Investigation of copper and zinc contaminations in bioponic systems using chicken manure fertilizer

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การตรวจสอบการปนเปื้อนของทองแดงและสังกะสีในระบบไบโอโปนิกส์โดยใช้ปุ๋ยมูลไก่

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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สัจจะ อักษร : การตรวจสอบการปนเปื้อนของทองแดงและสังกะสีในระบบไบโอโปนิกส์โดยใช้ปุ๋ยมูลไก่ (Investigation of copper and zinc contaminations in bioponic systems using chicken manure fertilizer) อ.ที่ปรึกษาหลัก : ดร.สุเมธ วงศ์เขียว

ระบบไบโอโปนิกส์เป็นระบบที่ใช้ประโยชน์จากของเสียทางชีวภาพเป็นแหล่งธาตุอาหารสำหรับการเจริญเติบโตของพืช ในปัจจุบันปุ๋ยมูลไก่มีการปนเปื้อนทองแดงและสังกะสี อาจส่งผลกระทบทางสุขภาพจากการรับสัมผัสทองแดงและสังกะสีผ่านการบริโภคผัก โดยวัตถุประสงค์ของการศึกษานี้คือ 1) ประเมินการเปลี่ยนรูปของไนโตรเจน การกู้คืนไนโตรเจน และการเจริญเติบโตของพืชในไบระบบไบโอโปนิกส์จากปุ๋ยมูลไก่ที่มีการเติมทองแดงและสังกะสีและ 2) ตรวจสอบผลกระทบของการเติมทองแดงและสังกะสีต่อการสะสมทางชีวภาพของพืช ชุมชนจุลินทรีย์ในรากพืชและความเสี่ยงต่อสุขภาพจากการบริโภคผัก โดยใช้ Inductively coupled plasma-optical emission spectrometry (ICP-OES) ในการวิเคราะห์ความเข้มข้นของโลหะหนักทองแดงและสังกะสีในตัวอย่างทั้งหมด ผลการศึกษาพบว่า ค่าเฉลี่ยความเข้มข้นของไนโตรเจนสำหรับ lettuce โดย TKN อยู่ในช่วง 11.2 ± 0.5 ถึง 19.4 ± 1.8 TAN อยู่ในช่วง 1.1 ± 0.8 ถึง 1.5 ± 1.8 และ NO_3^- อยู่ในช่วง 11.6 ± 4.0 ถึง 14.9 ± 4.7 มิลลิกรัมไนโตรเจนต่อลิตรและ pak choi พบว่า TKN อยู่ในช่วง 16.3 ± 0.1 ถึง 19.4 ± 1.8 TAN อยู่ในช่วง 0.7 ± 0.4 ถึง 0.9 ± 0.5 และ NO_3^- อยู่ในช่วง 8.8 ± 6.8 ถึง 10.1 ± 6.8 มิลลิกรัมไนโตรเจนต่อลิตร ตามลำดับ การศึกษาแสดงให้เห็นว่าทองแดงและสังกะสีที่เติมในปุ๋ยมูลไก่ไม่ส่งผลต่อความเข้มข้นและประสิทธิภาพการใช้ไนโตรเจนของพืชอย่างมีนัยสำคัญ ($p < 0.05$) โดยความเข้มข้นของทองแดงและสังกะสีที่ตรวจพบในใบและรากของ lettuce อยู่ในช่วง 5.6 ± 0.1 ถึง 7.2 ± 1.0 และ 64.8 ± 13.8 ถึง 89.9 ± 2.6 มิลลิกรัมต่อกิโลกรัมและ pak choi อยู่ในช่วง 1.3 ± 0.3 ถึง 4.1 ± 2.9 และ 43.3 ± 1.5 ถึง 119.8 ± 34.1 มิลลิกรัมต่อกิโลกรัม ตามลำดับ การวิเคราะห์ความชุกชุมสัมพัทธ์ของชุมขนจุลินทรีย์บริเวณรากพืช พบว่า ไฟลัมแบคทีเรีย *Proteobacteria* และ *Planctomycetes* มีความชุกชุมสูงที่สุด ตามลำดับ การประเมินความเสี่ยงต่อสุขภาพจากการรับสัมผัสทองแดงและสังกะสีผ่านการบริโภคผัก พบว่า ทองแดงและสังกะสีมีค่าระดับความเสี่ยงต่อสุขภาพอยู่ที่ HQ และ HI < 1 บ่งชี้ได้ว่าไม่ก่อให้เกิดความเสี่ยงทางสุขภาพจากการบริโภคผัก lettuce และ pak choi จากระบบไบโอโปนิกส์ที่ปนเปื้อนทองแดงและสังกะสีทั้งในเด็กและผู้ใหญ่

สาขาวิชา พิษวิทยาอุตสาหกรรมและการประเมินความเสี่ยง ลายมือชื่อนิสิต ....

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The bioponic system is a system that utilizes biological waste as a nutrient-rich source such as chicken manure fertilizer for plant growth integrated with aquaponic. At present, chicken manure fertilizer is contaminated with Cu and Zn may have the potential health effects through vegetable consumption. The objective of this study was to evaluate nitrogen transformation, nitrogen recovery, and plant growth in chicken manure-based bioponics at Cu (50–150 mg/kg) and Zn (200–600 mg/kg) supplementation and investigate the effects of Cu and Zn supplementations on plant bioaccumulation, root microbial community, and dietary health risk. Cu and Zn concentrations were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES). The result found that the average TKN was ranged from 11.2 ± 0.5 to 19.4 ± 1.8, TAN was 1.1 ± 0.8 to 1.5 ± 1.8 and NO_3^- was 11.6 ± 4.0 to 14.9 ± 4.7 mgN/L for lettuce cultivated, while pak choi cultivated TKN was ranged from 16.3 ± 0.1 to 19.4 ± 1.8, TAN was 0.7 ± 0.4 to 0.9 ± 0.5 and NO_3^- was 8.8 ± 6.8 to 10.1 ± 6.8 mgN/L respectively. These studies have shown that heavy metal contamination in chicken manure does not significantly affect nitrogen concentration and nitrogen use efficiency by plants (significant level $p < 0.05$). The concentrations of Cu and Zn were found in lettuce shoot and root ranged 5.6 ± 0.1 to 7.2 ± 1.0 mg/kg and 64.8 ± 13.8 to 89.9 ± 2.6 mg/kg, while in pak choi was ranged 1.3 ± 0.3 to 4.1 ± 2.9 mg/kg and 43.3 ± 1.5 to 119.8 ± 34.1 mg/kg, respectively. Furthermore, the relative abundance of bacterial communities of lettuce and pak choi roots-based bioponics at phylum level was found *Proteobacteria* and *Planctomycetes* are dominant highest relative abundance respectively. However, the result of the health risk assessment was showed that HQ and HI < 1, which does not exceed the acceptable level. This study indicated that consuming lettuce and pak choi-based bioponic systems did not cause any human health effects or health risks to children and adults.

Field of Study:	Industrial Toxicology and Risk Assessment	Student's Signature ....
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CHAPTER 1
INTRODUCTION

1.1 Background of the study

Bioponics is an emerging technology for conversion of biowaste to vegetable products using the symbiosis of microbes and plants. Bioponics system is an advanced version of the hydroponic system (Wongkiew et al., 2021). It requires a small area for plant growth and can reduce the water problem in agriculture. Bioponics recirculates and reuses water and nutrients; thus, reducing soil and water pollutions (Wongkiew et al., 2021). Chicken manure is a high potential biowaste substrate in bioponics as it contains high nitrogen and phosphorus concentrations and provides high nitrogen use efficiency. Studies showed that the productivity of plant in bioponics with chicken manure were comparable to soil agriculture, but higher nutrients was conserved and utilized by plants in bioponic systems. Key functions of bioponics are organic degradations of chicken manure, nutrient mineralization, and nitrification, which are carried out by microbial community in bioponics (Kechasov et al., 2021). Thus, chicken manure based bioponics can be further used in commercial and fulfill organic indoor vertical faming and sustainable agricultural method.

However, there are concerns of using chicken manure in bioponics due to heavy metal contaminations in chicken manure, especially copper (Cu) and zinc (Zn). In commercial chicken farms, Cu and Zn unavoidably contaminate in chicken manure because Cu and Zn are used as mineral additives in animal feed due to their antibacterial and growth-stimulating properties to support the growth of the animals. Chicken manure was found to contaminate with Cu and Zn in ranges of 51.6 to 81.1 mg/kg dry wt. and 268.2 to 384.2 mg/kg dry wt., respectively (Hejna et al., 2018). Moreover, chicken manure with high Cu and Zn levels can cause bioaccumulation in the environment/ ecosystem, leading to intoxication on plant growth and shift in microbial community. Cu and Zn uptake by human through vegetable consumption (ingestion route) also contributes significant adverse health effects to human body over a lifetime (Ahmad et al., 2021). Zn toxicity in plants is directly affects decreased in tissue water content, photosystem II efficiency, root and shoot fresh and dry biomass weight, and decreased plant growth (Kaya et al., 2018) and human body affect at the cellular level, for example, controlling apoptosis in a variety of cell types and playing a key part in neuronal death (Plum et al., 2010). Cu toxicity in plants can lead to membrane injury due to membrane proteins and to an increased peroxidase activity causing reduced plant biomass and plant growth (Marastoni et al., 2019) and disruptions in the homeostasis of Cu are associated with tissue damage and several diseases in humans (Gaetke et al., 2014). As the results, Cu and Zn contaminations in chicken manure used as substrate in bioponics must be evaluated in terms of effects on plant productivity, bioaccumulations, and health risk assessments along with the performance in nitrogen recovery.

Ecotoxicology and health risk assessment have been well integrated in several study to link toxic compound contamination levels to the ecosystem, which can be integrate with health risk assessment (e.g., oral exposure). Several studies used bioconcentrations factor (BCF) and distribution coefficient (K_d) to indicate the level of bioaccumulation of heavy metals in plants and adsorbent (e.g., soil) from polluting sources, which can be used to predict the average daily intake from consumption and evaluate in comparison to acceptable dose (e.g., reference dose, RfD). Studies have investigated heavy metal accumulation in leaf vegetables and associated potential health risks of vegetable consumption. The bioconcentration factors (BCF) of heavy metals from soil to vegetables were estimated, and the potential health risks of heavy metal exposure through consumption (Chang et al., 2014). Moreover, microbial community reveal the insights on ecology of biological systems and microbial interactions associated with plant growth, nitrogen transformation, and toxicant contaminations. The shift of microbial community structure and biomarkers can be the bioindicator of contaminating source and suggest the shift in specific functions of biological processes. Studies of the effects of heavy metals on microbial communities and bioindicators reported heavy metals significantly affected to microbial communities and bioindicators were predict the contamination status of heavy metals (Li et al., 2020a). However, there is lack of information in microbial community, bioaccumulation, and health risk assessment of using Cu and Zn contaminated in chicken manure in bioponics with respect on nitrogen recovery.

1.2 Objectives

1.2.1 Evaluate nitrogen transformation, nitrogen recovery, and plant growth in chicken manure-based bioponics at Cu (50–150 mg/kg) and Zn (200–600 mg/kg) supplementation.

1.2.2 Investigate the effects of Cu and Zn supplementations on plant bioaccumulation, root microbial community, and dietary health risk.

1.3 Hypotheses

1.3.1 Under low concentrations of Cu and Zn contaminated in the chicken manure, there will be a high rate of nitrogen uptake by plants. However, high concentrations of Cu and Zn contaminated in the chicken manure can cause a decrease in nitrogen uptake by plants.

1.3.2 Plants can accumulate a high amount of Cu and Zn when applied with a high Cu and Zn concentrations but could cause a negatively effect on plant growth.

1.3.3 Cu and Zn contaminated in chicken manure can cause different human health risk levels from low to high if operated at different Cu and Zn contamination levels.

1.4 Scopes of the study

1.4.1 The experiment was conducted using two plant varieties namely romaine lettuce (*Lactuca sativa* L. var. *longifolia*) and pak choi (*Brassica rapa* var. *chinensis*).

1.4.2 To prepare Cu and Zn concentrations in chicken manure, this study was added solutions of Cu and Zn directly to dry chicken manure in the unit of milligram heavy metal per kilograms of dry chicken manure.

1.4.3 This study was scope at increasing the Cu and Zn concentrations only, although there are relatively small amounts of other heavy metals. A constant load of chicken manure of 200 grams per system (8 plants) was added in bioponic system. This study was conducted using nutrient film technique systems (NFT) bioponics and 35 days of planting including seed germination.

1.5 Problem and significant of study

Recently global warming reached approximately 1.0 ± 0.2 °C above pre-industrial levels in 2017, increasing at 0.2 °C ± 0.1 °C per decade that affect climate change. Climate change may affect the water cycle and plants available water. The climate with high temperatures (annual mean of 25–35 °C) and low rainfall can affect nutrient concentration and moisture conditions in the soil that can impact on plant growth in agricultural food production (Li et al., 2020b). Organics waste recycling is the recycling of organic materials to a useful product, especially manure to fertilizer and plant biomass. Organics waste recycling provides a benefit such as nutrient source or fertilizer e.g., nitrogen and phosphorus that necessary nutrient element for plant growth in the agricultural food production process and soil amendment and environmentally friendly (Eden et al., 2017).

Bioponics system can solve the water problem in agriculture because the bioponic system will be reuse water in the system and the use of organic nutrients reduces soil and water pollution. It is one of highly efficient alternative technology for agricultural food production (Wongkiew et al., 2021). The global situation right now, there is an increasingly huge number of livestock farms, especially chicken farms. Most chicken farms use food additive that is added with essential heavy metals, mostly consisting of Cu and Zn for raising the growth rate of animals in the process of animal production (Hejna et al., 2018). That a reason causes essential heavy metals Cu and Zn to over contaminate in the animals or chicken manure at high concentrations and can represent a toxic substance and can completely enter the food chain. The essential heavy metals pollution Cu and Zn at high concentrations contaminate in the chicken manure can have potential adverse effects and bioaccumulation in plants cause toxicity on the living organisms in ecological and represent a risk on the environment. In this study conducted a research experiment to investigate the effects of essential heavy metals Cu and Zn in the chicken manure on plant uptake and plant growth in bioponics. In addition, this study can classify and indicate the potential ecological risk level of essential heavy metals contaminated in the chicken manure that applied use to cultivated plants in bioponic systems.

CHAPTER 2

LITERATURE REVIEW

2.1 Theoretical Backgrounds

2.1.1 Bioponic system

Bioponic system is related to hydroponics and aquaponics but focuses on the use of completely organic nutrients rather than adding fish and other aquatic creatures to supplement nutrition (Adriana, 2015). The growing method of organic bioponic has raise in only less than a decade. Bioponic systems use the same organic inputs, processes, and principles as field growers. Organic matter (plant and animal material) is added to the system to provide nutrition sources for the soil microbiology to flourish and provide nutrients source to the plant's growth (Fang, 2018). With this method, bioponic systems can shift from a conventional approach using inorganic fertilizers, towards a more sustainable way of employing organic fertilizers that are recycled from organic wastes; and thus, fit better into urban agriculture in pursuit of sustainable development (Hsieh et al., 2018).

2.1.2 Plants

Romaine lettuce (*Lactuca sativa* L. var. *longifolia*) is one of the most valuable fresh vegetables and is in the top ten most valuable crops. Romaine lettuce is also the most popular, commercially produced leafy vegetable worldwide. Romaine Lettuce is the primary ingredient of the increasingly popular, packaged, ready-to-eat salads and may ingredient other manure (Teng et al., 2019). However, lettuce contains health-promoting nutrients and biosynthesis of such phytochemicals varies depending on cultivar, leaf color and growing conditions. Thus, lettuce contains several dietary minerals important for human health such as iron (Fe), zinc (Zn), calcium (Ca), phosphorus (P), magnesium (Mg), manganese (Mn), and potassium (K) and other health-promoting bioactive compounds (Kim et al., 2016). Romaine lettuce consumption vegetables may be one of the most significant sources of trace metal intake through the diet (Dala-Paula et al., 2018) food contamination generates even greater concern, especially in vegetables. This vegetable stands out, as it is one of the most widespread crops in green leaf and presents the possibility of continuous cultivation throughout a single year, along with low production costs and low susceptibility (Franca et al., 2017). Lettuce is typically used to study in bioaccumulations. Lettuce can bioaccumulate significant amount of heavy metals in their edible parts than other vegetables, thus potentially provides a sensitive indicator for assessing the risk posed by soil heavy metals in relation to compliance with food standards (Cavanagh et al., 2019).

