}$
	2.0	7.45 ± 0.06^{A}	1.10 ± 0.66^{A}	0.72 ± 0.17^{A}	0.22 ± 0.08^{B}
	3.0	7.44 ± 0.04^{A}	1.24 ± 0.62^{A}	0.75 ± 0.13^{A}	0.22 ± 0.08^{B}
2/6.5	control	6.46 ± 0.04^{A}	1.09 ± 0.38^{A}	0.32 ± 0.11^{A}	0.26 ± 0.11^{A}
	1.0	6.43 ± 0.06^{A}	1.08 ± 0.32^{A}	0.34 ± 0.10^{A}	0.26 ± 0.10^{A}
	2.0	6.45 ± 0.05^{A}	1.12 ± 0.36^{A}	0.33 ± 0.08^{A}	0.26 ± 0.08^{A}
	3.0	6.45 ± 0.04A	1.11 ± 0.36^{A}	0.34 ± 0.09^{A}	0.24 ± 0.10^{A}
3/5.5	control	5.45 ± 0.04A	0.45 ± 0.12^{A}	0.45 ± 0.19^{A}	0.36 ± 0.12^{B}
	1.0	5.43 ± 0.06A	0.41 ± 0.14^{A}	0.44 ± 0.21^{A}	$0.41 \pm 0.14^{A, B}$
	2.0	5.46 ± 0.03A	0.39 ± 0.14^{A}	0.45 ± 0.17^{A}	0.43 ± 0.14^{A}
	3.0	5.45 ± 0.04 A	0.43 ± 0.10^{A}	0.47 ± 0.18^{A}	$0.38 \pm 0.10^{A, B}$

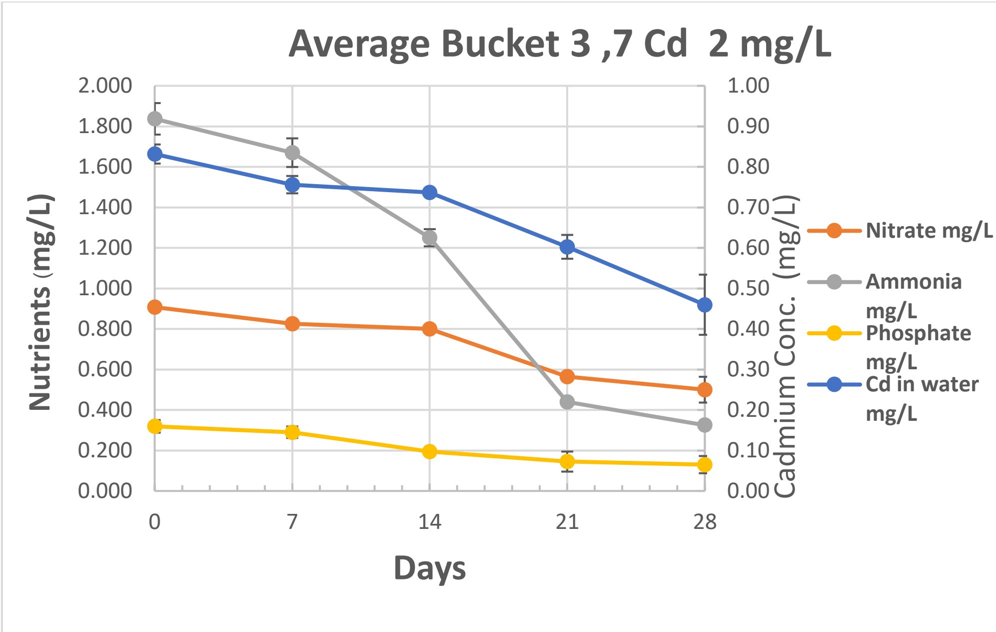
Note: Symbol * represents mean ± standard deviation of pH levels and ammonium, nitrate, phosphate concentrations in hydroponics (n = 10). Letters A to D indicate a significant difference of mean ($p < 0.05$) within each experiment.



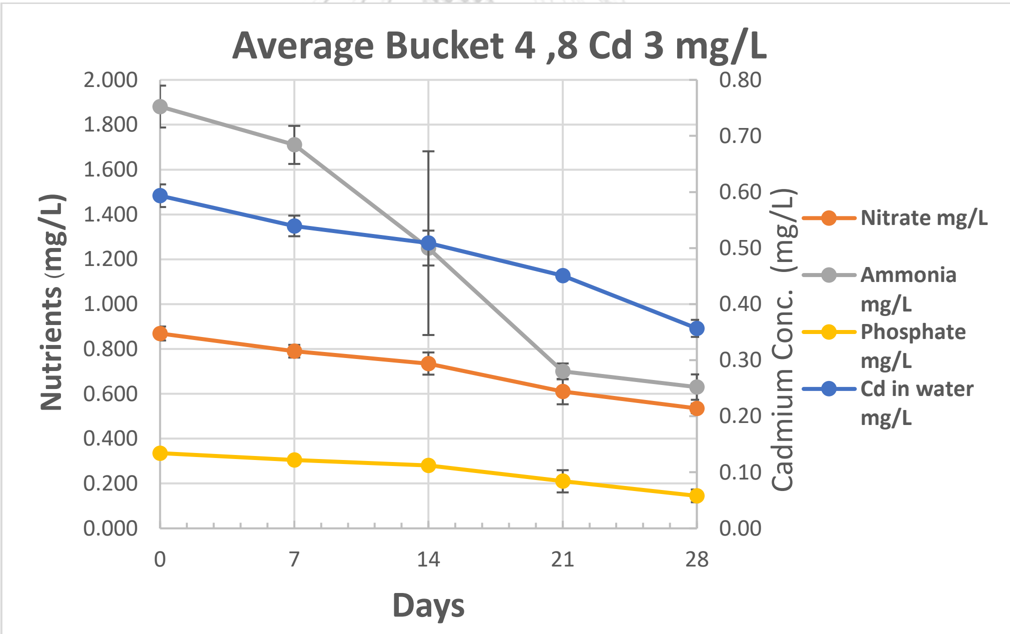
Figures 10 Tendency of relations between nutrients and days in cycle 1 (control)



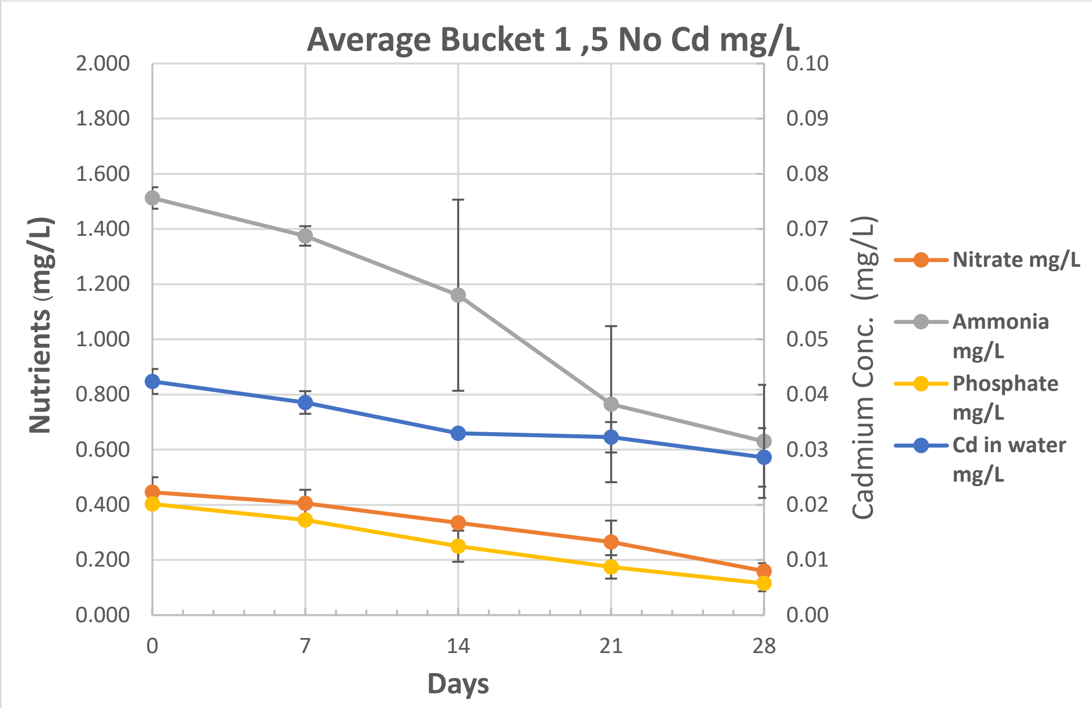
Figures 11 Tendency of relations between nutrients and days in cycle 1 (1 mg/L Cd added)