Pak choi (*Brassica rapa* var. *chinensis*) is important vegetables grown in Asia where earliest reports of their use are from the fifth century A.D. These green vegetables were made known around the world by the efforts of the travelers and immigrants (Balkaya et al., 2018). Pak choi is very important Brassica vegetable in East, Northeast, and Southeast Asia (Han et al., 2020). It accounts for 30-40% of the vegetable production area in China and Taiwan. Pak choi has thick white leaf stems and large, spoon-shaped, dark green leaves. Color and size of the stem and leaves vary with different types of pak choi are consumed in different forms. The plant is being used mostly for its leaves and leaf stalks (Ma et al., 2017). It’s a short growth cycle, low cost, and rich nutritional value. Pak choi can grow under contaminations of heavy metals possessing a high capacity for heavy metal accumulation in the edible parts (Li et al., 2018). Pak choi is well known to be tolerant of heavy metals such as Cd, Cu and Zn. It accumulates metal ions mainly in the leaves. Due to the high uptake coefficient for heavy metals, pak choi has been the subject of research on the risks of heavy metals contaminated soils and hydroponics (Pan et al., 2019).

2.1.3 Heavy metals

Copper (Cu) is one of the regularly utilized heavy metals for different applications such as food additive animal feeding and one of the most toxic heavy metals usually found in the environment (Labidi et al., 2016). $CuSO_4$ is the most common form of Cu that is added to animal feed. It is generally utilized in electronic chips, batteries, cell phones, semiconductors, water pipes, fertilizer industry, pulp, and paper industry, fungicides, insecticides, catalysts, and metal processing products (Vardhan et al., 2019). In recent decades, the economic boom has stimulated the demand for animal products such as pig and chicken products. It is a common practice to add minerals such as Cu to animal feeds via mineral additives because of growth-stimulating (Zhang et al., 2012). Copper is an essential trace element needed for the human body and well-known micronutrient for plants and animals, but it is toxic if it exceeds the limit specified (Sudha Rani et al., 2018). In addition, swine and poultry and chicken manure represent the most important sources of Cu pollution contaminated in environment. This is also linked to the additives used in animal feed in a livestock industry (Hejna et al., 2018). The higher Cu concentration exposure in the plants the toxicity of Cu can leads to membrane of plant injury due to the binding of the metal to the thiol-groups of membrane proteins and to an increased peroxidase activity causing reduced plant biomass and plant growth (Marastoni et al., 2019).

Zinc (Zn) is generally uncommon in nature; however, it has a long history of utilization due to its availability in restricted deposits. Zn is available in various minerals which include zinc oxide (ZnO), zinc sulfate (ZnS), zinc carbonate ($ZnCO_3$), zinc silicate ($Zn2SiO_4$) etc. (Vardhan et al., 2019). However, ZnO is the most common form of Zn that is added to animal feed. Zn is an essential nutrient element for plants and plays a role in several plant physiological process i.e., photosynthesis, respiration, and synthesis of protein (Mohammadi and Khoshgoftarmanesh, 2014). In addition, Zn pollution has become a general global problem. This is also linked to the additives used in animal feed. The livestock industry contributes to Zn pollution as it is widely used in animal feed additives and swine and poultry represent the most important sources of Zn pollution. This is related to the high content of Zn in animal additives, which have usually resulted in a higher concentration in the manure that can cause toxic if it exceeds the limit (Hejna et al., 2018). Higher Zn exposure concentration in the plants induce Zn toxicity directly affects decreased in tissue water content, photosystem II efficiency, root and shoot fresh and dry biomass weight, and decreased plant growth (Kaya et al., 2018). Zinc has beneficial effects for crops, since this microelement is involved in the synthesis of tryptophan, a precursor of indole acetic acid (IAA), responsible for growth stimulation. The sensitivity to Zn toxicity differed among other crops, being sensitivity higher in celery > Chinese cabbage > pak choi. But pak choi can accumulate high levels of Zn in their edible parts with negative impact for human health (Fatemi et al., 2020).

2.2 Cu and Zn contaminated in animal manure and bioaccumulation by plant

Pathogen antimicrobial and growth-stimulating properties, heavy metals, particularly Cu and Zn, are frequently over-added to animal feeds. Cu and Zn given to animal feeds can cause Cu and Zn to accumulate and reside in high concentrations in chicken manure. However, heavy metals such as Cu and Zn concentrations were found at high concentrations in chicken manure and plant absorption and accumulation in plant tissues when it was applied as a fertilizer for plant growth in the agriculture industry (Yazdankhah et al., 2014). The distribution coefficient or partition coefficient (K_d) is one of the key parameters for assessing the potential migration of a pollutant in the liquid phase that is in contact with sediment or suspended matter. This tool describes in quantitative terms the partitioning of a heavy metal's element or compound between sediments and the water column. The partition coefficient is the ratio of heavy metals pollutant concentrations between sediments (animals manure) and water (Gormley-Gallagher et al., 2015). The ability of egetables to absorb and accumulate heavy metals from soil, water, and manure fertilizer is known as the bioconcentration factor, which describes how much heavy metals are absorbed by plants (Cai and Song, 2019). The translocation factor, which indicates the plant's ability to translocate the pollutant heavy metals from the roots to the shoot of the plant (Coakley et al., 2019).

Table 1 Comparison of Cu and Zn content in chicken manure fertilizer (unit: mg/kg)

Element	Range concentration (mg/kg)	Reference
Cu	61.66	(Li et al., 2021)
Zn	424.11	
Cu	3.55~916	(Yang et al., 2017)
Zn	11.8~3692	
Cu	17.9–1726.3	(Wang et al., 2013b)
Zn	73.0–1827.3	
Cu	21.83–487.43	(Zhang et al., 2012)
Zn	152.17–1063.32	
Cu	225.50 ± 4.95	(Chen et al., 2015)
Zn	672.00 ± 8.49	
Cu	51.6~81.1	(Hejna et al., 2018)
Zn	268.2~384.2	

2.3 Nitrogen cycle and nitrogen uptake by plant

The nitrogen cycle is a biogeochemical process through which nitrogen is converted into many forms, the fate of the nitrogen passing from the atmosphere to the soil to the organism and back into the atmosphere. The nitrogen cycle that has been divided into fourth major processes including N_2 fixation, ammonification, nitrification, and denitrification and microorganisms play an important role in the nitrogen transformation in the nitrogen cycle (Stein and Klotz, 2016). The first processes are ammonification is the second step of mineralization by microorganism represents in the nitrogen transformation process. The organic nitrogen decomposition and is often referred to as nitrogen mineralization contained in the chicken manure are converted to ammonium (NH_4^+) (Hopkinson and Giblin, 2008). The second processes are nitrification is the oxidation nitrogen transformation process of NH_4^+ to NO_2^- and NO_3^- occurs readily in oxic environments through the activity of nitrifying bacteria. This process is important for nitrogen fertility as nitrate is readily assimilated by plants uptake (Qian et al., 2016). The fourth processes are denitrification describes the process of anaerobic respiration of microorganism to transform nitrite (NO_2^-), nitrate (NO_3^-), nitric oxide (NO), and nitrous oxide (N_2O) to nitrogen gas (N_2) microorganism that can directly couple these three reactions with the reduction of nitrate to nitrite and perform denitrification from NO_3^- to N_2 are referred to as classical denitrifiers (Stein and Klotz, 2016). The last processes are nitrogen fixation is the process by which nitrogen is taken from its molecular form (N_2) in the atmosphere and converted into biologically available forms of nitrogen (nitrogen compound) useful for other biochemical processes. Fixation can occur through atmospheric (lightning), industrial, or biological processes (Marino and Howarth, 2014).

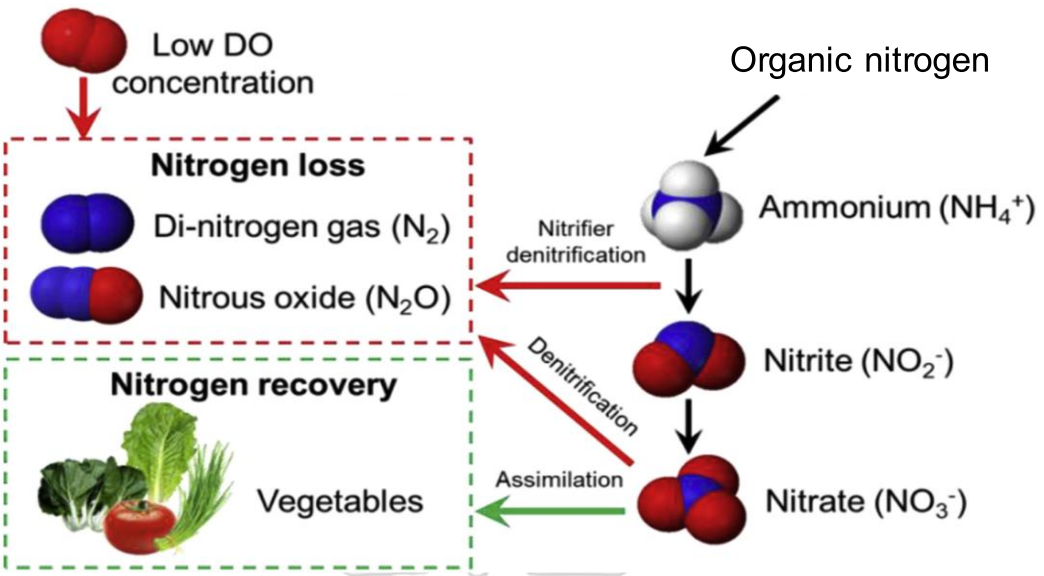


Figure 1 Nitrogen transformation cycle and picture modified from Wongkiew et al. (2018a)

Nitrogen (N) is a required nutrient for plant growth to complete their life cycles and is the most important nutrient acquired by roots assimilation. Mainly in the forms of nitrogen nutrient for plants assimilation is ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). Most forms of nitrogen uptake by plant species are NH_4^+ and NO_3^- according to both nitrogen forms root of the plant can uptake rapidly. In addition, pH values are important for the root of plants to uptake nutrients. Optimum pH can make the root of the plant uptake nutrients better (Feng et al., 2020).

2.4 Nitrogen recovery from organic waste recycling in agriculture

Øvsthus et al. (2017) studied the effects of nitrogen fertilizer from four waste-derived including anaerobically digested food waste (AD), shrimp shell (SS), algae meal (AM) and sheep manure (SM) and organic materials in a cropping sequence of broccoli, potato and lettuce grown. Effects of different N application rates and residual effects were tested on crop yield, N uptake, N recovery efficiency (NRE), N balance, N content in produce, mineral N in soil, product quality parameters and content of nitrate in lettuce. Mineral fertilizer (MF) served as control. Results showed that for crops fertilized with AD and SS were not significantly different from MF at the same N application rate, while AM, in agreement with its negative effect on N mineralization, gave negative or near-neutral effects compared to the control. No residual effect was detected after the year of application.

Wu et al. (2017) studied nitrogen fertilizer for grain yield and nitrogen recovery efficiency in a double rice (Oryza sativa L.) in subtropical China. Field plot experiments were conducted to evaluate the effects of N fertilizer placement on grain yield and N recovery efficiency (NRE). Different N application methods included: no N application (CK), N application (NBP), N deep placement (NDP) and NPK deep placement (NPKDP). Results showed that grain yield and apparent NRE significantly increased for NDP and NPKDP as compared to NBP. The experiment indicated that NDP could maintain a higher N supply in deep soil layers during rice growth. One important finding was that NDP and NPKDP significantly increased fertilizer NRE but did not lead to N declined in soil compared to NBP and NPKDP induced rice plants to absorb more fertilizer N rather than soil N.

2.5 Contamination in manures and bioaccumulation in plants of heavy metals

Zhen et al. (2020) studied heavy metal contamination in protected-field vegetable production and manure is one of the contamination sources. The experiment was conducted of three manure treatments (chicken manure: cattle manure = 3:1) with high (HMAR), medium (MMAR) and low (LMAR) applications to evaluate the long-term risks of heavy metal pollution. Results showed that high manure application rates significantly increased the total concentrations of soil Cd, Zn, Cr, and Cu rather than Pb, Ni, or As. The high application rate of manure also increased soil accumulation rates and available heavy metals although the soil organic matter was increased as well. Heavy metal accumulation risk (RAR), Zn, Cu, and Cr under HMAR and Cd and Zn under MMAR would exceed their soil threshold values and RAR could be a useful indicator for monitoring the long-term risk of soil heavy metal pollution.

Michalska and Asp (2006) studied plant uptake of heavy metals (Cd and Pb) by three lettuces (*Lactuca sativa* L.) in the hydroponic system. The experiment was added Cd and Pb to the nutrient solution following concentration (0; 0.05 mM Cd; 0.5 mM Cd; 0.05 mM Pb; 0.5 mM Pb; 0.5 mM Cd together with 0.5 mM Pb). Results showed that fresh and dry leaves and roots of plants were significantly reduced by the presence at concentration 0.5 mM Cd and 0.5 mM Cd together with 0.5 mM Pb. The higher the Cd or Pb concentration more Cd and Pb accumulated in the plants. Most of the accumulated heavy metals were in the roots. Roxette accumulated the least of Pb in the roots at 0.5mM Pb whereas Pia the least when both Cd and Pb.

Wan et al. (2020) studied heavy metal contamination of agricultural soils can cause the accumulation of heavy metals in food. A field experiment was carried out to investigate the effect of the continuous application of chicken or swine manure on the heavy metals (Pb, Cd, Cr and As) bioavailability, fractionation, and accumulation in soil and uptake by rice plants. Results showed that chicken or swine manure significantly reduced the Cd and Pb contents in rice grain with increasing application rates and the number of years; the exchangeable Cd and Pb fractions, and the Cd and Pb in the soil were also decreased. The application of chicken or swine manure substantially increased the As an accumulation in rice grain. Therefore, livestock manure can be used as soil amendments to decrease Cd and Pb accumulation in rice grains.

Wang et al. (2013a) studied about organic fertilizer application contaminated of Cu and Zn and their different bioavailability change in soil-rice system as affected by biowaste application. A field experiment was carried out to the accumulation and availability of Cu and Zn in soil, and their uptake by rice under the application of chicken manure, pig manure, and sewage sludge. Results showed that after applied chicken manure, pig manure and sewage sludge application, the soil Cu accumulation rates were higher Zn accumulation rates Compared to the control, the chicken- and pig manure treatments significantly decreased the Cu but increased the Zn in soils; thus decreased the Cu contents in rice grain and increased the grain Zn. The addition of sewage sludge significantly increased bioavailability of Zn in soil and its accumulation in rice.

2.6 Health risk assessment of heavy metals through vegetable consumption

Rahmdel et al. (2018) studied heavy metal contamination of vegetables (spinach, dill, cilantro, and cress) from the production sites of Shiraz Iran and its outskirts. Examined for lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), and cobalt (Co) content. The potential health risks of these metals to residents via the consumption of leafy vegetables were also estimated. Results showed that mean concentrations of Pb, Cd, Cu, Zn, Ni, and Co were 3.21, 0.28, 4.55, 40.44, 3.11, and 1.86 mg/kg dry weight, respectively. Cu and Zn was dominate high concentration in leafy of plants at the mean and 97.5 percentile levels. The estimated daily intake (EDI) was used to evaluated heavy metals consumption through leafy and all health risk index (HRI) values were less than the safe limit (< 1) of all heavy metals.

Mahmood and Malik (2014) studied associated soil pollution contaminated with heavy metals due to discharge of untreated urban and industrial wastewater. The presenting study aimed to determine human health risks associated via food chain contamination of heavy metals routing from irrigation of urban and industrial wastewater. Transfer factor (TF), daily intake of metals (DIM) and health risk index (HRI) were also calculated. Results showed that In food crops grown in wastewater, the extent of heavy metal concentration was in the order of $Cr^{2+} > Pb^{2+} > Cd^{2+} > Co^{2+} > Ni^{2+} > Cu^{2+} > Zn^{2+} > Mn^{2+}$, but Cr^{2+}, Pb^{2+} and Cd^{2+} in vegetables cultivated by wastewater exceeded the permissible limits, while others especially Cu and Zn was not exceeded the permissible limitsand HRI was found to be maximum for *Spinacia oleracea* (2.42 mg/kg) and *Brassica campestris* (2.22 mg/kg) cultivated by wastewater. *S. oleracea*, *B. campestris*, *Coriandrum sativum* posed a severe health risk with respect to Cd and Mn.

Singh et al. (2010) studied to human health risk assessment by heavy metals (Cd, Cu, Pb, Zn, Ni and Cr) through the intake of locally grown vegetables, cereal crops and milk from wastewater irrigated site. Results showed that concentration of heavy metals in vegetable leafy of Cd, Cu Pb, Zn, Ni and Cr were 7.17, 16.09, 10.01, 73.54, 2.70 and 3.86 mg/kg respectively. For health risk assessment found that Cd, Pb and Ni concentrations were above the ‘safe’ limits of Indian and WHO/FAO standards, while other heavy metals were below the permission limit in all the vegetables and cereals. The higher values of metal pollution index and health risk index indicated heavy metal contamination in the wastewater irrigated site that presented a significant threat of negative impact on human health. Health risk was greater due to higher contribution of cereals in the diet. The study suggests that wastewater irrigation led to accumulation of heavy metals in food stuff causing potential health risks to consumers.

Ali et al. (2021) studied to investigation heavy metals concentrations in the wastewater, soil, and consumed vegetables from the Gadoon Industrial Estate Swabi, Khyber Pakhtunkhwa Pakistan. Physicochemical parameters such as pH, electrical conductivity (EC), total dissolved solids (TDS), total suspended solids (TSS) and total solids (TS) and heavy metals such as Pb, Cr, Cd, Ni, Zn, Cu, Fe, Mn were determined using Atomic Absorption Spectrophotometer (AAS). Health risks due to the consumption of vegetables have also been estimated. Results showed that pH and TSS in wastewater were found to be higher than the permissible limit set by WHO (1996). Heavy metals concentration in vegetable of Pb, Cr, Cd, Ni, Zn, Cu, Fe, Mn was range 0.70–18.14, 0.13–17, 0.02–3.64, 0.02–26.85, 0.04–95.83, 0.05–25.83, 7.42–102.14, 7.03–44.16 mg/kg respectively. Health assessment via consumption of vegetables was higher level than the permissible limit (HRI > 1) for Pb and Cd, while Cr, Ni, Zn, Cu, Fe, Mn was lower than the permissible limit children and adults. Based on the findings of this study, there would be a significant risk to the consumers of Pb and Cd associated with consumptions of vegetables. Therefore, strict regulatory control measures are highly recommended for the safety of vegetables originated from the study area.

2.7 Human health risk assessment

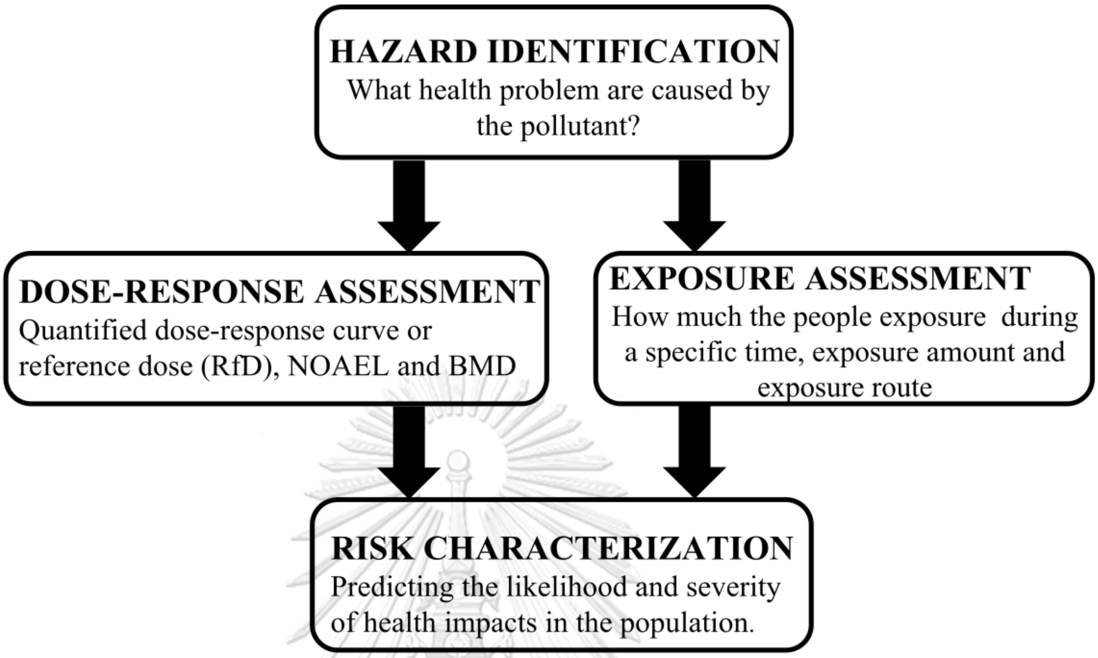


Figure 2 A four step of human health risk assessment framework

Source: U.S.EPA (2014)

A human health risk assessment is the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future. The health risk assessment process is typically described as consisting of four basic steps including hazard identification, dose response assessment, exposure assessment and risk characterization (U.S.EPA, 2014). These steps are defined below.

2.7.1 Hazard identification

Hazard identification is the process of identifying the type of hazard to human health (e.g., cancer, birth defects) posed by the exposure of interest for a given risk assessment. Hazard identification may be focused on health risks of exposure to specific individual chemicals or identification of groups of chemicals with common MOAs (e.g., heavy metals and pesticides). Chemical agents are a subset of all stressors (e.g., chemical, biological, social, or physical). In the case of chemical agents, the process examines the available scientific data specific to individual chemical i.e., material safety data sheet (SDS) for a given chemical properties, toxicity, and a characterization of hazard. This step requires identification, evaluation, and synthesis of information to describe the health effects of individual chemicals or chemical mixtures (U.S.EPA, 2014).

2.7.2 Dose-response assessment

The relationship between a contaminant's exposure or dose and the occurrence of specific health effects or outcomes is assessed in this component of impacts dose-response characterization. Dose-response characterization can describe the magnitude of a response e.g., magnitude of IQ loss. The assessment also may include the derivation of an established metric, such as EPA’s reference doses (RfD) and reference concentrations (RfC). Toxicokinetic information also is described; in data-rich situations, measured or modeled target tissue dose may be used in the dose-response calculations. In some cases, multiple chemicals may be included in a single dose-response assessment, with decisions made about the grouping of chemicals, as well as how the chemicals will be combined e.g., common MOA, common toxic effect, estimation of cancer potency factors, specific data for chemical mixtures, likelihood of simultaneous exposure (U.S.EPA, 2014).

2.7.3 Exposure assessment

Exposure assessment is one of the primary components of risk assessment; it describes how humans come into contact with hazards substants. The use of exposure science has been instrumental in forecasting, preventing and mitigating exposures that lead to adverse human health outcomes (U.S.EPA, 2014). The average daily dose (ADD_{Ing}) oral exposure is the lifetime average daily dose from ingestion exposure (mg/kg/day) of heavy metals from consumption vegetable (Kim and Han, 2011). The exposure assessment of human intake can estimate by following Ep1.

$$ADD_{Ing} = (C_{metal} \times IngR \times DW \times EF \times ED)/AT \qquad (1)$$

where;

ADD_{Ing} is the average daily dose (mg/kg-day)

C_{metal} is the heavy metal concentration (Cu or Zn) in plants of the edible part (mg/kg)

IngR is the ingestion rate (g/kg-day)

DW is conversion factor dry weight to wet weight of plants

EF is the exposure frequency (365 days/year)

ED is the exposure duration (years)

AT is the average time (365 days/year x ED)

2.7.4 Risk characterization

Risk characterization is the final step, integrative step of risk assessment. This step integrates heavy metals exposure assessment and effects assessment into quantitative and qualitative estimates of risk (U.S.EPA, 2014). The hazard quotient (HQ) is one of the most used methods for determining the levels of concern for pollutant exposure of an individual heavy metals. The Hazard index (HI) shows that overall heavy metals can pose a human health risk (Chonokhuu et al., 2019). However, the HQ values lower than the permitted limit of 1, indicating that adults and children who consume heavy metal do not pose a non-carcinogenic risk in human health. Whereas the HQ and HI values over than the permission limit of 1, indicates the potential of non-carcinogenic to health impact of individual heavy metals may affected to the human health. The HQ and HI values can estimate by following below Eq 2. And Eq 3. respectively.

$$HQ = ADD_{Ing}/RfD \quad (2)$$

$$HI = HQ_{Cu} + HQ_{Zn} \quad (3)$$

where;

HQ is the hazard quotient

HQ_{Cu} is the hazard quotient of Cu

HQ_{Zn} is the hazard quotient of Zn

ADD_{Ing} is the average daily dose (mg/kg-day)

RfD is the daily reference dose (mg/kg/day) of Cu and Zn

CHAPTER 3

METHODOLOGY

3.1 Bioponic system setup

Eight chicken manure-based nutrient film technique (NFT) bioponic systems were operated at the Department of Environmental Science, Faculty of Science, Chulalongkorn University. Each bioponic system consisted of a water recirculating tank (volume: 28.3 L), biofilter tank (volume: 24.7 L), and grow bed (length: 1.8 m) Figure 3. Water in the recirculating tank flowed continuously (600 L/h) to a bioponic grow bed channel and back to the recirculating tank. Biofilter tank connected to the water circulating tank for purifying water suitable for plant growth in grow bed. The biofilter tank also contained a mesh bag of chicken manure (200 g dry wt. of chicken manure) placed between the layers of the aquarium filter pad, leaching organic and inorganic nutrients into the water and then flowed to recirculating tank (600 L/h). Eight plants in each grow bed channel assimilated nutrients from recirculating water and recovery nutrients for their growth. All bioponic systems were operated for 28 days after 10 days of seedling. To acclimate microbes and nutrients, each bioponic system without plants and heavy metal supplementation was operated with 200 g dry wt. of chicken manure before starting each experimental phase for two weeks.

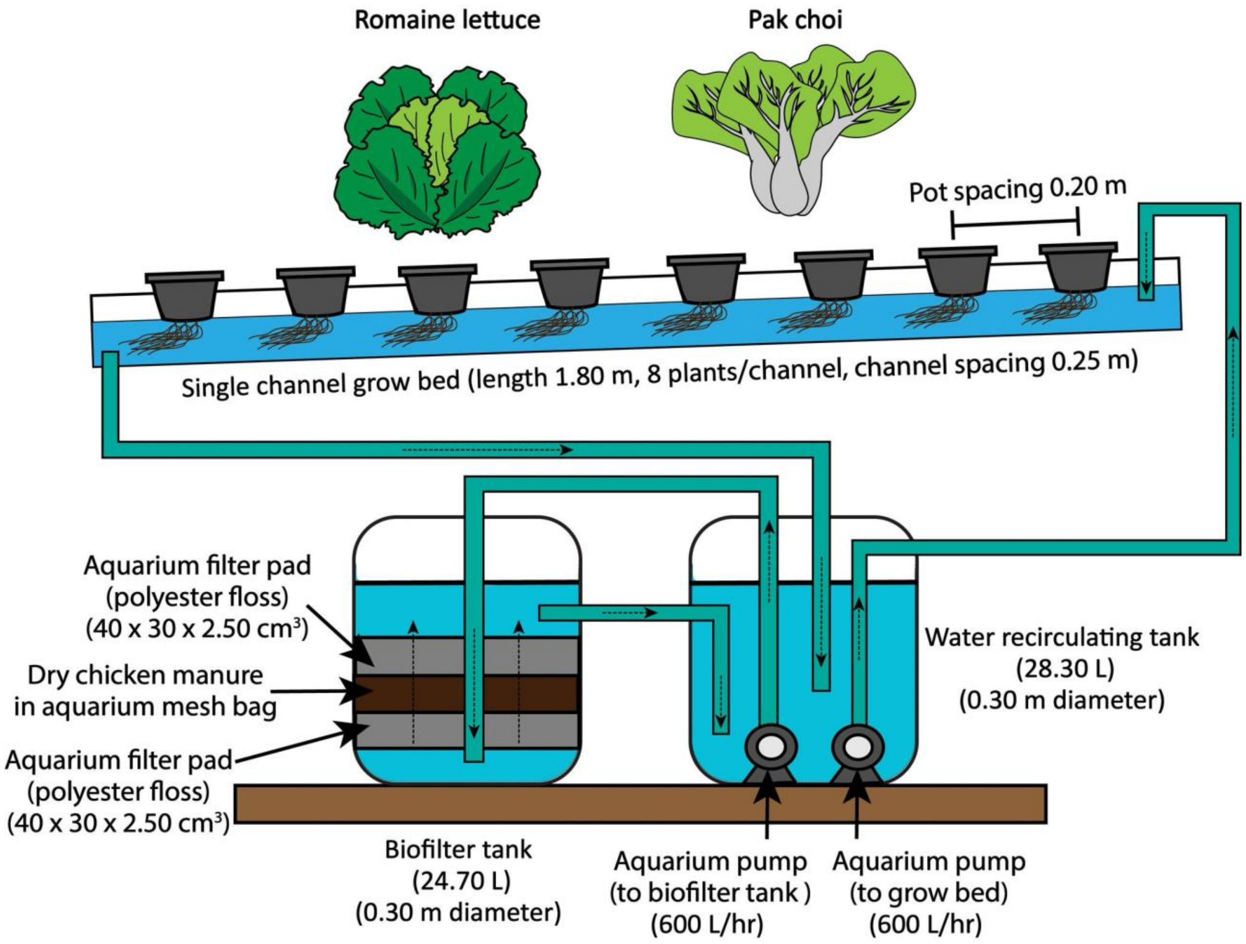


Figure 3 Construction diagram of the bioponic system

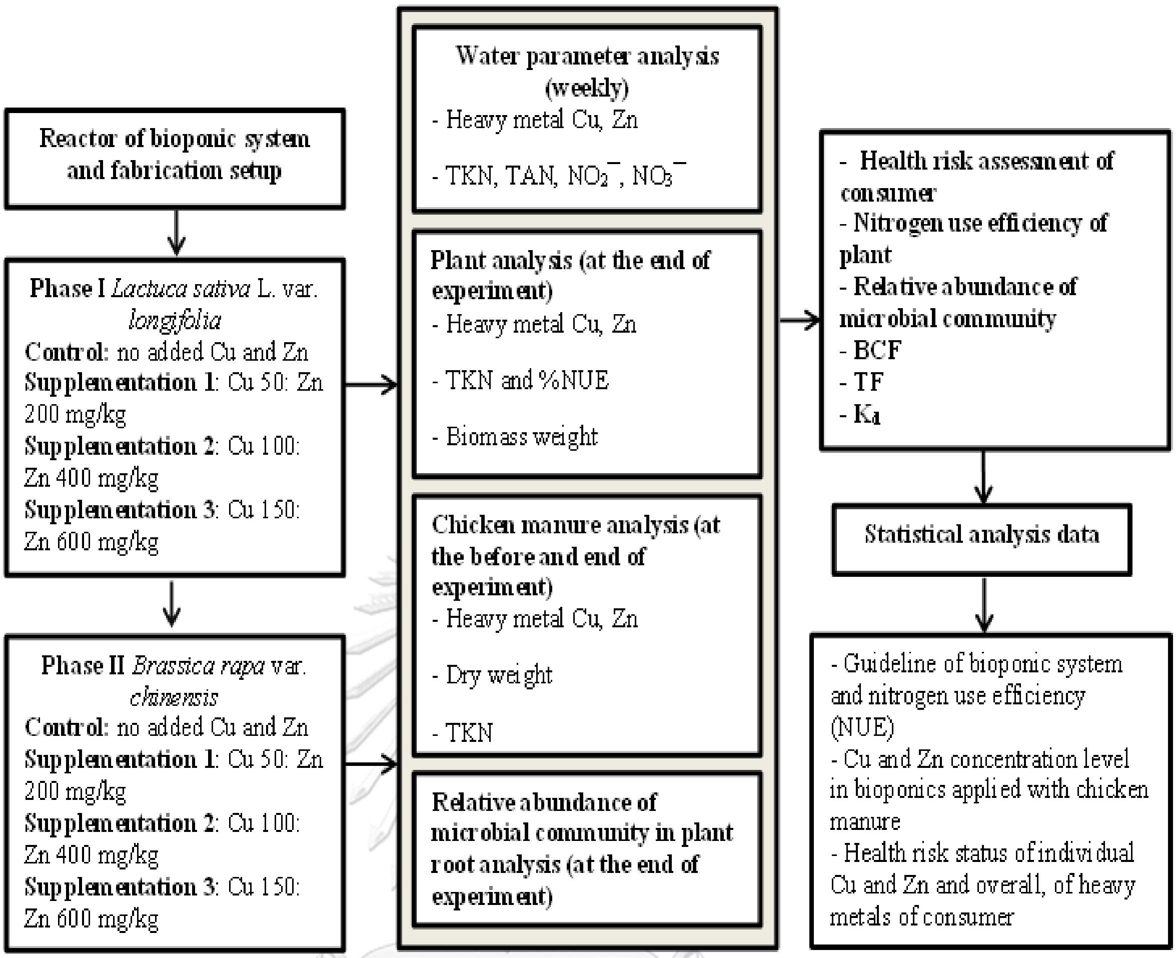


Figure 4 Experimental design and conceptual framework diagram of bioponic system.