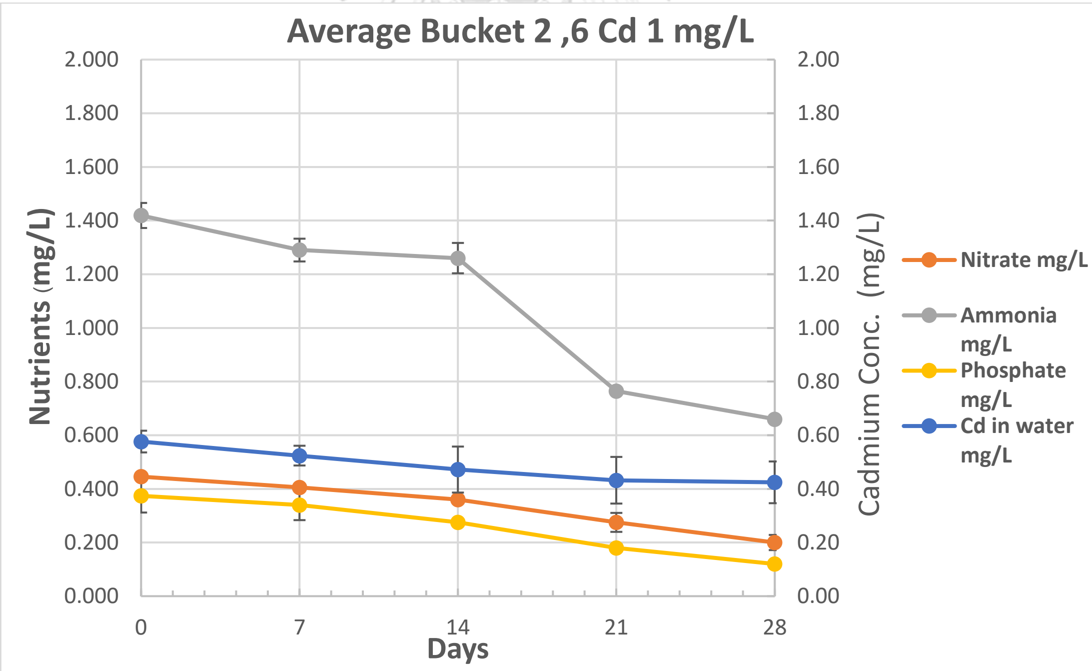
Figures 12 Tendency of relations between nutrients and days in cycle 1 (2 mg/L Cd added)



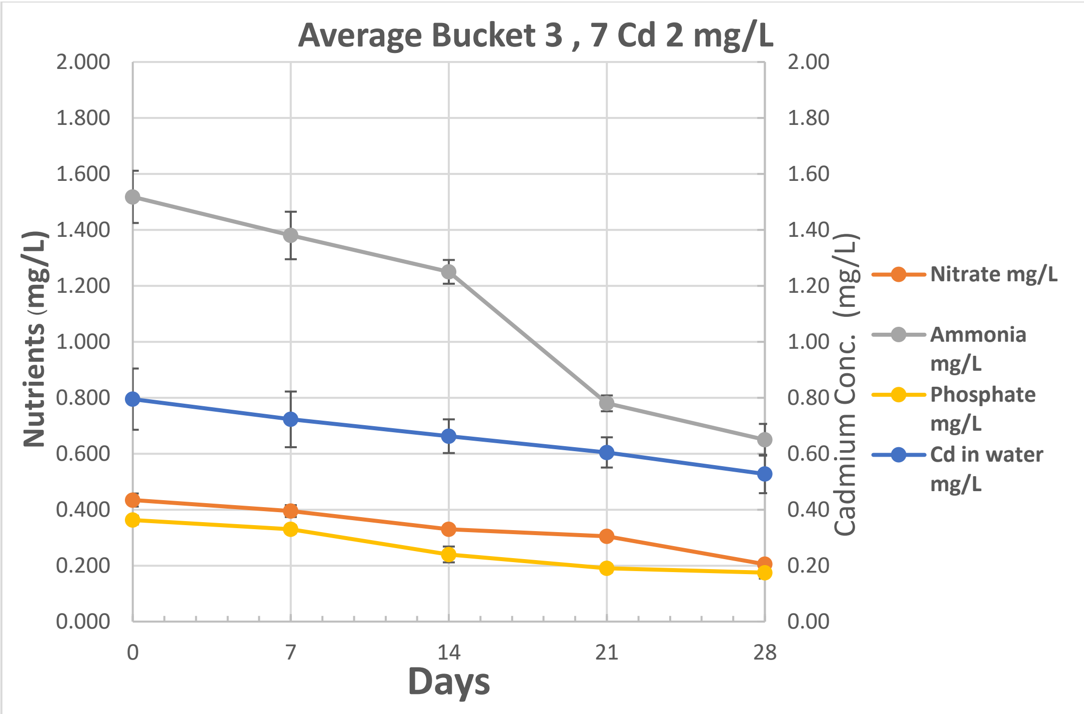
Figures 13 Tendency of relations between nutrients and days in cycle 2 (3 mg/L Cd added)



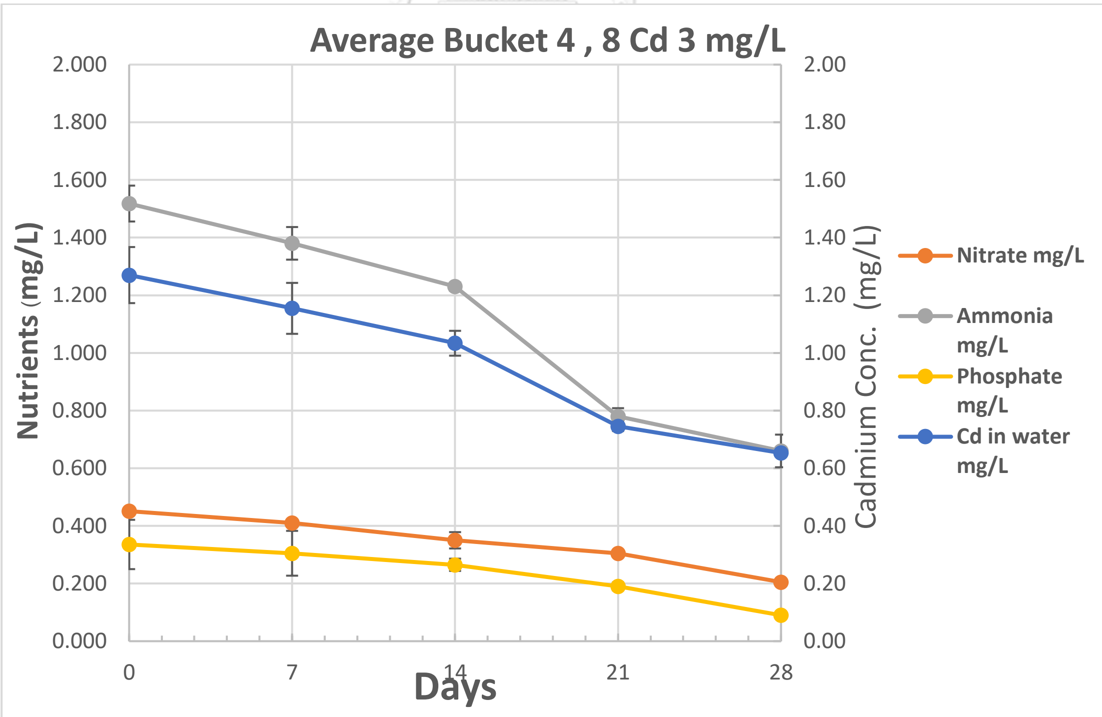
Figures 14 Tendency of relations between nutrients and days in cycle 2 (control)



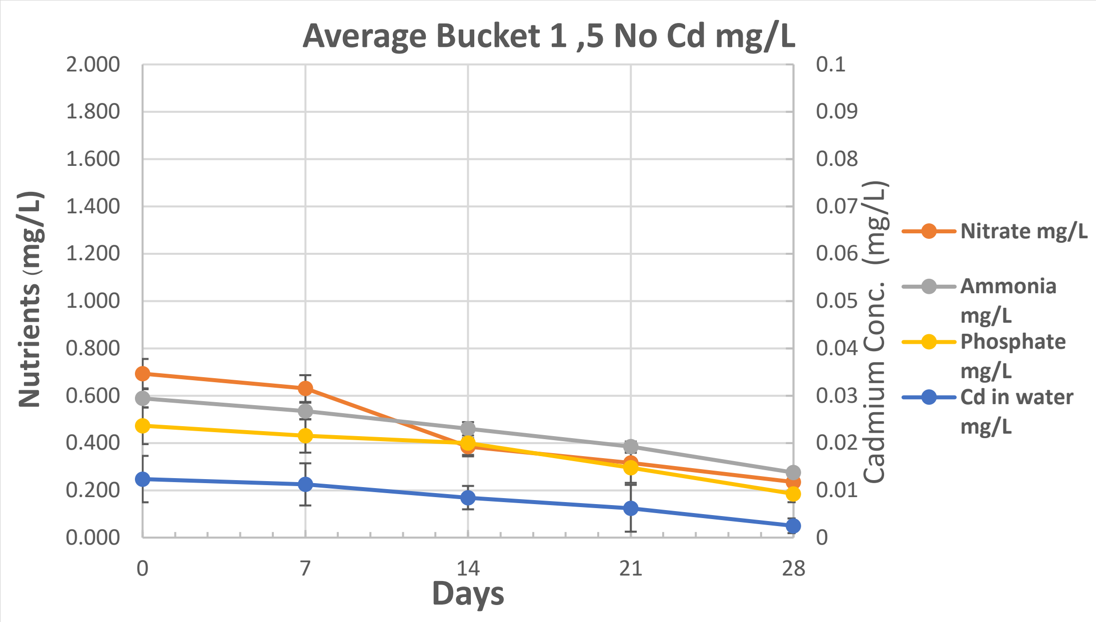
Figures 15 Tendency of relations between nutrients and days in cycle 2 (1 mg/L Cd added)