3.2 Experimental design

The experiments were separated into two phases using romaine lettuce (*Lactuca sativa* L. var. *longifolia*) in phase I and pak choi (*Brassica rapa* var. *chinensis*) in phase II. Raw chicken manure fertilizer was produced and purchased from a local commercial farm in dry weight form. The nutrient characteristics of the chicken manure fertilizer was purchased from local farm including organic matter (OM) was 29.8 ± 2.6% (n = 3) (Walkley and Black Method), total nitrogen (N) was 3.1 ± 0.2% (n = 3) (Kjeldahl M ethod), phosphorus (P_2O_5) was 7.3 ± 0.3% (n = 3) (Spectrophotometric Molybdovana dophosphate Method) and potassium(K_2O) was 2.5 ± 1.2% (n = 3) (Flame Photometric Method) respectively. Chicken manure was used in this experiment have a Cu and Zn contaminated background was 49.3 ± 26.3 and 370.5 ± 188.6 mg/kg respectively. This study was conducted at four Cu ($CuSO_4•5H_2O$) and Zn ($ZnSO_4•7H_2O$) supplementation including contaminated background of chicken manure fertilizer, namely (1) Cu: Zn = 0: 0 mg/kg dry wt. (control is background contaminated), (2) Cu: Zn = 50: 200 mg/ kg dry wt., (3) Cu: Zn = 100: 400 mg/kg dry wt., and (4) Cu: Zn = 150: 600 mg/kg dry wt. Before chicken manure was added to biofilter tank the Cu and Zn solution was added to 200 g dry wt. of chicken manure fertilizer in supplementation condition. Then, chicken manure was oven-dried at 60°C overnight before added 200 g dry wt. in biofilter tank of each system. The ranges of Cu (50–150 mg/kg dry wt.) and Zn (200–600 mg/kg dry wt.) concentrations were selected based on possible concentrations of Cu and Zn in chicken manure from previous study (Hejna et al., 2018). All experiments were conducted in duplicate.

3.3 Sampling and preparation

Water samples were collected weekly from the recirculation tanks and filtered through filter paper Whatman No.42 before analyses. For Cu and Zn analyses, the water samples were preserved by adding concentrated HNO_3 to adjust pH level below 2 and stored at 4 °C. Plants were harvested at the end of each experiment and were separated into two parts: shoot (whole edible part) and roots. The plant parts were then analyzed for the total wet weight and dry weight using 70°C for 24 h., respectively (Montiel-Rozas et al., 2016). Dry chicken manure stock was sampled before each experiment (n = 3), and digested chicken manure residue in each biofilter was also taken at the end of each experiment. The manure samples were oven-dried for 24 h. at 105 °C. Finally, the dry plant roots, shoot, and chicken manure samples were ground using a ceramic mortar and pass through a 2 mm sieve (Lamine et al., 2019). The homogenized plant and manure samples were stored in zip lock bags and kept in desiccator at room temperature before analyses.

Prior to the analyses for Cu and Zn concentrations, dry plant shoot and roots and chicken manure were digested. The samples (~ 1 gram per sample) were digested in digestion vessel using 10 mL concentrated HNO_3 at 95 °C for approximately 2 h (until the HNO_3 volume decreased to about 5 mL). Then, 2 mL of deionized water and 5 mL of 30% H_2O_2 were added, and the samples were continuously heated until brown fumes disappeared, and a clear color solution observed. Finally, 5mL of concentrated HCl and 10 mL of deionized water were added and heated at 95 °C for 15 minutes. The samples were rested to room temperature and filtered before adjusted to 50 mL for Cu and Zn analysis (U.S.EPA, 1996).

Because microbial communities in plant roots apparently reflect the shift in microbial community from pollutant exposure, lettuce and pak choi roots in all bioponics were collected at the end of the experiment for microbial community analyses ($n = 2$). This is to assess the shift in microbial communities in plant roots affected by exposure to Cu and Zn concentrations.

3.4 Analytical methods

3.4.1 Physical and chemical analysis

pH, temperature, and dissolved oxygen (DO) concentration in bioponic systems were measured on-site weekly at recirculating tank using the pH meter (Multi 9620 IDS) and DO meter equipped with temperature probe (InoLab® Oxi 7310). Total

Kjeldahl Nitrogen (TKN), total ammonia nitrogen (TAN), nitrite (NO_2^-), and nitrate (NO_3^-) in recirculating water were analyzed using titrimetric Kjeldahl (APHA, 2017), Nessler (Jeong et al., 2013), spectrophotometric (APHA, 2017) and sodium salicylate (M.I.C. Monteiro, 2003) methods, respectively. TKN contents in plant roots, shoot, and dry chicken manure were analyzed using acid digestion method (USEPA 3052) followed by Kjeldahl method (AOAC 955.04D). Moisture content in plant samples was determined based on the standard method (APHA, 2017). Cu and Zn concentrations in water samples and plant roots, shoot, and dry chicken manure digested samples were analyzed using inductive coupled plasma-optical emission spectroscopy (ICP-OES, PlasmaQuant 9100 Elite, Analytik Jena, Germany).

3.4.2 Next-generation sequencing analysis

Bacterial 16S rRNA gene sequencing was used to investigate bacteria community of plant roots in the bioponics. TIANamp Soil DNA Kit (Tiangen Biotech, China) was used to extract bacterial rRNA genes from plant roots. In this study, 341F (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 805R (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) primers (V3 V4 regions) and sparQ HiFi polymerase chain reaction (PCR) master mix (Quantabio, USA) were used to amplify the target gene. A denaturation (94°C, 3 min) followed by a total of 25 cycles of desaturation (98°C, 20 min), annealing (60°C, 30 min), and extension (72°C, 30 min), and a final extension (72°C, 5 min) were used to amplify bacterial genes (Wongkiew et al., 2021). Following that, the amplified genes were purified with AMPure XP beads and indexed in a 50-ml PCR reaction with 5 ml of each Nextera XT index primer and followed by 8–10 cycles of the same PCR. The final PCR products were then cleaned, pooled, and diluted to a concentration of 6 pM for final loading to Illumina MiSeq to generate clusters of sequences of 250 bp paired-end reads. Bioinformatic analysis for the microbial community was performed using QIIME 2-2019.10 (Bolyen et al., 2019) with q2-demux plugin for demultiplex/quality filtering of raw sequences and DADA2 (through q2-dada2) for denoising (Callahan et al., 2016). The SEPP q2-plugin and sepp-refs-gg-13-8.qza reference was used to generate a phylogeny (Janssen et al., 2018). The q2-feature-classifier (Bokulich et al., 2018) and scikit-learn naïve Bayes classifier were used to classify operational taxonomic units (OTUs) of microbial community against Greengenes 13_8 reference at 99% similarity (McDonald et al., 2012). Using the qiime taxa filter-table command in the q2-taxa plugin, chloroplast DNA sequences were filtered out.

3.5 Calculations

3.5.1 Nitrogen use efficiency

Nitrogen use efficiency (NUE) was used to evaluate the efficiency of nitrogen uptake by plants (Dobermann, 2005). Since chicken manure is the main source of nitrogen for plant growth in the bioponics, NUE was calculated using Ep. 4.

$$NUE = (U_N - U_0)/F_N \times 100 \qquad (4)$$

where;

NUE is nitrogen use efficiency (%)

U_N is nitrogen assimilated in whole plants at the end of each experiment (gN)

U_0 is nitrogen before the experiment in plant tissues, which was negligible in this study (gN = 0)

F_N is nitrogen released from chicken manure

3.5.2 The bioconcentration factor (BCF), translocation factor (TF), and distribution coefficient (K_d)

The bioconcentration factor (BCF) was calculated based on the heavy metal contents in plant tissues (shoot and roots) per the heavy metal concentrations in bioponic recirculating water (Taghipour and Jalali, 2019). BCF can be determined using the slope of Eq. 5.

$$BCF = C_{plant}/C_{water} \qquad (5)$$

where;

BCF is the bioconcentration factor

C_{plant} is the Cu or Zn content in plants tissues (mg/kg)

C_{water} is the Cu or Zn concentrations in water (mg/L).

Translocation factor (TF) was calculated as the ratio of the heavy metal concentration in plant shoot divided by roots. TF values higher than 1 indicate that the plants effectively translocate metals from the root to shoot (Dinu et al., 2020). TF can be determined using the slope of Eq. 6.

$$TF = C_{plant}/C_{root} \qquad (6)$$

where;

TF is the translocation factor

C_{plant} is the Cu or Zn contents in shoot (mg/kg)

C_{root} is the Cu or Zn contents in root (mg/kg)

The distribution coefficient (K_d) was used to evaluate the potential distribution of heavy metal in chicken manure relative to water (Sedeño-Díaz et al., 2019). K_d can be determined using the slope of Eq. 7.

$$K_d = C_{CM}/C_{water} \qquad (7)$$

where;

K_d is distribution coefficient

C_{CM} is the Cu or Zn contents in chicken manure at the end of experiment (mg/kg)

C_{water} is the Cu or Zn concentrations in bioponic water (mg/L)

3.5.3 Average daily dose of Cu and Zn

The average daily dose (ADD_{Ing}) through ingestion route pathway was used to evaluate human (Wei et al., 2020) exposure of Cu and Zn from consuming lettuce and pak choi grown by bioponics with Cu and Zn contaminations during a time span of vegetable consumption Eq. 8.

$$ADDIng = (C_{metal} \times IngR \times DW \times EF \times ED)/AT \qquad (8)$$

Where;

ADD_{Ing} is the average daily dose (mg/kg-day)

C_{metal} is the Cu or Zn contents in the edible part of plants (mg/kg)

IngR is the ingestion rate of vegetable (children 8.1 g/kg-day and adult 5.9 g/kg-day at 95th percentile)

DW is conversion factor dry weight to wet weight of lettuce (0.050) and pak choi (0.113)

EF is the exposure frequency (365 days/year)

ED is the exposure duration (10 years for children, and 64 years for adults)

AT is the average time (365 days/year × ED) (U.S.EPA, 2018).

3.5.4 Noncarcinogenic risks assessment

Hazard quotient (HQ) was used to assess long-term non-carcinogenic health risk from exposure of a heavy metal via ingestion. HQ was calculated using a ratio of ADD_{Ing} of Cu or Zn per a reference dose (RfD) (Eq. 9). The hazard index (HI) represents the overall potential of non-carcinogenic health risk of total heavy metals (Eq. 10). This study did not perform carcinogenic health risk assessment because Cu and Zn were not classified as carcinogen. In this study, only Cu and Zn were detected and supplemented in the bioponics. Thus, HI was calculated based on Cu and Zn (Wei et al., 2020).

$$HQ = ADD_{Ing}/RfD \quad (9)$$

$$HI = HQ_{Cu} + HQ_{Zn} \quad (10)$$

where;

HQ_{Cu} and HQ_{Zn} are the hazard quotient of Cu and Zn, respectively

HI is the overall of Cu and Zn health risk assessment

RfD is the daily reference dose (mg/kg/day) of Cu and Zn (0.04 and 0.3 mg/kg/day for Cu and Zn, respectively) (Tepanosyan et al., 2018)

HQ and HI above 1 identify the possible non-carcinogenic health risk from consuming vegetable during a lifetime of ingestion (10 years for children, and 64 years for adults). HQ and HI values below 1 suggest no non-carcinogenic health risk from the vegetable consumption (Njuguna et al., 2019).

3.6 Statistic analysis

Difference of means in a compared group (> 2 groups) were analyzed by one-way ANOVA and ANCOVA for data without covariance and with covariance (e.g., days of operation), respectively. ANOVA and ANCOVA were followed by Tukey-Kramer post-hoc test at a significant level of 0.05. Linear regression and Pearson’s correlation was used to evaluate a slope and significant correlation of two sets of variables respectively. Minitab software version 19.1 was used for statistical analyses. Heatmap visualization and biostatistical analyses of the bioponic root microbiota were performed using Statistical Analysis of Metagenomic Profiles (STAMP) software version 2.1.3.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Bioponics performance and nitrogen recovery

The bioponic systems with and without Cu and Zn supplementations showed a good performance for nutrient mineralization and transformation. Operating parameters (DO concentrations, pH levels, and water temperature) were maintained equally in all bioponic systems and weekly was measured of water in the recirculating tank was found DO concentrations ranged from 4.35 ± 0.4 to 4.52 ± 0.5 mg/L, pH levels ranged from 8.12 ± 0.2 to 8.24 ± 0.2, and water temperatures ranged from 28.3 ± 1.3 to 28.9 ± 1.7 °C for lettuce grown in phase I. While the experiment in phase II, pak choi grown was measured of DO concentrations was found ranged from 4.03 ± 0.3 to 4.10 ± 0.4 mg/L, pH levels ranged from 8.11 ± 0.1 to 8.24 ± 0.2, and water temperatures ranged from 28.1 ± 1.3 to 29.4 ± 1.3 °C. was shown in Table 2 and Table 3 of each phase experiment respectively.

The pH values in this study are slightly alkaline and might be a direct effect on nitrogen transformation in the nitrification process of NH_4^+ and NO_3^- (plant assimilation), and heavy metals concentration and uptake by plants was decreased. This study utilized 200 g dry wt. of chicken manure and nutrient characterization consisting of high organic matter 29.8 ± 2.6 percent, phosphorus (P_2O_5) 7.3 ± 0.3 percent, and potassium (K_2O) 2.5 ± 1.2 percent was released, resulting in pH was high (alkaline pH buffer condition) in bioponics water. However, the focus of this study was on nitrogen recovery from chicken manure with 200 g dry wt., and phosphorus and potassium in bioponics water should be measured in future research. It was suggested that 1.0 M

H_2SO_4 be used to adjust pH values and maintain optimal pH levels (pH 6.0-7.5) for available nutrients and plants grown in bioponics (Zou et al., 2016). Previous research study operated bioponics with pH 8.38-8.45 is also negative effect to plant yield cultivated in bioponic systems (Wongkiew et al., 2021). Because the alkaline condition might induce a high rate of organic compound precipitation is not good for organic compounds and inorganic compounds dissolution (ion) into water. Optimal pH levels condition in bioponics water can enhance capable dissolution of organic and inorganic compounds including nitrogen use efficiency (NUE) and Cu and Zn absorption and bioavailable forms (ion) for plant uptake because might increasing capable of ion exchangeable of plant root in the bioponic system (Neina, 2019). According to previous research, the highest nitrogen use efficiency uptake by plants was 50.9 percent at pH 6, while 47.3 percent and 44.7 percent at pH 7.5 and pH 9 in aquaponic systems, respectively (Zou et al., 2016) obtained at acidity condition have the highest performance organic and inorganic compounds dissolution in water rather than alkaline condition. However, in statistical analysis of DO concentrations, pH levels, and the water temperature was not significantly different from each other condition at $p = 0.671$, 0.236 and 0.982 for DO concentrations, 0.775, 0.940 and 0.000 for pH levels, and 0.828, 0.651 and 0.084 for temperature in phases I and II respectively. One-way ANOVA was used to determine the difference of means in a compared group (> 2 groups) for data of parameters without covariance days of operation (e.g., pH, DO, and temperature), followed by Tukey-Kramer post-test represent statistical differences with comparisons were made separately within each parameter at a significant level of 0.05. The result indicates that range of Cu and Zn concentrations in chicken manure at loadings of 200 g dry wt. per system did not significantly affect nutrients for plant growth and NUE during the period time of operation.

Plant biomass yields (g wet wt.) and nitrogen use efficiency of plants in bioponic system were highest plant biomass and nitrogen use efficiency at control, while Cu and Zn supplementations at highest supplementation condition were affected to plant biomass and nitrogen use efficiency of lettuce and pak choi-based bioponics applied with chicken manure loading 200 g dry wt. as nutrients source was shown in Figure 5 and Figure 6 respectively. In phase I, lettuce cultivated had the highest edible biomass yield performance was shown at Cu 50: Zn 200 mg/kg supplementation and biomass yield was ranged from 144.2 ± 19.6 to 194.8 ± 22.7 g wet wt. and % NUE was ranged from 7.3 ± 3.3 and 13.9 ± 3.3 respectively. Pak choi cultivated had the highest edible biomass yield performance was shown at control condition and biomass yield was ranged from 163.2 ± 50.1 to 253.8 ± 14.4 g wet wt. and the % NUE of phase II was ranged from 35.8 ± 29.7 and 71.2 ± 3.1 was shown in Table 2 and Table 3 respectively.

Lettuce-based bioponics of Cu and Zn concentration in root and shoot highest was shown at highest (Cu 150: Zn 600 mg/kg) supplementation condition was 20.6 ± 2.8 mg/kg and 395.6 ± 13.9 mg/kg for root, and 7.2 ± 1.0 mg/kg and 89.9 ± 2.6 mg/kg for shoot and the highest for %Cu and %Zn uptake of lettuce was 0.7 ± 0.4 and 1.51 ± 0.2 respectively. Pak choi cultivated-based bioponic system the average highest bioconcentration of Cu and Zn concentration in root and shoot was shown at Cu 150: Zn 600 mg/kg supplementation similarity with phase I was 19.2 ± 1.5 mg/kg and 280.4 ± 17.3 mg/kg for root, and 3.8 ± 2.6 mg/kg and 119.8 ± 34.1 mg/kg for shoot and highest for %Cu and %Zn uptake of pak choi was ranged 1.4 ± 0.6 and 4.5 ± 01.5 respectively, Cu and Zn highest accumulated are present in highest supplementation condition (Cu 150: Zn 600 mg/kg) was shown in Table 4 and Table 5 respectively.

However, the DO concentrations in this However, the DO concentrations in this study two phases cycle of the experiment operated with lower optimal concentrations were ranged from 4 to 5 mg/L and water temperature was ranged from 28 to 29.4 °C. The results indicate that DO concentrations were lower than the optimal range for bioponic system and the optimal was ranged 6 to 7 mg/L. Because at the beginning of the experiment, chicken manure was added to the bioponics, causing a flow rate of water turbulence was affected the organic matter released from chicken manure at highest concentration into bioponics water resulting in decreasing at low DO concentration in bioponics. Based on the experience of the experiment suggested that to prevent turbulence of water in bioponic system chicken manure should be added to a biofilter tank before adding water to bioponics. The operational water temperature and the ability of solubility of dissolved oxygen together with microbial communities on roots are respiration to increase during the plant growth process and recirculation rate in the bioponics obtained affect to the DO concentration to drop (Wongkiew et al., 2018a). Previous studies have suggested that optimal range recommendation to avoid anaerobic conditions in the biofilter and rot disease in the plant roots (Wongkiew et al., 2021). According to the experiment for plant production in bioponics suggested that should be operated with optimal DO concentration in bioponics to avoid nitrogen loss and anaerobic conditions in bioponics, it can affect plants grown in bioponics. It is suggested that aeration and increasing the flow rate of water in the recirculating tank be used to enhance the dissolved oxygen in bioponics water.

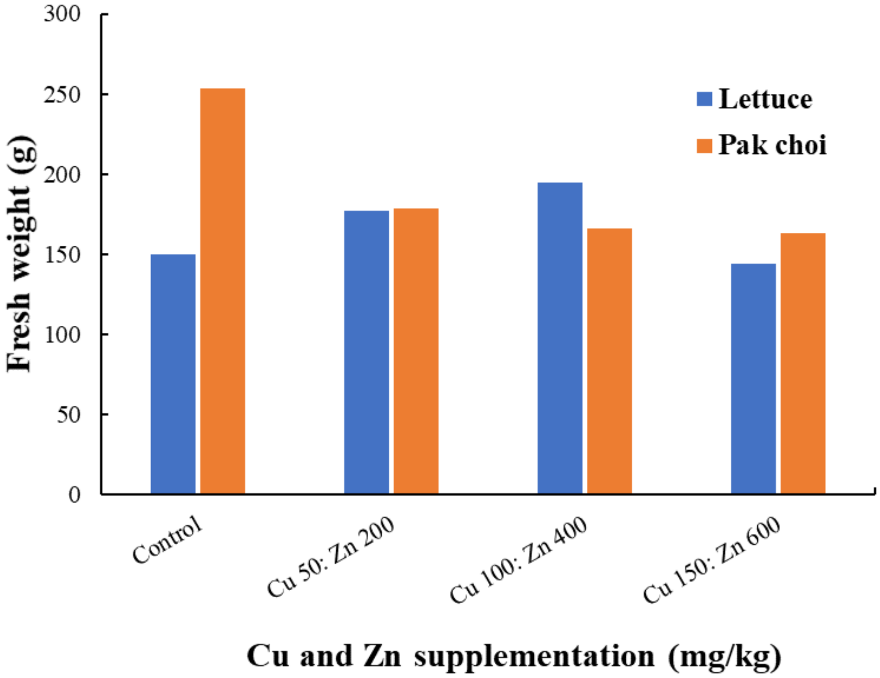


Figure 5 Fresh weight of lettuce and pak choi cultivated with different Cu and Zn supplementation of the bioponics system.

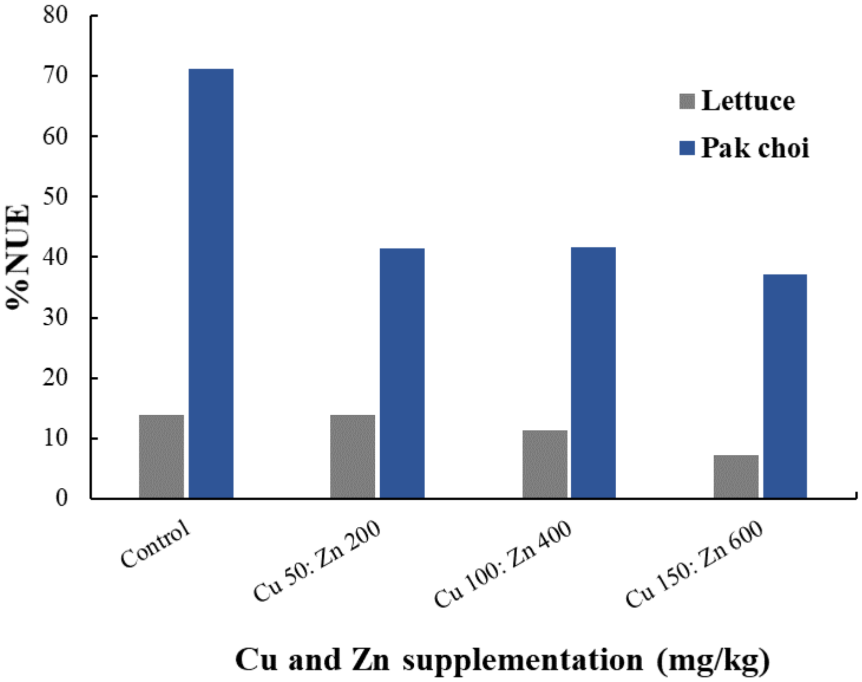


Figure 6 %NUE of lettuce and pak choi cultivated with different Cu and Zn supplementation of the bioponics system.

Table 2 Operating parameters (pH, temperature, DO concentration), nitrogen concentrations, fresh weight and %NUE in lettuce-based bioponics at Cu and Zn supplementation.

Phase I: Lettuce-based bioponics									
Supplementation condition (mg/kg)	**pH**	**Temperature (ºC)**	**DO (mg/L)**	**TKN (mgN/L)**	**TAN (mgN/L)**	**NO_2^- (mgN/L)**	**NO_3^- (mgN/L)**	**Fresh weight (g wet wt.)**	**%NUE**
Control	8.2 ± 0.2^{a}	28.8 ± 1.3^{a}	4.4 ± 0.4^{a}	11.2 ± 0.5^{a}	1.1 ± 0.8^{a}	0.02 ± 0.03^{a}	11.6 ± 4.0^{a}	150.2 ± 16.7^{a}	13.8 ± 3.5^{a}
Cu 50: Zn 200	8.2 ± 0.2^{a}	28.9 ± 1.7^{a}	4.5 ± 0.5^{a}	13.6 ± 0.7^{a}	1.2 ± 1.1^{a}	0.02 ± 0.03^{a}	12.0 ± 5.5^{a}	176.9 ± 34.9^{a}	13.9 ± 3.3^{a}
Cu 100: Zn 400	8.1 ± 0.3^{a}	28.7 ± 1.9^{a}	4.4 ± 0.4^{a}	19.4 ± 1.8^{a}	1.5 ± 1.8^{a}	0.04 ± 0.04^{a}	14.9 ± 4.7^{a}	194.8 ± 22.7^{a}	11.4 ± 8.6^{a}
Cu 150: Zn 600	8.2 ± 0.2^{a}	28.3 ± 1.7^{a}	4.4 ± 0.4^{a}	16.3 ± 1.1^{a}	1.1 ± 1.0^{a}	0.02 ± 0.02^{a}	12.9 ± 4.8^{a}	144.2 ± 19.6^{a}	7.3 ± 3.3^{a}

Values reported as mean ± standard deviation ANOVA and ANCOVA. The superscripts a, b and c represent statistical differences by Tukey-Kramer post-test ($p < 0.05$). Comparisons were made within each column.

Table 3 Operating parameters (pH, temperature, DO concentration), nitrogen concentrations, fresh weight and %NUE in pak choi-based bioponics at Cu and Zn supplementations.

	Phase II: Pak choi-based bioponics								
Supplementation condition (mg/kg)	**pH**	**Temperature (^oC)**	**DO (mg/L)**	**TKN (mgN/L)**	**TAN (mgN/L)**	**NO_2^- (mgN/L)**	**NO_3^- (mgN/L)**	**Fresh weight (g wet wt.)**	**%NUE**
Control	8.2 ± 0.2^a	29.4 ± 1.3^a	4.0 ± 0.3^a	19.0 ± 0.5^a	0.8 ± 0.3^a	0.02 ± 0.04^a	10.0 ± 6.5^a	253.8 ± 14.4^a	71.2 ± 3.1^a
Cu 50: Zn 200	8.2 ± 0.1^a	28.2 ± 1.4^a	4.1 ± 0.4^a	17.2 ± 0.7^a	0.7 ± 0.4^a	0.02 ± 0.04^a	10.1 ± 6.8^a	178.7 ± 13.9^a	41.4 ± 8.5^a
Cu 100: Zn 400	8.1 ± 0.1^a	29.0 ± 1.2^a	4.0 ± 0.3^a	19.4 ± 1.8^a	0.9 ± 0.5^a	0.01 ± 0.01^a	8.8 ± 6.8^a	166.1 ± 59.3^a	41.6 ± 24.6^a
Cu 150: Zn 600	8.2 ± 0.2^a	28.7 ± 1.4^a	4.1 ± 0.3^a	16.3 ± 1.1^a	0.7 ± 0.5^a	0.01 ± 0.01^a	9.7 ± 6.9^a	163.3 ± 50.1^a	37.2 ± 29.7^a

Values reported as mean ± standard deviation ANOVA and ANCOVA. The superscripts a, b and c represent statistical differences by Tukey-Kramer post-test ($p < 0.05$). Comparisons were made within each column.

Table 4 %Cu and %Zn uptake by plants, Cu and Zn content in plant roots, shoot and chicken manure residue in lettuce-based bioponics under Cu and Zn supplementation.

Phase I: Lettuce-based bioponics								
Supplementation condition (mg/kg)	**Roots and root**		**Roots**		**Shoot**		**CM_{after}**	
	%Cu uptake	**%Zn uptake**	**Cu (mg/kg)**	**Zn (mg/kg)**	**Cu (mg/kg)**	**Zn (mg/kg)**	**Cu (mg/kg)**	**Zn (mg/kg)**
Control	0.8 ± 0.4^{a}	1.5 ± 0.2^{a}	12.8 ± 4.1^{a}	283.7 ± 10.9^{b}	5.6 ± 0.1^{a}	66.7 ± 13.3^{a}	87.1 ± 8.9^{c}	779.6 ± 31.2^{b}
Cu 50: Zn 200	0.5 ± 0.2^{a}	1.2 ± 0.3^{a}	21.6 ± 6.6^{a}	345.4 ± 11.9^{a}	6.0 ± 0.2^{a}	64.8 ± 13.8^{a}	$136.4 \pm 7.6^{b,c}$	919.4 ± 33.5^{a}
Cu 100: Zn 400	0.2 ± 0.3^{a}	0.9 ± 0.6^{a}	20.2 ± 5.6^{a}	381.2 ± 13.6^{a}	5.8 ± 2.4^{a}	74.5 ± 2.6^{a}	$187.6 \pm 26.4^{a,b}$	996.0 ± 32.2^{a}
Cu 150: Zn 600	0.1 ± 0.02^{a}	0.5 ± 0.3^{a}	20.6 ± 2.8^{a}	395.7 ± 13.9^{a}	7.2 ± 1.0^{a}	90.0 ± 2.6^{a}	222.9 ± 18.8^{a}	1034.8 ± 27.9^{a}

Values reported as mean ± standard deviation ANCOVA and ANOVA. The superscripts a, b and c represent statistical differences by Tukey-Kramer post-hoc test ($p < 0.05$). Comparisons were made within each column.

Table 5 %Cu and %Zn uptake by plants, Cu and Zn content in plant roots, shoot and chicken manure residue in pak choi-based bioponics under Cu and Zn supplementation.