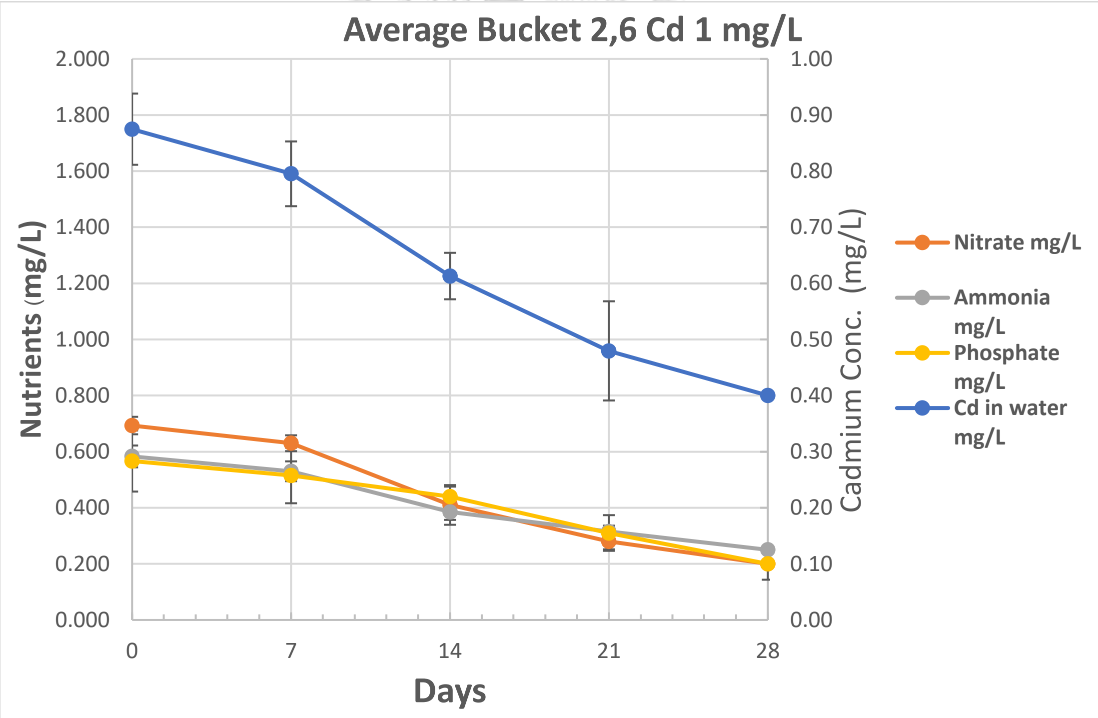
Figures 16 Tendency of relations between nutrients and days in cycle 2 (2 mg/L Cd added)



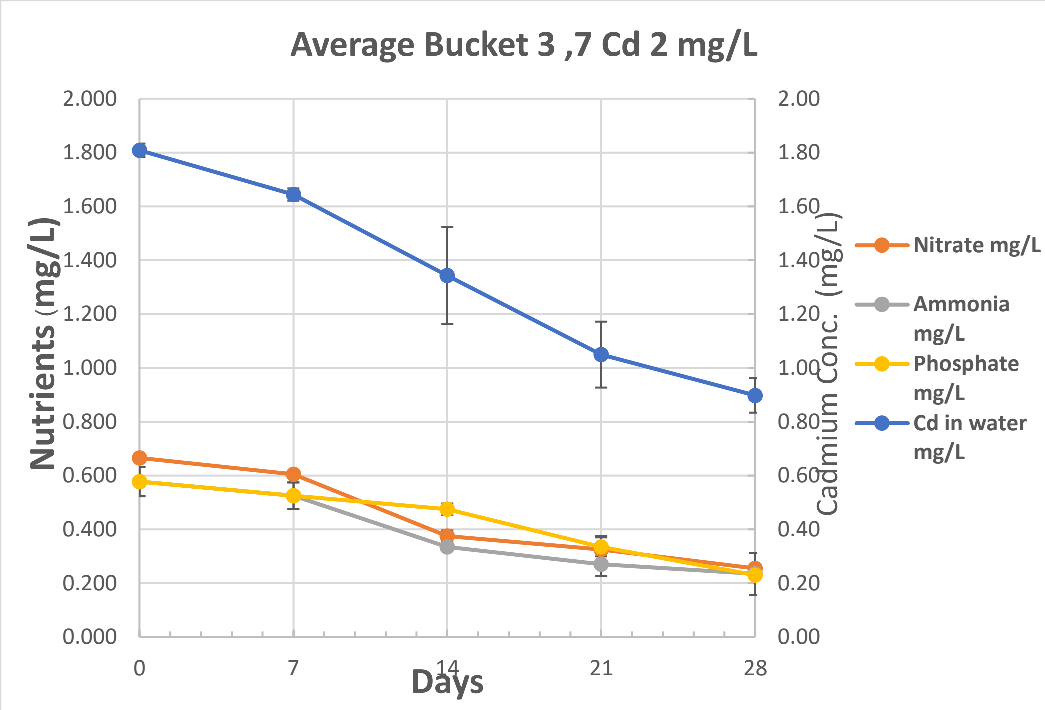
Figures 17 Tendency of relations between nutrients and days in cycle 2 (3mg/L Cd added)



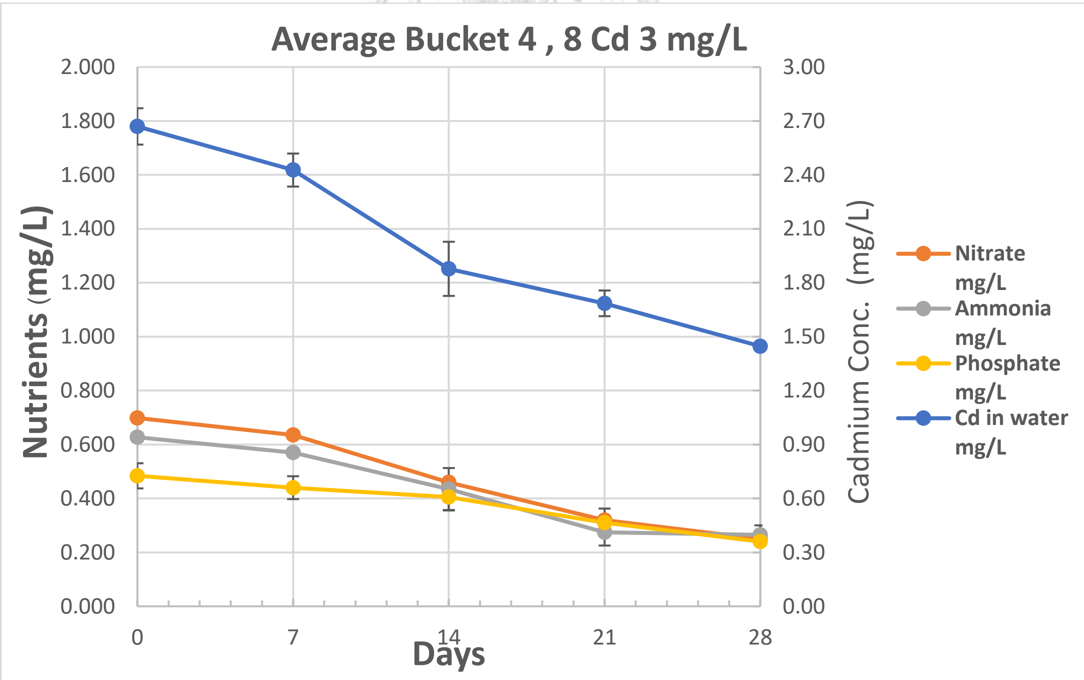
Figures 18 Tendency of relations between nutrients and days in cycle 3 (control)



Figures 19 Tendency of relations between nutrients and days in cycle 3 (1 mg/L Cd added)



Figures 20 Tendency of relations between nutrients and days in cycle 3 (2 mg/L Cd added)



Figures 21 Tendency of relations between nutrients and days in cycle 3 (3 mg/L Cd added)

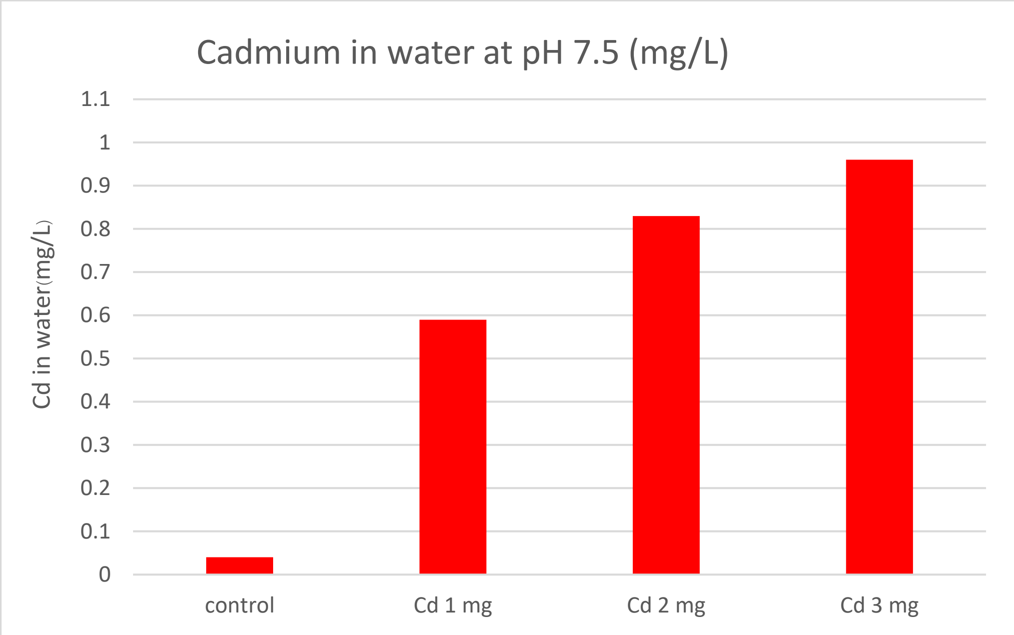
Different pH levels affected the cadmium dissolution and were available in water (Table 3). At low pH, there were high soluble cadmium concentrations more than that at higher pH levels (e.g., 6.5 and 7.5), as shown in Table 3. Particularly, at 3 mg/L cadmium added, the % cadmium availability was found to increase toward low pH levels. For example, at a cadmium dose of 3 mg/L, the pH level of 5.5 showed the highest cadmium available in water (89.0%) compared to pH levels of 6.5 (42.3%) and 7.5 (32.0%). The result could be because other ions in hydroponics such as bicarbonate and other nutrient minerals (e.g., nitrate, sulfate, chloride, ionic strength, etc.) contributed to the precipitation of some cadmium ions and resulted in a loss of calcium soluble in water (Kubier, Wilkin, & Pichler, 2019). Another reason could be due to cadmium uptake by plants that translocate cadmium in water to cadmium in plant tissues such as roots and leaves(Wei et al., 2019). High cadmium doses resulted in higher cadmium concentrations in hydroponic water. However, the higher concentrations caused lower percent cadmium available in water because cadmium concentration is the factor that positively affects the precipitation of cadmium species. Therefore, cadmium was found to dissolve toward low pH levels, while higher cadmium concentrations in water could result in precipitation of cadmium ions, leading to lower percent cadmium available for plant uptake. The results suggested that plant grown with hydroponics has high tendency to absorb cadmium at a low pH level, and precipitation of cadmium could be found proportional to high cadmium concentration.