	Phase II: Pak choi-based bioponics							
Supplementation condition (mg/kg)	**Roots and shoot**		**Roots**		**Shoot**		**CM$_{after}$**	
	%Cu uptake	**%Zn uptake**	**Cu (mg/kg)**	**Zn (mg/kg)**	**Cu (mg/kg)**	**Zn (mg/kg)**	**Cu (mg/kg)**	**Zn (mg/kg)**
Control	1.4 ± 0.6^{a}	4.6 ± 1.5^{a}	11.4 ± 2.9^{a}	138.5 ± 15.0^{b}	1.4 ± 0.3^{a}	43.4 ± 7.8^{a}	84.7 ± 1.5^{c}	525.6 ± 34.5^{a}
Cu 50: Zn 200	0.6 ± 0.1^{a}	4.2 ± 0.4^{a}	16.9 ± 2.6^{a}	160.4 ± 22.0^{b}	2.5 ± 1.2^{a}	68.5 ± 19.3^{a}	146.4 ± 15.9^{b,c}	649.5 ± 73.2^{a}
Cu 100: Zn 400	0.6 ± 0.05^{a}	4.3 ± 0.7^{a}	17.1 ± 3.6^{a}	207.7 ± 24.9^{a,b}	3.3 ± 2.9^{a}	112.9 ± 33.2^{a}	176.8 ± 30.4^{a,b}	605.3 ± 21.5^{a}
Cu 150: Zn 600	0.4 ± 0.01^{a}	4.0 ± 0.9^{a}	19.3 ± 1.5^{a}	280.5 ± 17.3^{a}	3.8 ± 2.6^{a}	119.8 ± 34.1^{a}	218.1 ± 1.5^{a}	651.3 ± 72.6^{a}

Values reported as mean ± standard deviation ANCOVA and ANOVA. The superscripts a, b and c represent statistical differences by Tukey-Kramer post-hoc test ($p < 0.05$). Comparisons were made within each column.

Table 6 %Nitrogen output distribution of lettuce-based bioponic system with 200 g dry weight of chicken manure.

Phase I: Lettuce-based bioponics			
Supplementation condition (mg/kg)	**Lettuce (gN)**	**Nitrogen loss (gN)**	**CM$_{after}$ (gN)**
Control	0.14 ± 0.03^{a}	4.76 ± 0.03^{a}	25.7 ± 1.5
Cu 50: Zn 200	0.14 ± 0.03^{a}	4.76 ± 0.03^{a}	
Cu 100: Zn 400	0.11 ± 0.08^{a}	4.78 ± 0.08^{a}	
Cu 150: Zn 600	0.07 ± 0.03^{a}	4.82 ± 0.03^{a}	

Values reported as mean ± standard deviation ANOVA. The superscripts a represent statistical differences by Tukey-Kramer post-test ($p < 0.05$). Comparisons were made within each column.

Table 7 %Nitrogen output distribution of pak choi-based bioponic system with 200 g dry weight of chicken manure.

Phase II: Pak choi-based bioponics			
Supplementation condition (mg/kg)	**Pak choi (gN)**	**Nitrogen loss (gN)**	**CM$_{after}$ (gN)**
Control	0.71 ± 0.03^{a}	4.18 ± 0.03^{a}	25.7 ± 1.5
Cu 50: Zn 200	0.41± 0.08^{a}	4.48 ± 0.08^{a}	
Cu 100: Zn 400	0.42± 0.24^{a}	4.48 ± 0.03^{a}	
Cu 150: Zn 600	0.37 ± 0.29^{a}	4.52 ± 0.03^{a}	

Values reported as mean ± standard deviation ANOVA. The superscripts a represent statistical differences by Tukey-Kramer post-test ($p < 0.05$). Comparisons were made within each column.

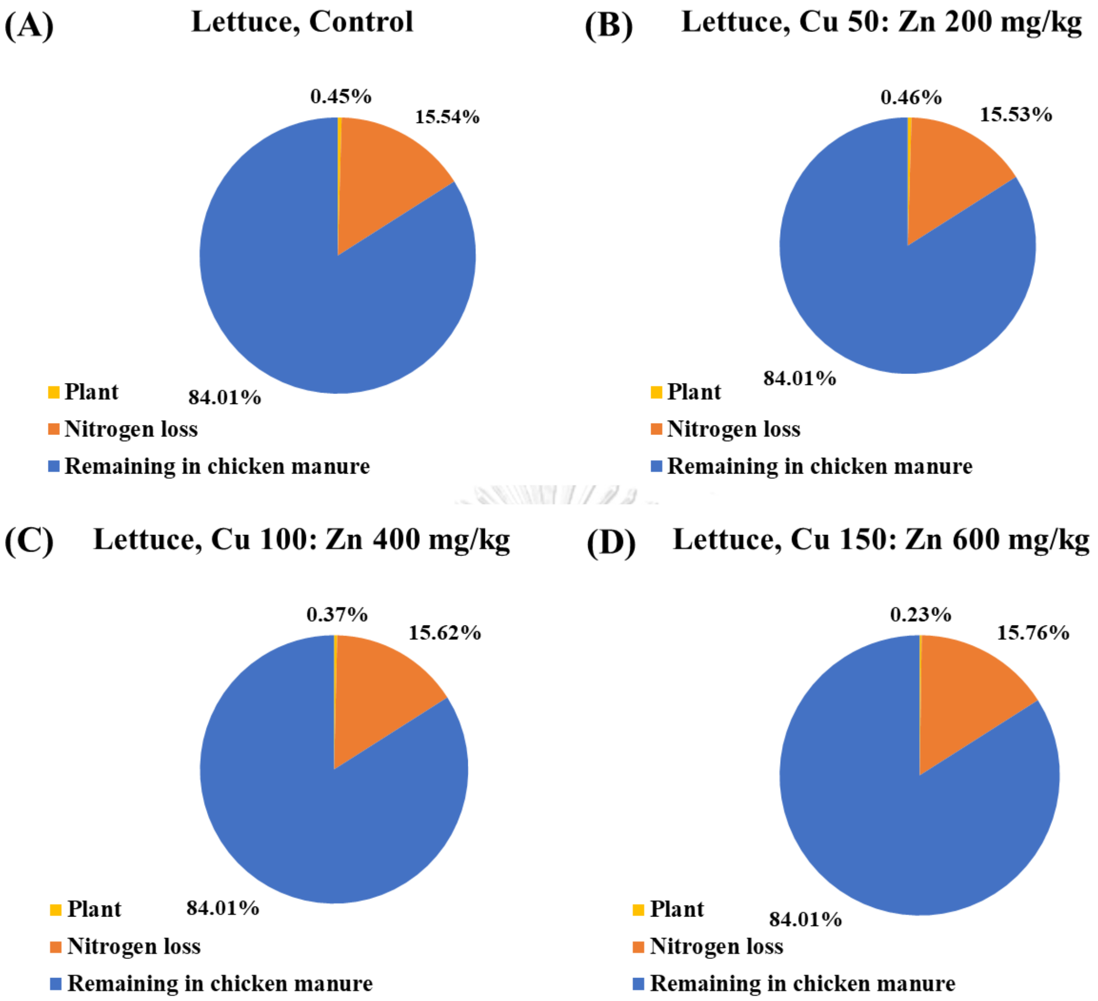


Figure 7 %Nitrogen output distribution at different Cu and Zn supplementation (A–D) of lettuce-based bioponic system.

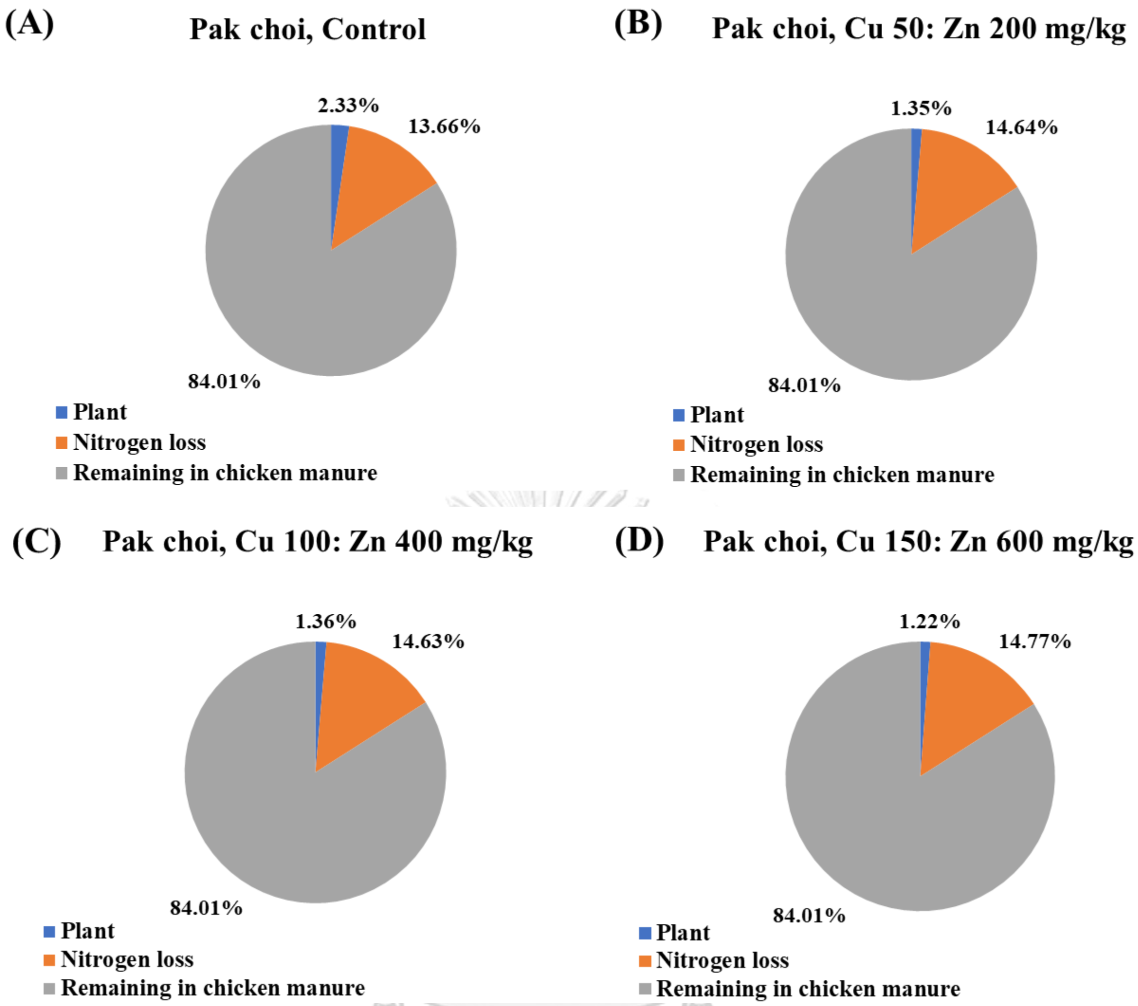


Figure 8 %Nitrogen output distribution at different Cu and Zn supplementation (A–D) of pak choi-based bioponic system.

The nutrient characteristics of the chicken manure fertilizer before the experiment consist of macronutrients is organic matter 29.8 ± 2.6%, total nitrogen (N) 3.1 ± 0.2%, phosphorus (P_2O_5) 7.3 ± 0.3% and potassium (K_2O) 2.5 ± 1.2% respectively. Nutrient concentration (nitrogen, phosphorus, and potassium) was high enough for the plant to require in the growth process, and no symptoms of nutrient deficiency of lettuce and pak choi growth in bioponics were observed (Wongkiew et al., 2021). was range from 15.89 ± 6.9 to 19.38 ± 11.2, 1.11 ± 0.8 to 1.48 ± 1.8, 0.02 ± 0.2 to 0.04 ± 0.04 and 11.59 ± 4.0 to 14.89 ± 4.7 mgN/L, followed by phase II pak choi-based bioponics were range from 18.87 ± 8.5 to 20.15 ± 11.8, 0.70 ± 0.4 to 0.94 ± 0.5, 0.01 ± 0.01 to 0.02 ± 0.04 and 8.80 ± 6.8 to 10.13 ± 6.8 mgN/L was shown in Table 2 and Table 3 respectively. The trend nitrogen parameter of TKN, TAN, NO_2^-, and NO_3^- at phases I and II of the experiment was shown highest concentration at day zero of the experiment and then, which continually decreased from the first week (7 days) of operation until at the end of the experiment in all experimental supplementation conditions. Because TKN was converted to TAN, NO_2^-, and NO_3^-, plants uptake and accumulation, nitrogen loss, absorbed by the biofilter, re-absorbed by chicken manure, and microorganisms in the bioponic system also (Anjana and Iqbal, 2007; Farrell et al., 2014)

The table of %nitrogen output distribution of lettuce and pak choi-based bioponics was show in Table 6 and Table 7 respectively. In the control, nitrogen accumulation in plant tissues was higher than the highest Cu 150: Zn 600 mg/kg supplementation. However, the composition of residue of nitrogen in chicken manure after the experiment was highest nitrogen accumulation concentration and followed by nitrogen loss and lowest nitrogen concentration in plant tissues of lettuce and pak choi-based bioponics was show in Figure 7 and Figure 8 respectively. The bioaccumulation of Cu and Zn in lettuce, pak choi and nitrogen concentration in plants tissues was not significantly different and Cu and Zn supplementation had no effect on nitrogen concentration in a bioponic system not significantly different at $p < 0.05$ using ANCOVA with covariance (days operation). The samples size in this study was taken to Cu and Zn analysis (n = 2), resulting in a large standard deviation and for increasing accuracy and significantly of statistical analysis should the size sample (n = 3). Furthermore, the concentration of A previous study of Cu and Zn supplementation was reported that Cu and Zn can accumulate in plant tissues at a significant level ($p < 0.0001$) using ANOVA (Ondo J.A, 2012). The nitrogen mass balance of chicken manure 200 g dry wt. per system of bioponic system was remaining highest concentration in chicken manure after experiment following with nitrogen loss and lowest nitrogen concentration in plants tissues of all condition of the experiment in phases I and II was shown in Figure 9 - 17 respectively. However, in a previous study plants uptake heavy metals from soil the relationship between root and soil, but in this study in the bioponics system the relationship between root and water for heavy metals uptake. Thus, the chicken manure loading rate of 200 g dry wt. per system could be optimal level for nitrogen required by plants was enough associate to nitrogen released concentration from chicken manure fertilizer. This indicates that nitrogen has a low potential to release and soluble from chicken manure fertilizer to bioponics water but does not affect the nitrogen necessary for plant growth because the nitrogen concentration in water is more enough for plant growth and plants have no symptoms of nitrogen deficiency during the experiment period time (Wongkiew et al., 2021).

At the beginning time of operation, soluble organic nitrogen compounds could hydrolyze by heterotrophs organisms into TAN (e.g., proteins in chicken manure are hydrolyzed). Then, ammonia oxidizing bacteria (AOB) and nitrite oxidizing (NOB) converted TAN to NO_2^{-} and then NO_2^{-} to $NO_3^{-\,-}$ respectively. The anaerobic condition in the bioponic system can release N_2 and N_2O to the atmosphere via the denitrification process by the denitrification organism. This process has the capability of removing nitrogen from the bioponic system (Zhu et al., 2013). N_2O, is a greenhouse gas, was one significant contributor to the ozone layer-depleting that causes the global warming phenomenon (Wu and Mu, 2019). However, there is still a lack of research on N_2O loss via denitrification process from bioponics systems. Previous research study of N_2O emissions from aquaponic systems (similar system with bioponics) resulted found that total N_2O emission in lettuce and pak choi cultivated was 25.1 ± 2.7 and 22.2 ± 1.9 mgN/day respectively (Wongkiew et al., 2018b). It was suggested that the biopoonic system should be run at the optimal DO concentration along the experiment times to minimize nitrogen loss from the bioponic system. However, there were no symptoms of nutritional inadequacy of lettuce and pak choi when nitrogen concentrations decreased. The nitrogen concentrations in the bioponic systems applied with 200 g dry wt. of chicken manure were enough for the growth of both plant species. (Wongkiew et al., 2021).