Table 4 Cadmium concentrations in hydroponics at varying cadmium and pH levels

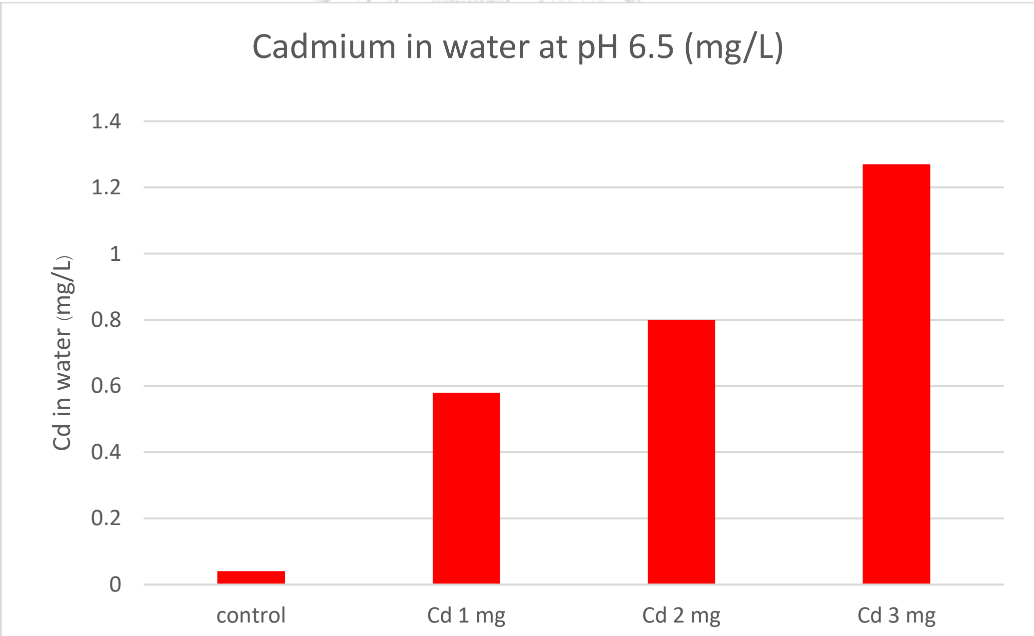
Exp no./pH	**Cadmium dose (mg/L)**	**pH Level**	**Cadmium in water (mg/L)**	**% Cadmium available**
1/7.5	control	7.49 ± 0.03^{A}	0.04 ± 0.001^{D}	-
	1.0	7.46 ± 0.04^{A}	0.59 ± 0.01^{C}	59
	2.0	7.45 ± 0.06^{A}	0.83 ± 0.02^{B}	41.5
	3.0	7.44 ± 0.04^{A}	0.96 ± 0.02^{A}	32.0
2/6.5	control	6.46 ± 0.04^{A}	0.04 ± 0.0064^{D}	-
	1.0	6.43 ± 0.06^{A}	0.58 ± 0.04^{C}	58
	2.0	6.45 ± 0.05^{A}	0.80 ± 0.11^{B}	40.0
	3.0	6.45 ± 0.04^{A}	1.27 ± 0.10^{A}	42.3
3/5.5	control	5.45 ± 0.04^{A}	0.01 ± 0.005^{D}	-
	1.0	5.43 ± 0.06^{A}	0.87 ± 0.06^{C}	87
	2.0	5.46 ± 0.03^{A}	1.81 ± 0.02^{B}	90.5
	3.0	5.45 ± 0.04^{A}	2.67 ± 0.10^{A}	89.0

Note: Symbol *represents mean ± standard deviation of pH levels and cadmium concentrations in hydroponics (n = 10). ** Percent cadmium soluble was calculated using average cadmium detected in hydroponics divided by cadmium dosage supplemented at the beginning of each experiment. Letters A to D indicate a

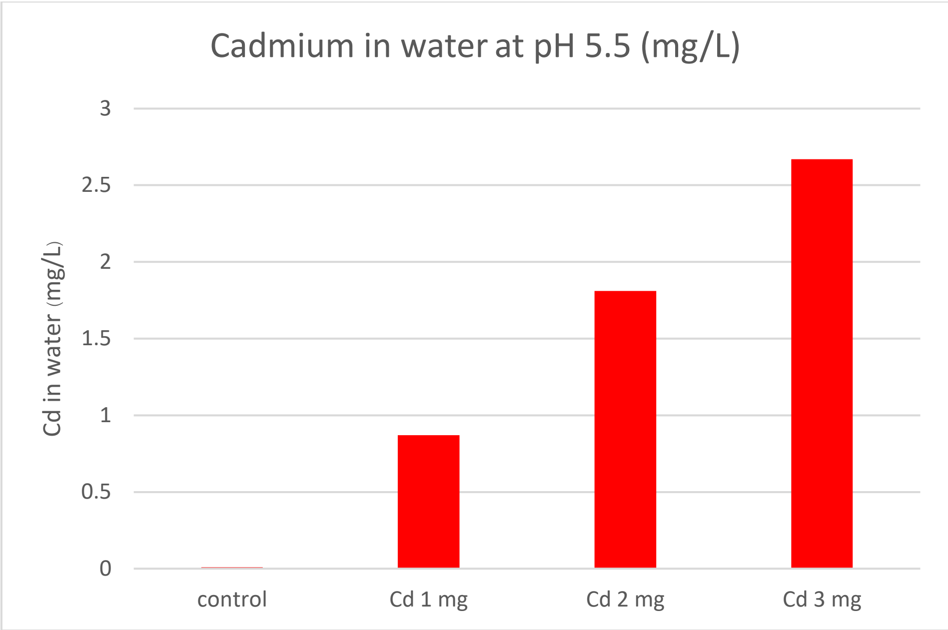
significant difference of mean ($p < 0.05$) within each experiment.



Figures 22 Cadmium in water at pH 7.5 (mg/L)



Figures 23 Cadmium in water at pH 6.5 (mg/L)



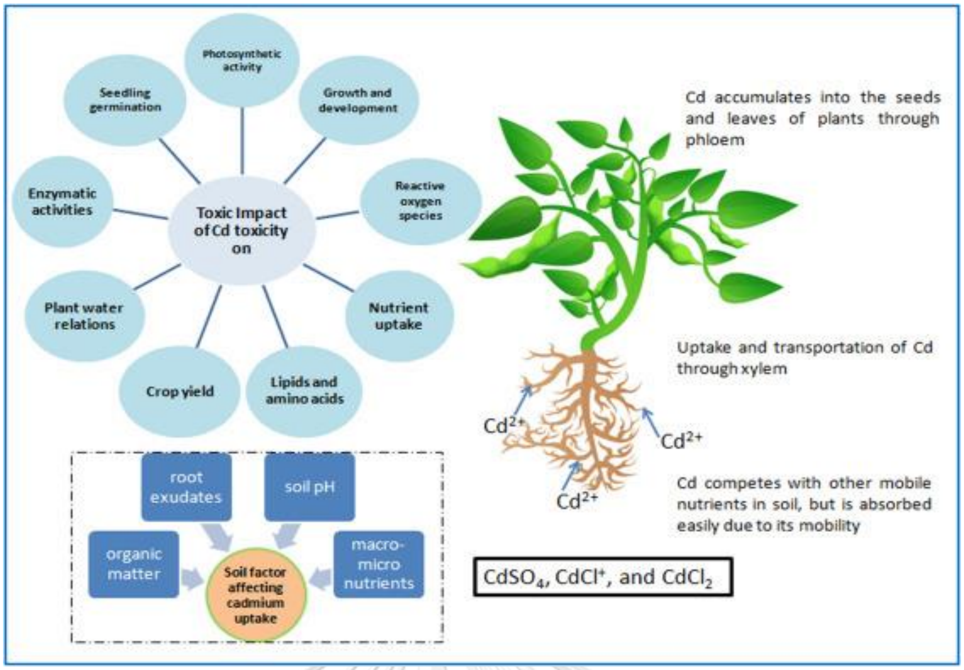
Figures 24 Cadmium in water at pH 5.5 (mg/L)

4.2. Effects of cadmium concentrations on plant growth

Cadmium concentrations affected plant and root growth. Significant differences of means ($p < 0.05$) show that plant growth was high in the control condition. Results show that increasing doses of cadmium resulted in an increased adverse response of pak choi by inhibiting plant and root growth. Compared with the control condition, wet weight of pak choi decreased by 23.9%–58.8%, 11.4%–39.6%, and 6.3–24.0% at cadmium doses of 1 mg/L, 2 mg/L, and 3mg/L, respectively. Pak choi growth also decreased at pH levels. For example, at cadmium dosage of 3 mg/L, the highest inhibition was found at pH 5.5 (93.7%) followed by pH 6.5 (90.7%), and pH 7.5 (76.0%), respectively. Studies also reviewed that cadmium inhibited plant growth, and induced other toxic effects such as germination inhibition, reduction in root elongation, reduction in protein synthesis and nitrogen content in leaves, decrease in photosynthesis, and chlorosis in leaves. Cadmium was also reported to

induce reactive oxygen species that stimulate oxidative stress and negatively plant productivity(Haider et al., 2021). Cadmium inhibition depends on plant species with toxic levels from about 1–50 mg/L. Inhibition level of plant growth was found at cadmium concentration about 10 mg/L and 6 mg/L for lettuce and pak choi, respectively(Li, Tang, Qiao, & Huang, 2020). In this study, it was found that the lowest-observed-adverse-effect level (growth reduction) of pak choi at the pH range of 5.5–7.5 was below 0.59 mg/L of cadmium in hydroponic water (or below 1 mg/L of total cadmium in the system). Although these cadmium concentrations were not at severe inhibition level, pak choi reduced in growth rate, and the results could suggest that growth reduction could be a bioindicator for the plant in hydroponics that adversely responds to cadmium contamination in growth media.

Absorption, transit, accumulation, and toxicity of cadmium (Cd) in plant species. The absorption of Cd in plants is influenced by root exudates, soil pH, organic matter, and micro- and macronutrients in the soil. Cd absorption in roots can take the form of inorganic compounds (such as CdSO4, $CdCl^+$, and $CdCl_2$). Cd toxicity slows plant growth and development, lowering nutrient and water intake and, as a result, lowering photosynthetic rate. Cadmium toxicity disrupts the balance between antioxidant synthesis and reactive oxygen species (ROS) formation in plants, resulting in increased ROS buildup and oxidative stress. Overabundance of reactive oxygen species (ROS) in plants Changes protein and lipid synthesis, inhibits enzymatic activity that leads to lipid peroxidation, and lowers cell division, all of which have a detrimental influence on agricultural output(Haider et al., 2021).



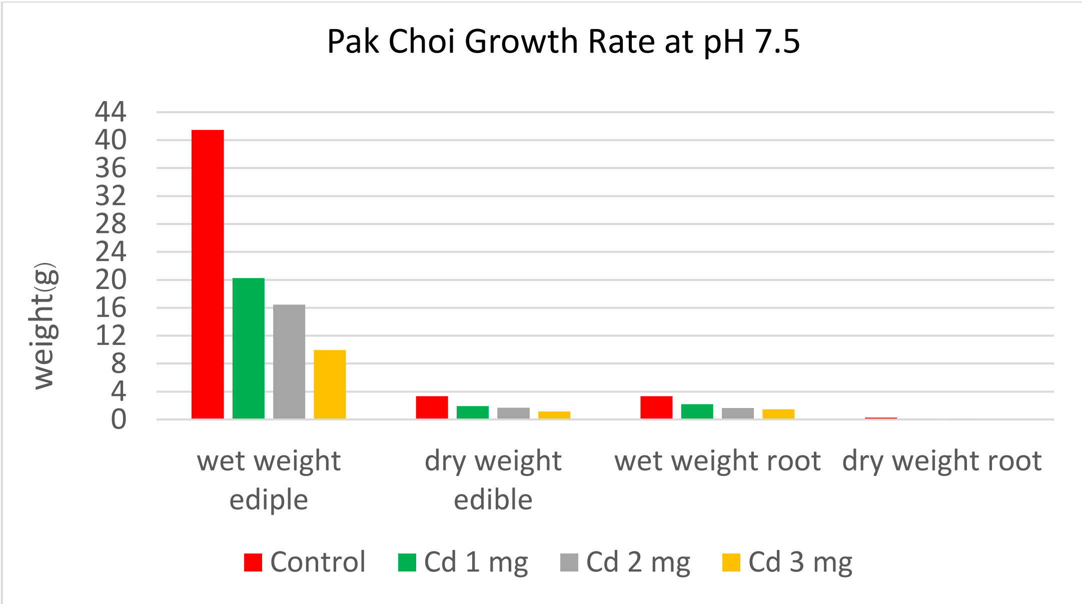
Figures 25 Cadmium (Cd) absorption, transportation, accumulation, and toxicity in plant species.

Figures 26 Difference of growth rate of pak choi for no Cd added, 1,2 and 3mg/L Cd added

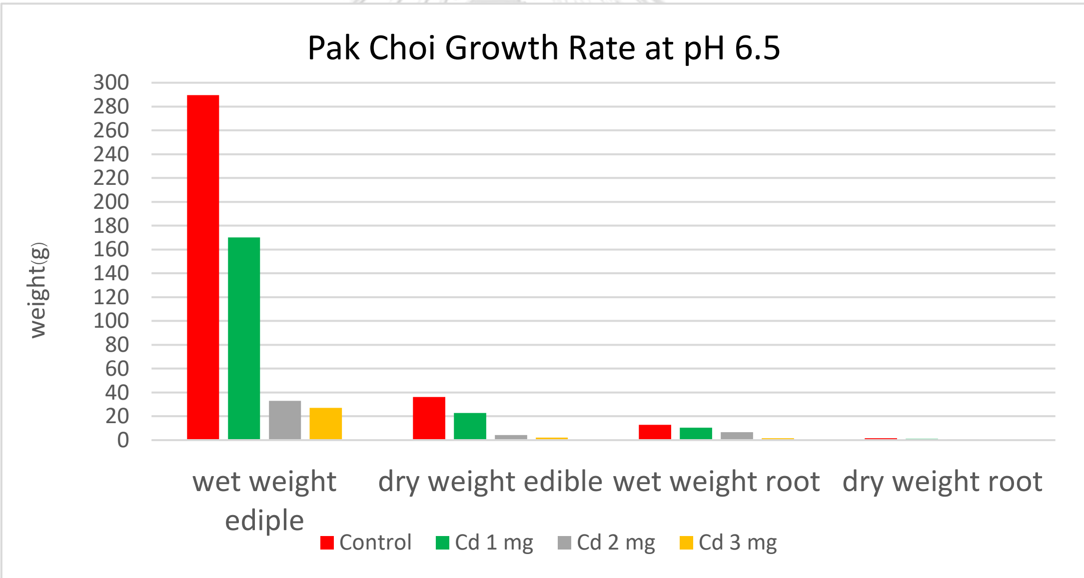
Table 5 Pak choi growth and effect of cadmium inhibition during 28 days at different cadmium concentrations and pH levels

Exp no.	Cadmium dose (mg/L)	Wet weight (Edible part) (g)	Dry weight (Edible part) (g)	Wet weight (root) (g)	Dry weight (root) (g)
1/7.5	control	41.49 ± 1.36^{A}	3.35 ± 0.42^{A}	3.35 ± 0.36^{A}	0.26 ± 0.02^{A}
	1.0	20.25 ± 0.44^{B}	1.94 ± 0.02^{B}	2.21 ± 0.33^{B}	0.14 ± 0.02^{B}
	2.0	16.43 ± 1.49^{C}	1.71 ± 0.20^{C}	1.66 ± 0.007^{C}	0.12 ± 0.01^{B}
	3.0	9.96 ± 0.51^{D}	1.16 ± 0.33^{D}	1.48 ± 0.05^{D}	0.09 ± 0.007^{C}
2/6.5	control	289.63 ± 2.02^{A}	36.10 ± 1.76^{A}	12.94 ± 1.46^{A}	1.56 ± 0.54^{A}
	1.0	170.26 ± 4.16^{B}	22.85 ± 1.23^{B}	10.42 ± 2.00^{AB}	1.04 ± 0.13^{A}
	2.0	33.09 ± 7.36^{C}	4.20 ± 0.35^{C}	6.70 ± 0.82^{C}	0.39 ± 0.32^{B}
	3.0	26.99 ± 2.96^{C}	2.20 ± 0.49^{D}	1.48 ± 0.94^{D}	0.12 ± 0.04^{B}
3/5.5	control	236.59 ± 2.52^{A}	27.42 ± 3.13^{A}	37.74 ± 5.89^{A}	4.10 ± 0.60^{A}
	1.0	56.65 ± 1.59^{B}	5.33 ± 3.45^{B}	11.82 ± 3.25^{B}	0.62 ± 0.24^{B}
	2.0	40.95 ± 2.58^{C}	4.22 ± 2.12^{B}	5.81 ± 5.74^{B}	0.61 ± 0.23^{B}
	3.0	14.90 ± 0.77^{C}	1.88 ± 0.96^{B}	1.27 ± 0.70^{C}	0.19 ± 0.04^{C}

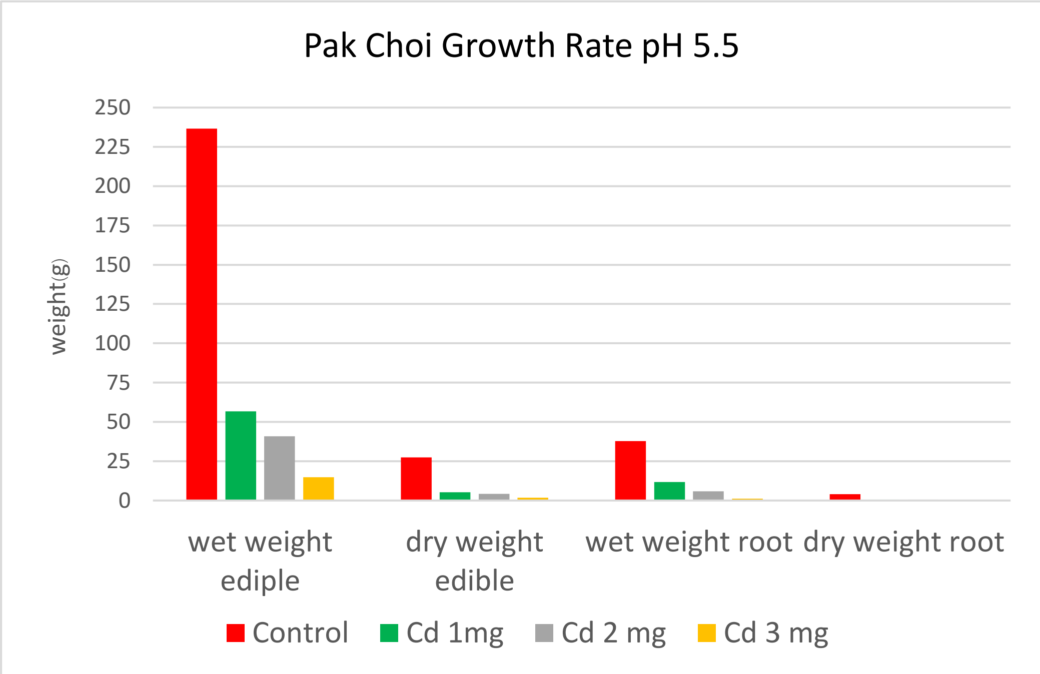
Note: Symbol * represents mean ± standard deviation of plant weight in hydroponics (n = 16). Letters A to D indicate a significant difference of mean ($p < 0.05$) within each experiment.