The nitrogen transformation process is important for transforming organic nitrogen to NH_3^{+}, NO_2^{-}, and NO_3^{-} by nitrifying microbial group, which are available easy forms for plant assimilation and the dynamic of nitrogen concentration of bioponics water in this study was assessed as TKN, TAN, NO_2^{-}, and NO_3^{-}. The dynamic of nitrogen parameter consists of the major forms such as TKN, TAN, NO_2^{-}, and NO_3^- of phase I lettuce, phase II pak choi cultivated of 5 weeks of period time of experiment under four supplementation conditions of Cu and Zn was shown in Figure 17 and Figure 18 respectively. These all two phases of the experiment indicate that TKN was found at high concentration than others because the nitrogen release from chicken manure into bioponics water as organic nitrogen degradation form (presented by TKN in this study), while NO_2^- was found at a low concentration from beginning to the end of experiment of all experiment Cu and Zn supplementations. This is the advantage for plants and microorganisms in bioponics because NO_2^- of the plant is needed at very low concentration assimilation. However, if NO_2^- concentration are raised too high, this can cause toxicity to plants and microorganisms (Wongkiew et al., 2017). In this study, NO_3^- and TAN were found as the major organic nitrogen for plant uptake.

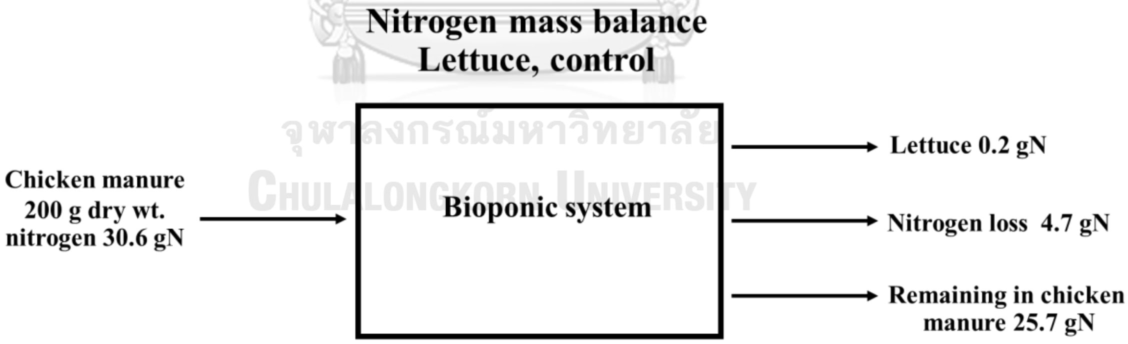


Figure 9 Nitrogen mass balance of lettuce-based bioponics (phase I) at control condition.

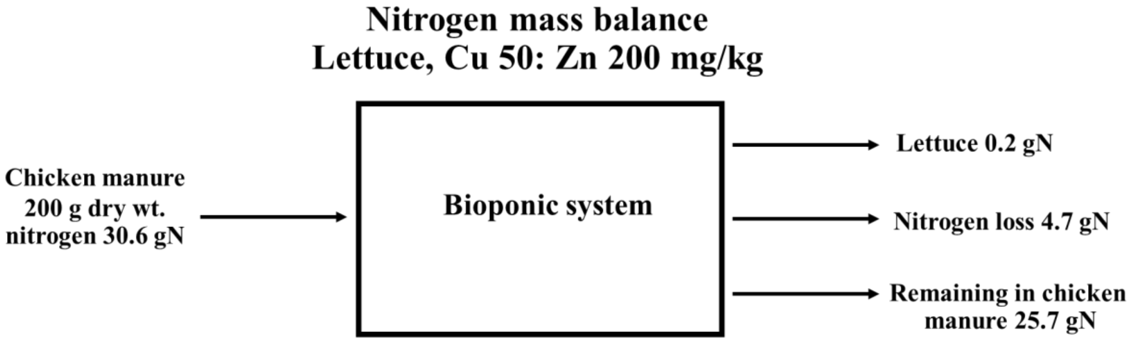


Figure 10 Nitrogen mass balance of lettuce-based bioponics (phase I) at Cu 50: Zn 200 mg/kg supplementation condition.

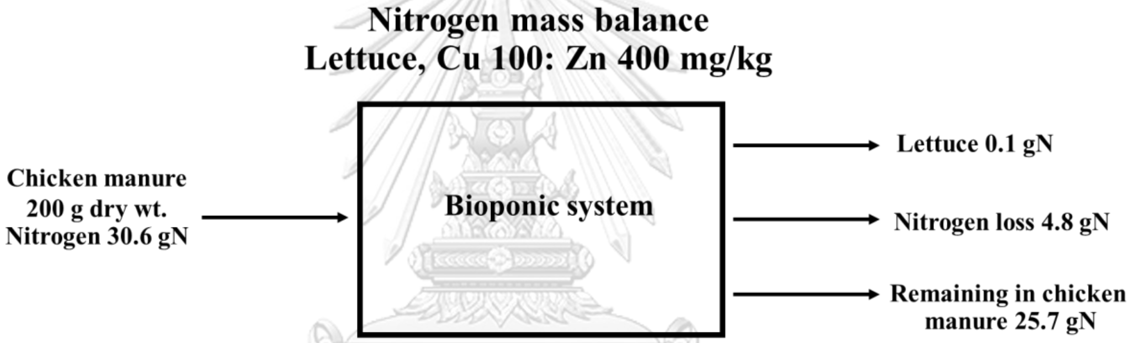


Figure 11 Nitrogen mass balance of lettuce-based bioponics (phase I) at Cu 100: Zn 400 mg/kg supplementation condition.

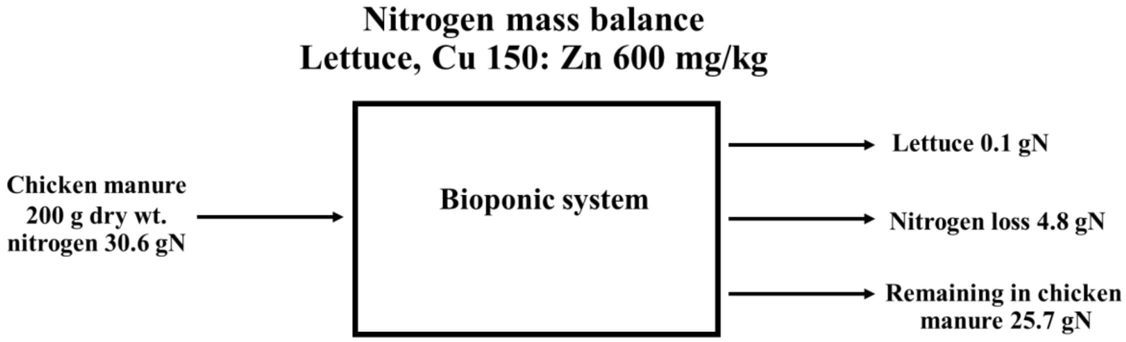


Figure 12 Nitrogen mass balance of lettuce-based bioponics (phase I) at Cu 150: Zn 600 mg/kg supplementation condition.

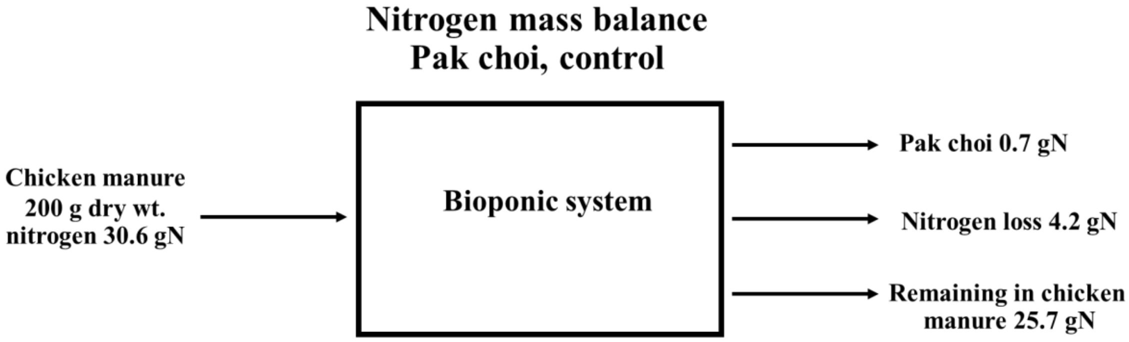


Figure 13 Nitrogen mass balance of pak choi-based bioponics (phase II) at control condition.

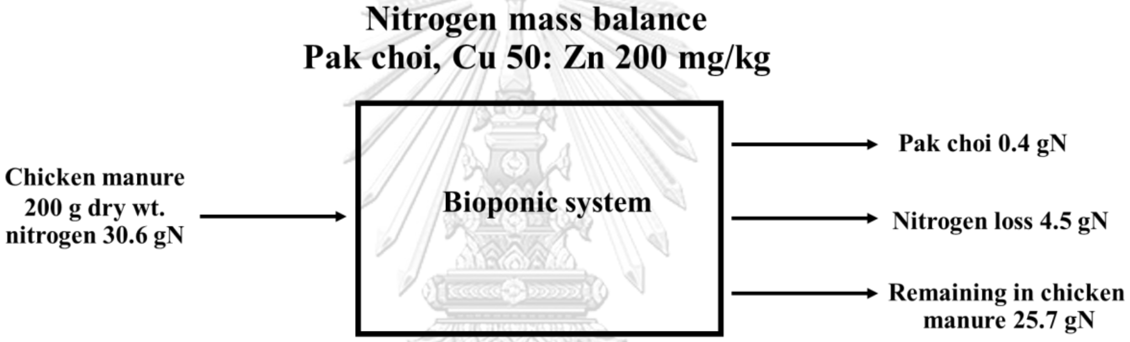


Figure 14 Nitrogen mass balance of pak choi-based bioponics (phase II) at Cu 50: Zn 200 mg/kg supplementation condition.

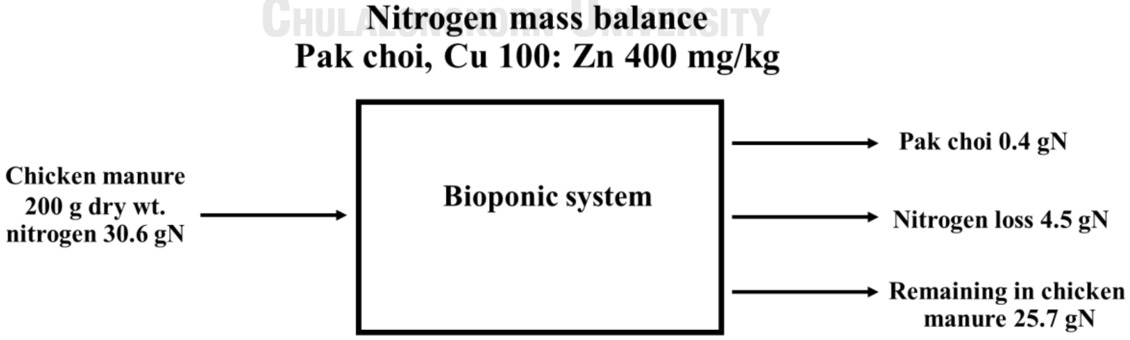


Figure 15 Nitrogen mass balance of pak choi-based bioponics (phase II) at Cu 100: Zn 400 mg/kg supplementation condition.

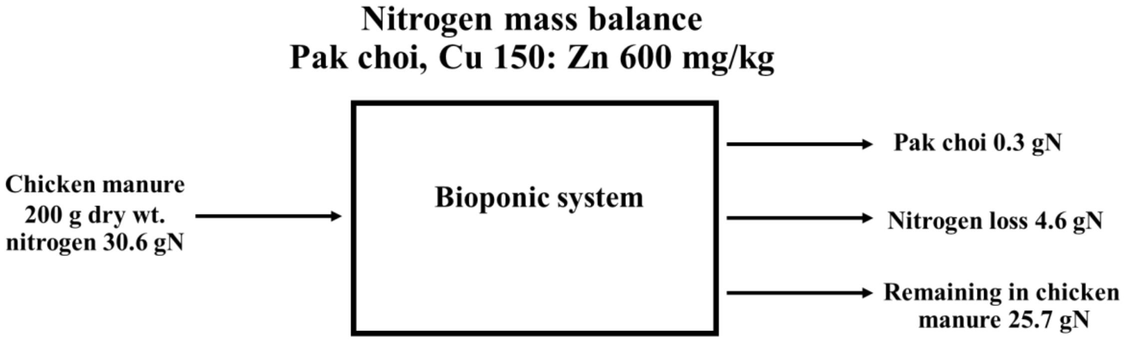


Figure 16 Nitrogen mass balance of pak choi-based bioponics (phase II) at Cu 150: Zn 600 mg/kg supplementation condition.

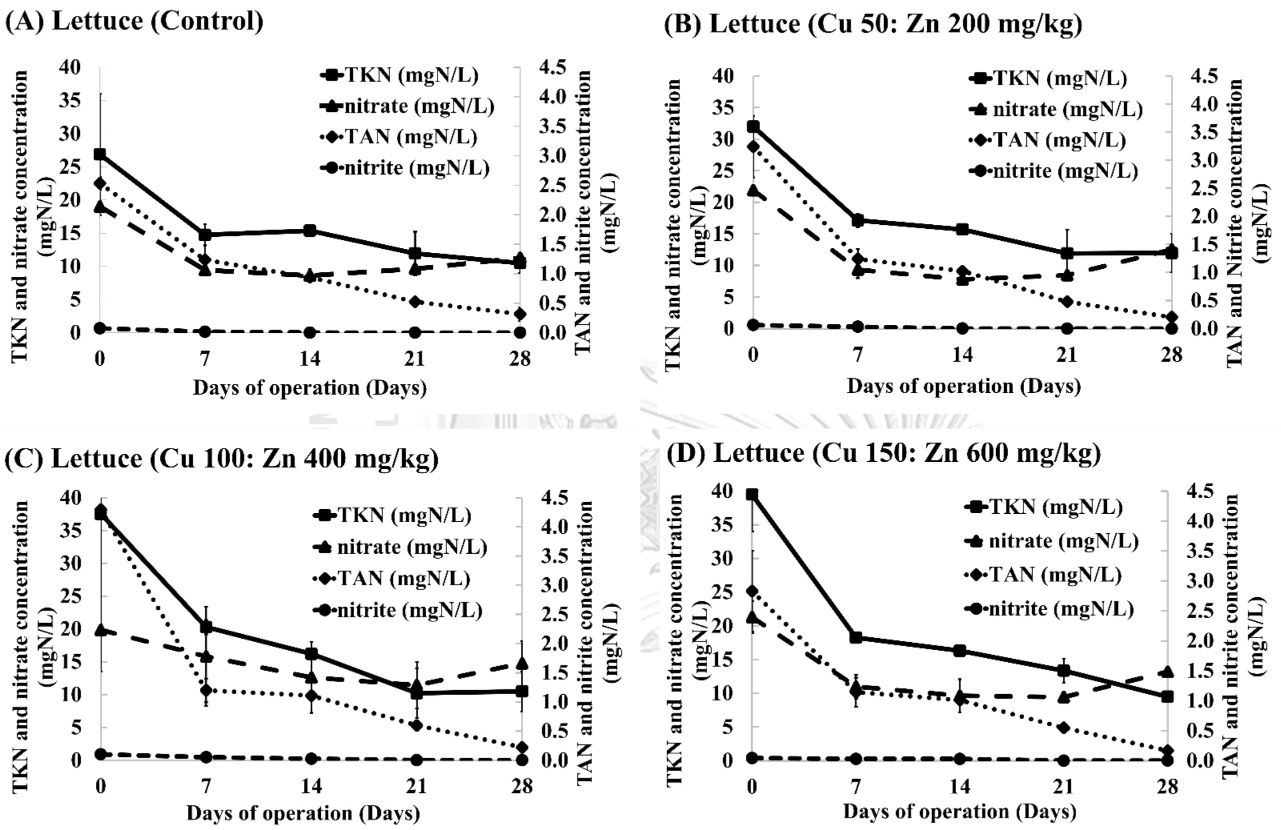


Figure 17 Dynamics of TKN, TAN, nitrite, and nitrate concentrations in lettuce-based bioponics at Cu and Zn supplementations. Error bars represent standard deviation (n = 2).