Figures 27 Pak Choi Growth Rate at pH 7.5



Figures 28 Pak Choi Growth Rate at pH 6



Figures 29 Pak Choi Growth Rate at pH 5.5

4.3 Bioaccumulation of cadmium in pak choi at high cadmium concentrations

Cadmium was found to accumulate in roots and plants with a positive tendency toward dosing concentrations (Table 5). For example, cadmium dosing of 3 mg/L was significantly higher than control, which has the lowest cadmium accumulation. The highest concentrations of cadmium in plants and roots were found at a dosing of 3 mg/L followed by 2 mg/L, 1 mg/L, and control, respectively. Moreover, cadmium was likely to accumulate in plants at low pH levels where plant growth was enhanced. The results were found to agree with other studies that the amount of cadmium accumulated in plants increases with a tendency to accumulate more in pH at 5.5 greater than 6.5 and 7.5(Khan & Rahman, 1996).

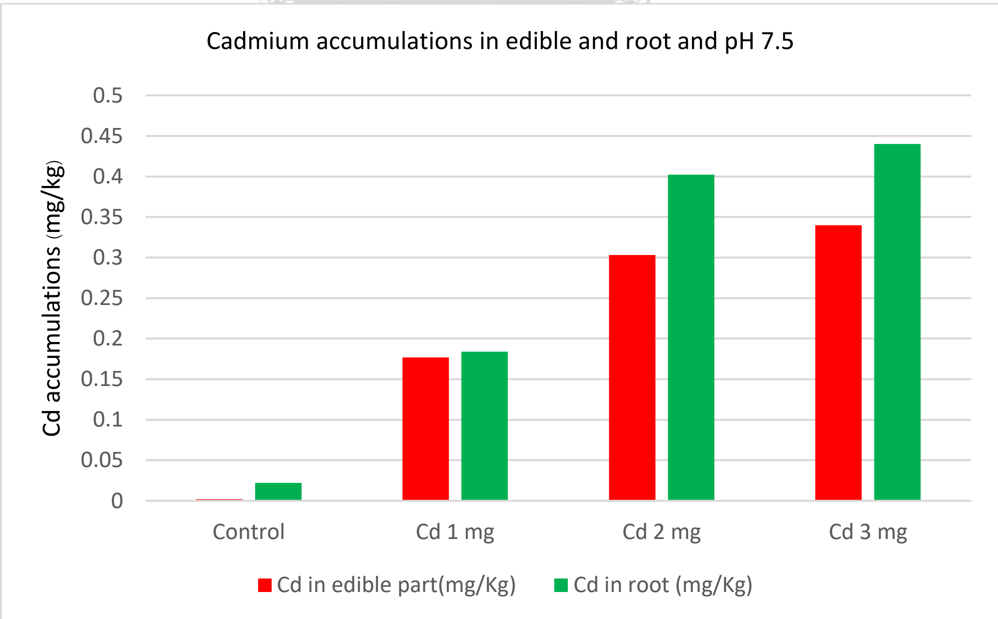
At cadmium concentrations below 1 mg/L, cadmium tended to accumulate in plant leaves and stems (Table 5). However, when cadmium concentration increased above 2 mg/L, cadmium tended to accumulate more in roots. This can be implied that plants could accumulate cadmium in leaves and stems. Usually, roots store most toxins absorbed from the soil or water in roots before translocating to stems and leaves. At stress conditions, which the plant could not tolerate and accumulate cadmium in the upper (edible) part, it could result in accumulation in roots due to inhibition in translocation; thus, high cadmium concentrated in roots(Woraharn, Meeinkuirt, Phusantisampan, & Avakul, 2021). The results were found to agree with other studies that roots contained higher concentrations of all heavy metals than leaves (Page et al., 2006). Another study also showed that, at cadmium level in industrial wastewater (0.5–40 mg/L), cadmium can accumulate in seagrass *Cymodocea nodosa* (leaves and stems) and roots with accumulation kinetics significant to Michaelis–Menten-type(Malea, Kevrekidis, Chatzipanagiotou, & Mogias, 2018).

Table 6 Cadmium accumulations in Pak choi and roots at varying cadmium and pH levels

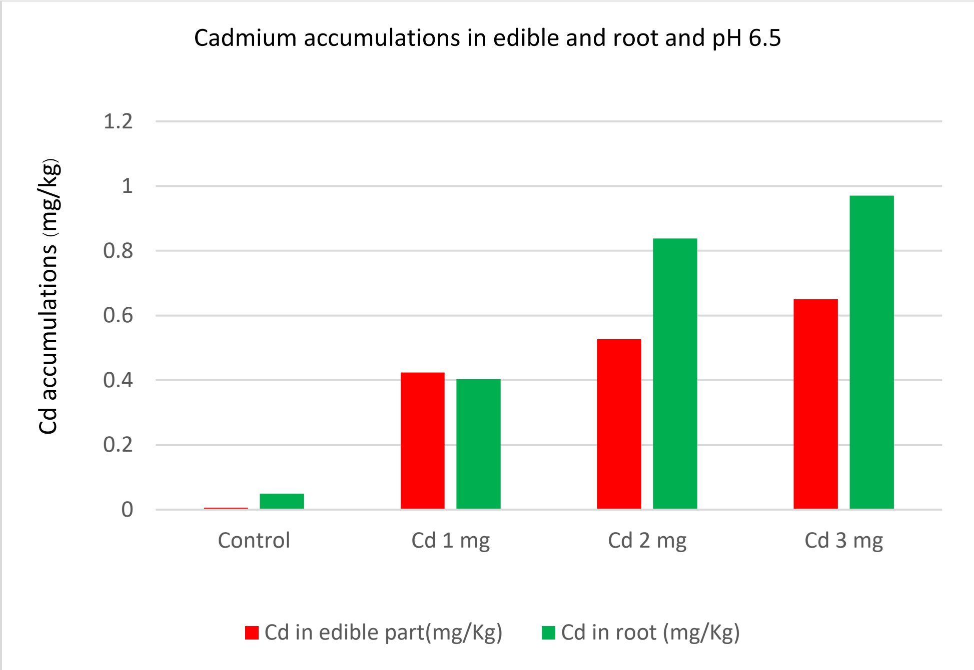
Exp no./pH	**Cadmium dose (mg/L)**	**Cd in edible part (mg/kg DW)**	**Cd in root (mg/kg DW)**
1/7.5	control	0.029 ± 0.005^{A}	0.022 ± 0.007^{A}
	1.0	0.177 ± 0.002^{B}	0.184 ± 0.07^{B}
	2.0	0.303 ± 0.070^{BC}	0.402 ± 0.07^{C}
	3.0	0.340 ± 0.015^{C}	0.440 ± 0.05^{C}

2/6.5	control	0.060 ± 0.001[A]	0.049 ± 0.02[A]
	1.0	0.424 ± 0.077[B]	0.403 ± 0.08[B]
	2.0	0.527 ± 0.068[B]	0.838 ± 0.07[C]
	3.0	0.650 ± 0.007[C]	0.970 ± 0.08[C]
3/5.5	control	0.002 ± 0.001[A]	0.016 ± 0.001[A]
	1.0	0.400± 0.001[B]	0.352 ± 0.07[B]
	2.0	0.900 ± 0.063[C]	0.800 ± 0.17[C]
	3.0	1.450 ± 0.004[D]	0.920 ± 0.18[C]

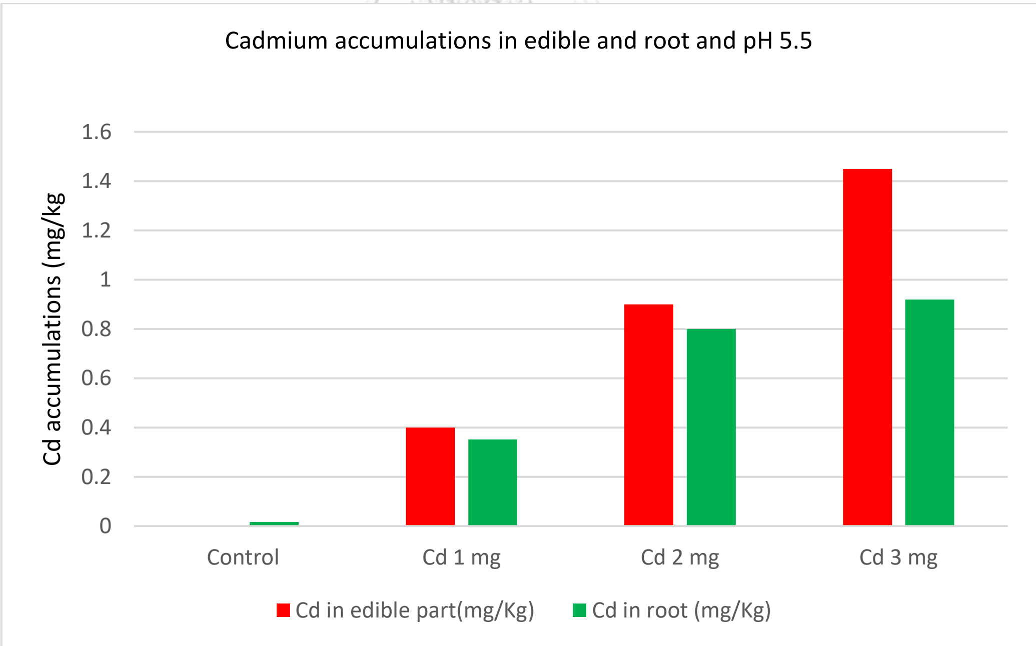
Note: Symbol * represents mean ± standard deviation of plant weight in hydroponics (n = 16). Letters A to D indicate a significant difference of mean ($p < 0.05$) within each experiment.



Figures 30 Cadmium accumulations in edible and root and pH 7.5



Figures 31 Cadmium accumulations in edible and root and pH 6.5



Figures 32 Cadmium accumulations in edible and root and pH 5.5

The highest BCFs in edible part and root were found at pH levels of 6.5 followed by 5.5 and 7.5, respectively, although the highest cadmium accumulation in plants was found at the pH of 5.5 (Table 6). This is because BCF is relative to cadmium concentration in water. The pH of 6.5–7.5 resulted in lower cadmium solubility in hydroponic water compared to the pH of 5.5. In this study, BCF in pak choi was relatively low compared to plants used in phytoremediation such as *Typha angustifolia* (BCF = 28.8–285), *Pandanus amaryllifolius* (BCF = 12.2–60.0), and *Acorus calamus* (BCF = 6.3–398.2). However, the BCFs from this study were higher than BCF from pak choi (BCF = 0.24–0.37) grown in cadmium contaminated soils (0.47–0.73 mg/kg) because hydroponics provides higher available cadmium for plant uptake directly rather than being absorbed by soil minerals and organic nutrients (Cao, Wahbi, Ma, Li, & Yang, 2009). Thus, plants grown with the hydroponic method could accumulate a higher amount of cadmium than soil methods because all ions are freely available. Reuse of cadmium contaminated water/wastewater for agriculture and farming using hydroponics should be considered more seriously than soil methods as the high possibility of cadmium bioaccumulation in hydroponics plants could be relatively high and cause a potential health risk.

Table 7 Mass balance for Cadmium precipitated in NFT system

Exp no./pH	Cadmium dose (mg/L)	Cd in plants (%)	Cd available in water (%)	Cd precipitate (%)
1/7.5	control	-	-	-
	1.0	0.0031	59	41
	2.0	0.0033	41.5	58.5
	3.0	0.0040	32.0	68
2/6.5	control	-	-	-
	1.0	0.0034	58.0	42
	2.0	0.0035	40.0	60
	3.0	0.0041	42.3	57.7
3/5.5	control	-	-	-
	1.0	0.0029	87.0	13
	2.0	0.0033	90.5	9.5
	3.0	0.0037	89.0	11

Table 8 Bioconcentration factor of Cadmium in Pak choi and roots at different pH

Exp no./pH	Plant – Water		Root – Water	
	BCF	R^2	BCF	R^2
1/7.5	0.501	0.965	0.035	0.809
2/6.5	1.038	0.962	2.963	0.996
3/5.5	0.434	0.97	0.574	0.976

4.4 Health risk analyses of consuming pak choi from cadmium contaminated hydroponics

Consumption of pak choi grown at total cadmium doses above 1 mg/L could not cause non-carcinogenic (HQ < 1, Table 7) but could cause carcinogenic ($R_c > 10^{-6}$) health risks to consumers during 30-year consumption. Average non-carcinogenic risk levels were varied from 0.005 to 0.303 and 3×10^{-5} to 1.95×10^{-3}. The level of risk increased with cadmium contents in the edible part of pak choi, which positively corresponded with cadmium doses in water. In this study, it was found that cadmium can be contaminated with hydroponic nutrient solutions (0.01–0.04 mg/L), which was found in the control condition. Although cadmium contaminated at control condition did not cause non-carcinogenic risk, consuming pak choi at control condition and at cadmium supplemented conditions were identified to cause carcinogenic risk. Therefore, growing vegetables with cadmium contaminated in 0-3 mg/L should not be eaten because it can result in cancer.

Table 9 non-carcinogenic risk assessment via ingestion route of Pak choi grown by different total cadmium doses and pH levels over 30-year consumption

Exp no./ pH	Cadmium dose (mg/L)	ADD (mg/g-day)	HQ Average	HQ (95% CI)
1/7.5	control	0.0089	0.009	0.006–0.010
	1.0	0.0865	0.086	0.056–0.101
	2.0	0.0947	0.095	0.062–0.111
	3.0	0.1052	0.105	0.082–0.116
2/6.5	control	0.0153	0.015	0.006–0.020
	1.0	0.1259	0.126	0.090–0.144
	2.0	0.2615	0.261	0.227–0.278
	3.0	0.3026	0.303	0.264–0.321
3/5.5	control	0.0051	0.005	0.004–0.006
	1.0	0.1099	0.110	0.079–0.125
	2.0	0.2488	0.249	0.172–0.287
	3.0	0.2870	0.287	0.207–0.326

According to the Industrial Effluent Standard of Thailand, maximum cadmium concentrations in effluent must be below 0.03 mg/L (Muttamara & Leong, 1997). However, with this level of effluent standard, carcinogenic risk could occur with the population consuming pak choi from hydroponics that directly utilizes reused water

from industrial discharge. Based on this study, possible cadmium concentrations of 0.01–0.04 mg/L could lead to cancer risk at a 95% confidence interval of 4×10^{-5} to 2.36×10^{-3}, which is higher than safe level (10^{-6}) for consumption via oral intake. Considering other possible heavy metals in vegetables, the carcinogenic risk level could be higher than the levels from this study.

Studies also reported similar results of both non-carcinogenic and carcinogenic risk of cadmium contamination in rice grown by contaminated soil (cadmium content in soil = 0.325–26 mg/kg) in China (Zeng et al., 2015). Despite soil could adsorb cadmium ions, long-term heavy metal exposure from brown rice consumption poses both non-carcinogenic and carcinogenic health hazards to the local population. Therefore, in terms of health risk assessment cadmium contaminations in soil and water must be considered before reusing in irrigation and agriculture. For hydroponics, heavy metal removal processes are needed to purify water for reusing in hydroponics. Moreover, regulation and standard of inorganic chemical fertilizers used for hydroponics must be strictly controlled to prevent carcinogenic risk from such low levels of cadmium concentrations

Table 10 Carcinogenic risk assessment via ingestion route of Pak choi grown by different total cadmium doses and pH levels over 30-year consumption

Exp no./ pH	Cadmium dose (mg/L)	ADD (mg/g-day)	R_C Average	R_C (95% CI)
1/7.5	control	0.0038	0.00006	0.00004–0.00008
	1.0	0.0371	0.00056	0.00036–0.00075
	2.0	0.0406	0.00061	0.00040–0.00082

	3.0	0.0451	0.00068	0.00053–0.00082
2/6.5	control	0.0065	0.00010	0.00004–0.00015
	1.0	0.0539	0.00081	0.00058–0.00104
	2.0	0.1121	0.00168	0.00146–0.00190
	3.0	0.1297	0.00195	0.00170–0.00219
3/5.5	control	0.0022	0.00003	0.00003–0.00004
	1.0	0.0471	0.00071	0.00051–0.00090
	2.0	0.1066	0.00160	0.00110–0.00210
	3.0	0.1230	0.00184	0.00133–0.00236

CHAPTER 5
CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

From this study, pak choi as a model plant in a hydroponic setup was used to access cadmium bioaccumulation and the health risk of vegetable consumption. The experiment was conducted using four initial cadmium doses (control, 1 mg/L, 2 mg/L, and 3 mg/L) in a four-week operation. Three initial pH levels (7.5, 6.5, and 5.5) were used for experiments I, II, and III. Results show that cadmium concentrations of 1–3 mg/L significantly inhibited pak choi growth ($p < 0.05$) compared with control. Bioaccumulation in plant roots (0.035–2.963) and edible part (0.434–1.038) occurred with an increase in cadmium concentration ($p < 0.05$). n That means Cd can be mostly contamination in root more than in leaf. Based on USEPA average daily intake and health risk assessment, it was found that consumption of pak choi grown by the cadmium contaminated water resource in the range of 1 to 3 mg/L will not likely cause chronic non-carcinogenic (HQ < 1) risk but will cause carcinogenic risk (cancer risk > 10^{-6}) over 30-year consumption. Moreover, at low cadmium concentration (0.01–0.04 mg/L), consuming pak choi grown by cadmium contaminated water, based on the level of industrial effluent standard of Thailand, was found to cause carcinogenic risk from. Therefore, it is recommended that the monitoring of cadmium in water for agricultural use must be checked every time.

5.2 Recommendation

Future study can be developed by change the type of vegetables in the experiment. Moreover, not only cadmium but also change heavy metals to another one to compare the absorption rate of other heavy metals that affect plant growth and the rate of heavy metal deposition that how its difference. Maybe a difference

types of heavy metal is also affected to risk assessment in non-cancer risk and cancer risk too.

REFERENCES

Achakzai, A., & Bazai, Z. (2006). Phytoaccumulation of heavy metals in spinach (Spinacea oleraceac L.) irrigated with wastewater of Quetta City. *Journal of the Chemical Society of Pakistan, 28*(5), 473-477.

Akün, M. E. (2020). Heavy Metal Contamination and Remediation of Water and Soil with Case Studies From Cyprus. In *Heavy Metal Toxicity in Public Health*: IntechOpen.

Alexopoulos, A. A., Marandos, E., Assimakopoulou, A., Vidalis, N., Petropoulos, S. A., & Karapanos, I. C. (2021). Effect of Nutrient Solution pH on the Growth, Yield and Quality of Taraxacum officinale and Reichardia picroides in a Floating Hydroponic System. *Agronomy, 11*(6), 1118.

Anwar, S., Naz, A., Ashraf, M. Y., & Malik, A. (2020). Evaluation of inorganic contaminants emitted from automobiles and dynamics in soil, dust, and vegetations from major highways in Pakistan. *Environmental Science and Pollution Research, 27*, 32494-32508.

Arumugam, T., Sandeep, G., & Maheswari, M. U. (2021). Soilless farming of vegetable crops: An overview.

Athar, R., & Ahmad, M. (2002). Heavy metal toxicity: effect on plant growth and metal uptake by wheat, and on free living Azotobacter. *Water, Air, and Soil Pollution, 138*(1), 165-180.

Bawa, B., Sharif, N., & Hashim, T. (2015). Concentrations of some Heavy Metals in Lycopersicon esculentum and Allium cepa Grown in Abarma River Bank, Gusau-Nigeria. *International Journal Of Science for Global Sustainability, 1*(1).

Cao, X., Wahbi, A., Ma, L., Li, B., & Yang, Y. (2009). Immobilization of Zn, Cu, and Pb in contaminated soils using phosphate rock and phosphoric acid. *Journal of Hazardous Materials, 164*(2-3), 555-564.

Chove, B., Ballegu, W., & Chove, L. (2006). Copper and Lead levels in two popular leafy vegetables grown around Morogoro Municipality, Tanzania. *Tanzania Journal of Health Research, 8*(1), 37-40.

da Silva Cerozi, B., & Fitzsimmons, K. (2016). The effect of pH on phosphorus availability and speciation in an aquaponics nutrient solution. *Bioresource technology, 219*, 778-781.

Debnath, M., Chejara, V., Vijaya, B. K., Bika, D. S., Kumar, S., Dixit, A., . . . Jain, V. (2014). Evaluation of quality and impact of untreated wastewater for irrigation. *Am. J. Res. Commun., 2*, 1-43.

Fang, H., Li, W., Tu, S., Ding, Y., Wang, R., Rensing, C., . . . Feng, R. (2019). Differences in cadmium absorption by 71 leaf vegetable varieties from different families and genera and their health risk assessment. *Ecotoxicology and environmental safety, 184*, 109593.

Haider, F. U., Liqun, C., Coulter, J. A., Cheema, S. A., Wu, J., Zhang, R., . . . Farooq, M. (2021). Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicology and environmental safety, 211*, 111887.

Kabata-Pendias, A. (2004). Soil–plant transfer of trace elements—an environmental issue. *Geoderma, 122*(2-4), 143-149.

Khan, M., & Rahman, A. (1996). *The influence of zinc and copper fertilizer application on zinc, copper and cadmium concentration in mixed pasture: a thesis presented in partial fulfilment of the requirements for the degree of Master of Applied Science in Soil Science at Massey University.* Massey University,

Kubier, A., Wilkin, R. T., & Pichler, T. (2019). Cadmium in soils and groundwater: a review. *Applied Geochemistry, 108*, 104388.

Li, R., Tang, T., Qiao, W., & Huang, J. (2020). Toxic effect of perfluorooctane sulfonate on plants in vertical-flow constructed wetlands. *Journal of Environmental Sciences, 92*, 176-186.

López-Millán, A.-F., Sagardoy, R., Solanas, M., Abadía, A., & Abadía, J. (2009). Cadmium toxicity in tomato (Lycopersicon esculentum) plants grown in hydroponics. *Environmental and experimental botany, 65*(2-3), 376-385.

Malea, P., Kevrekidis, T., Chatzipanagiotou, K.-R., & Mogias, A. (2018). Cadmium uptake kinetics in parts of the seagrass Cymodocea nodosa at high exposure concentrations. *Journal of Biological Research-Thessaloniki, 25*(1), 1-11.

Manjón, I., Ramírez-Andreotta, M. D., Sáez, A. E., Root, R. A., Hild, J., Janes, M. K., &

Alexander-Ozinskas, A. (2020). Ingestion and inhalation of metal (loid) s through preschool gardening: an exposure and risk assessment in legacy mining communities. *Science of the Total Environment, 718*, 134639.

Mehta, P. (2018). Advanced Engineering of Contact Lens Coatings using Electrohydrodynamic Atomization.

Mishra, T., & Pandey, V. C. (2019). Phytoremediation of red mud deposits through natural succession. In *Phytomanagement of polluted sites* (pp. 409-424): Elsevier.

Muttamara, S., & Leong, S. T. (1997). Environmental monitoring and impact assessment of a solid waste disposal site. *Environmental Monitoring and Assessment, 48*(1), 1-24.

Organization, W. H. (2004). *Cadmium in drinking-water: background document for development of WHO guidelines for drinking-water quality*. Retrieved from

Prazeres, A. R., Albuquerque, A., Luz, S., Jerónimo, E., & Carvalho, F. (2017). Hydroponic system: A promising biotechnology for food production and wastewater treatment. In *Food Biosynthesis* (pp. 317-350): Elsevier.

Quispe, N., Zanabria, D., Chavez, E., Cuadros, F., Carling, G., & Paredes, B. (2021). Health risk assessment of heavy metals (Hg, Pb, Cd, Cr and As) via consumption of vegetables cultured in agricultural sites in Arequipa, Peru. *Chemical Data Collections, 33*, 100723.

Rana, S., Bag, S., Golder, D., Roy, S. M., Pradhan, C., & Jana, B. (2011). Reclamation of municipal domestic wastewater by aquaponics of tomato plants. *Ecological Engineering, 37*(6), 981-988.

Singh, R., Singh, D., Kumar, N., Bhargava, S., & Barman, S. (2010). Accumulation and translocation of heavy metals in soil and plants from fly ash contaminated area. *Journal of Environmental Biology, 31*(4), 421-430.

STRAŽANAC, D., MATIJEVIĆ, L., PETRIĆ, J., ČAGALJ, D., BABIĆ, J., & HENGL, B. Croatian Journal of Food Science and Technology.

Wei, R., Guo, Q., Tian, L., Kong, J., Bai, Y., Okoli, C. P., & Wang, L. (2019). Characteristics of cadmium accumulation and isotope fractionation in higher plants. *Ecotoxicology and environmental safety, 174*, 1-11.

Woraharn, S., Meeinkuirt, W., Phusantisampan, T., & Avakul, P. (2021). Potential of ornamental monocot plants for rhizofiltration of cadmium and zinc in hydroponic systems. *Environmental Science and Pollution Research*, 1-14.

Woraharn, S., Meeinkuirt, W., Phusantisampan, T., & Chayapan, P. (2021). Rhizofiltration of Cadmium and Zinc in Hydroponic Systems. *Water, Air, & Soil Pollution, 232*(5), 1-17.

Zeng, F., Wei, W., Li, M., Huang, R., Yang, F., & Duan, Y. (2015). Heavy metal contamination in rice-producing soils of Hunan province, China and potential health risks. *International journal of environmental research and public health, 12*(12), 15584-15593.

APPENDIX

APPENDIX A

A1. Nutrient parameter method

1.1 Nitrate in water sample

- Micropipette 0.5 ml of each standard and sample into suitably marked test tubes.
- Add 1.0 ml of salicylic acid solution to each test tube, mix well immediately the acid is added and leave for 30 minutes.
- Add 10.0 ml of sodium hydroxide solution to each test tube, mix well and leave for 1hr for color development.
- Read each sample absorbance at 410 nm by spectrophotometer.

1.2 Phosphate in water sample

- Measure for 50 ml sample and dilute 10 times. Pipette 5ml from diluted sample into suitably marked test tubes
- Add 10.0 ml of Vanadate-Molybdate.
- Read each sample absorbance at 470 nm by spectrophotometer.

1.3 Ammonia in water sample

- Prepare a water sample for 5ml, adjust volume to 50ml with distilled water and put into Nessler tube.
- Add 1-2 drops of EDTA Solution, 2ml of Nessler solution, mix well and carefully shake 5-6 times.
- Leave for 15 mins for development, read each sample absorbance at 410 nm by spectrophotometer.

APPENDIX B

B1. Safety Data Sheet Summary of Cadmium

1. CAS Number: 7440-43-9
2. EC Number: 231-152-8
3. International Chemical Safety Cards: 0020
4. Related diseases: Cadmium chronic toxic effect, Osteomalacia, Tubular disfunction, Abdominal pain Liver dysfunction, Pneumonitis Lung dysfunction
5. BEI: Cd in urine = 5 ug/g creatinine, Cd in blood = 5 ug/L, monitoring in blood should be preferred during the initial year of exposure and whenever changes in the degree of exposure are suspected.
6. Skin Designation: No
7. Bioaccumulates: No
8. TWA (AVGIH): 0.01 mg/m3 total, 0.002 mg/m3 resp.
9. PEL (OSHA): 0.005 mg/m3
10. IDLH: 9 mg/m3
11. Hazard Statement: May cause cancer, suspected of causing genetic defects, suspected of damaging fertility, suspected of damaging the unborn child, fatal if inhaled, causes damage to organs through prolonged or repeated exposure and very toxic to aquatic life in ling term effects.

VITA

NAME	Piyachet Jinsart
DATE OF BIRTH	23 Sep 1997
PLACE OF BIRTH	Bangkok
HOME ADDRESS	79/172 ม.ธารารมร์ ซอย รามคำแหง 150 ถนน สุขาภิบาล 3 เขต สะพานสูง แขวง ราษฎรพัฒนา กทม 10240