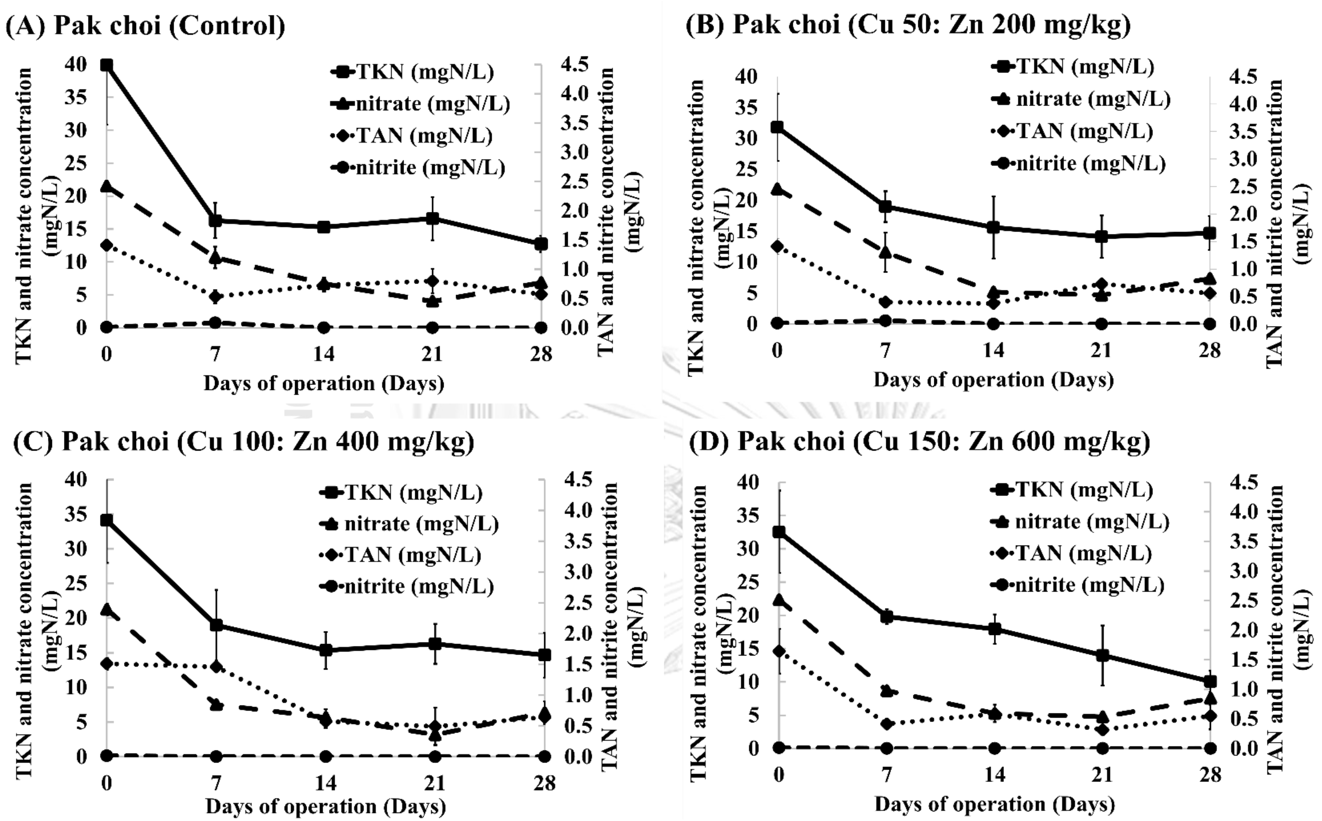


Figure 18 Dynamics of TKN, TAN, nitrite, and nitrate concentrations in pak choi-based bioponics at Cu and Zn supplementations. Error bars represent standard deviation (n = 2).

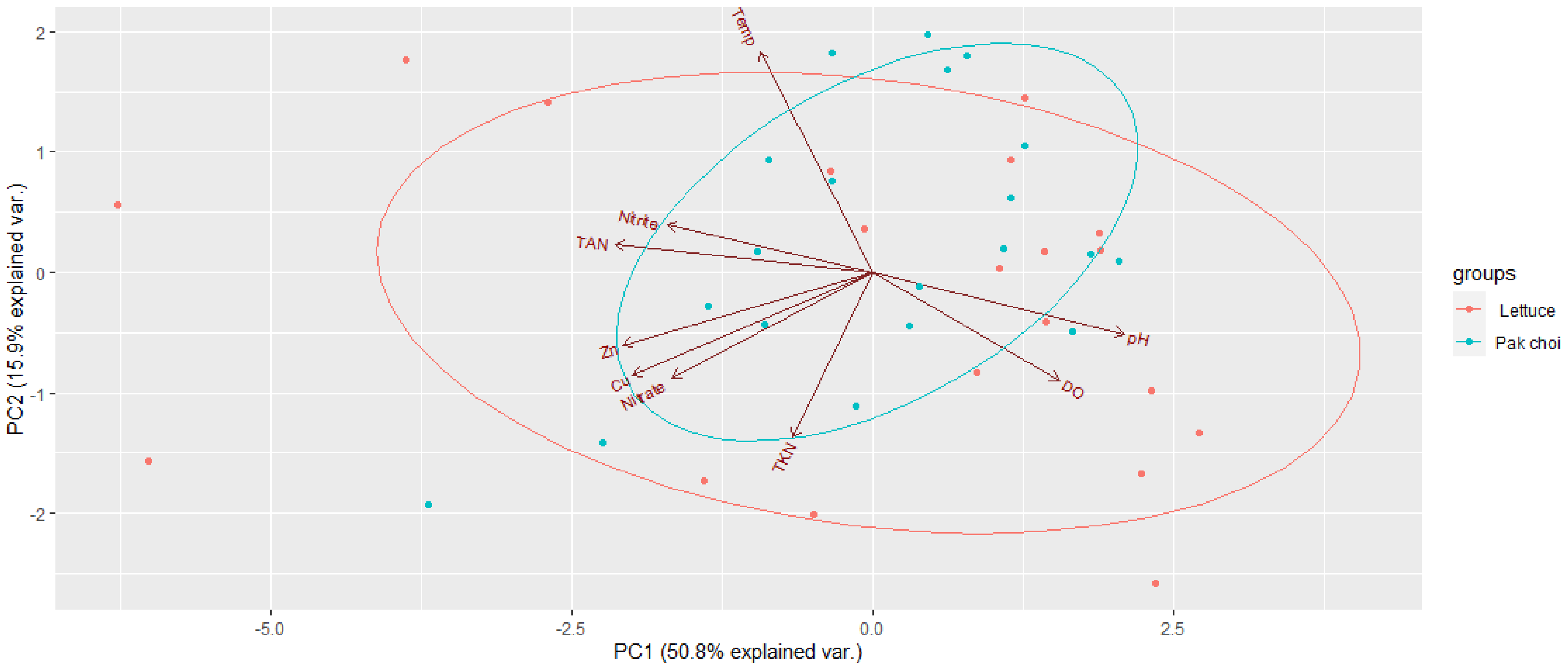


Figure 19 Relationships of the lettuce and pak choi to operating parameters (pH, DO, and temperature), nitrogen concentration (TKN, TAN, NO_2^-, and NO_3^-) and heavy metal (Cu and Zn).

4.2 Dissolution of Cu and Zn in bioponic system

In the control condition of the experiment (contaminated with Cu and Zn at background concentration level) because Cu and Zn are regarded as essential elements that animals require nutrition for growth in commercial livestock (e.g., poultry and swine). Thus, Cu and Zn are used as mineral additives in animal feed due to their antibacterial and growth-stimulating properties to support the growth of the animals (Hejna et al., 2018) causing Cu and Zn unavoidably contaminated in chicken manure. The result showed that Cu and Zn concentrations in water were higher than those at control conditions during the first week of operation. Trend of the concentrations of Cu and Zn in water of all experimental supplementation tanks including control, Cu 50: Zn 200, Cu 100: Zn 400, and Cu 150: Zn 600 mg/kg during the experiment period of lettuce and pak choi-based bioponics was shown in Figure 23 and Figure 24 respectively. Lettuce and pak choi cultivated was found to uptake and accumulate Cu and Zn in biomass, which is proportional to the level of Cu and Zn contaminated in chicken manure and water. Cu and Zn in chicken manure fertilizer can dissolve in bioponics water in complex (binding with another organic substance e.g., organometallic form) and anion forms. Thus, plants can adsorb organometallic and anion (biodegradable by a microbe), and microorganisms (e.g., bioaccumulation) can cause toxicity to plants and microorganisms in bioponics (Briffa et al., 2020). However, Cu and Zn supplementation was carried out in this work using $CuSO_4$•$5H_2O$, and $ZnSO_4$•$7H_2O$, it does not form complexes has a higher capacity to dissolve in bioponics water in ion form than organometallic form (Wuana and Okieimen, 2011).

The dissolution of Cu and Zn concentration from chicken manure into water of lettuce-based bioponics (phase I) at the beginning of the experiment was ranged from

0.003 to 0.762 mg/L for Cu and 0.037 to 1.955 mg/L for Zn, while pak choi based-bioponics (phase II) range from 0.035 to 0.743 mg/L for Cu and 0.177 to 1.355 mg/L for Zn. The highest dissolution was found in the Cu 150: Zn 600 mg/kg supplementation of all phases that was contaminated with the highest Cu and Zn contents in chicken manure. Cu and Zn in chicken manure at control (no Cu and Zn added), Cu 50: Zn 200 mg/kg, Cu 100: Zn 400 mg/kg, and Cu 150: Zn 600 mg/kg supplementation conditions of phases I and II had no effect on plant biomass weight and growth. This indicates that the Cu and Zn concentrations in chicken manure of four Cu and Zn supplementations and plant accumulation in tissues from each Cu and Zn supplementation conditions were not significantly different at $p < 0.05$. However, phases I and II of the experiment Cu and Zn concentrations were highest at the first week of operation (week 0), and then continually decreased in the first week (7 days) to the end of the operation and then remain or stable at low concentration levels because Cu and Zn concentrations in water decreased over the operation period because of the plant uptake and heavy metals were absorb by biofilter/precipitation and re-absorb in chicken manure.

The highest ability of plant uptake and accumulation of Cu and Zn in lettuce-based bioponics was 0.8% and 1.5%, while pak choi-based bioponics was 0.4% and 4.6% respectively was shown in Table 4 and 5. Pak choi have high ability to uptake and accumulation Cu and Zn better than lettuce obviously. Cu and Zn dramatically decreasing and which plants can assimilate/accumulate Cu and Zn in plant tissues, chicken manure and microbial biofilms in biofilter could adsorb heavy metals. Thus, which this reason were significantly reduce the Cu and Zn concentration in the water (Sharifan et al., 2019). In addition, pH value is major factor that affects the capability of heavy metals dissolution into water. Where slightly acidic condition in boponics can increasing the capability of Cu and Zn dissolution into bioponics water. On the other hand, the average overall pH values in this study were ranged 8.11 to 8.24. The pH in bioponics water was alkaline values not good for Cu and Zn and which could reduce the capability of Cu and Zn dissolution into the bioponics water. In alkaline condition can induce Cu and Zn binding with organic compound and precipitation rather than dissolution (Jalali and Najafi, 2018). The result from this study indicates that Cu and Zn content in chicken manure could be bound in an organic and residual fraction of chicken manure and microbial biofilm in biofilter when operated the bioponics with alkaline condition (Wierzbowska et al., 2018). This indicates that when applied chicken manure fertilizer was contaminated Cu and Zn as a nutrient source of plants production in bioponics can not affect the health risk of a consumer of plant from bioponics.

The relationship of solubility of heavy metals of metal hydroxide and metal sulphide at different pH level was shown in Figure 20. Metal hydroxide precipitation is optimal at pH 8.0-9.5 for Cu and pH 9-9.5 for Zn, while metal sulphide precipitation is optimal at pH 8 for Cu and pH 10-10.5 for Zn respectively (Nur, 2019). Heavy metals (Cu and Zn) have a higher potential for solubility at low pH than at high pH. However, this study was conducted and measurable at approximate pH 8-8.5 in bioponics water of phases I and II. This indicates that in this study, pH 8-8.5 may completely induce Cu and Zn precipitation in bioponic system water.

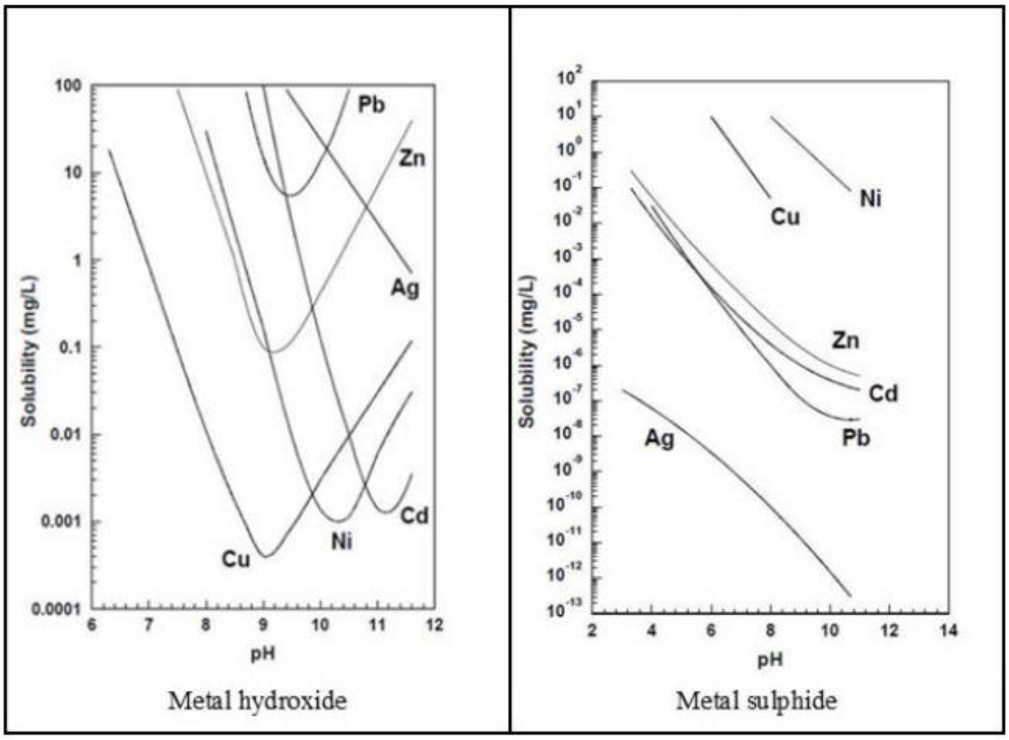


Figure 20 The solubility of heavy metals (Cu, Zn, Cd, Ag, Pb and Ni) at different pH

Source: Nur (2019**)**

The table of %Cu and Zn output distribution at different Cu and Zn supplementation of lettuce and pak choi-based-bioponics was show in Table 8 and Table 9 respectively. The composition of Cu and Zn distribution was the most remaining accumulation in chicken manure and water and the lowest Cu and Zn concentration in plants tissues, but Zn can have a higher potential bioaccumulate in plant tissues than Cu of lettuce and pak choi-based bioponics was show in Figure 21 and Figure 22 respectively. The heavy metal mass balance of chicken manure 200 g dry wt. per system and difference Cu and Zn supplementations of bioponic system. Cu mass balance was shown in Figure 25-32 and Zn mass balance was shown in Figure 33-40 of phases I and II respectively. The result indicates that Cu and Zn concentration did not affect nitrogen concentration for plant uptake, but the major factor was affected to the nitrogen concentration and nitrogen transformation is temperature, pH levels and DO concentration (Grzyb et al., 2021) in the water of bioponic system. Suggesting nitrification process was occurred in bioponic systems. One-way ANCOVA was used to determine the difference of means in a compared group (> 2 groups) for data of parameters with covariance days of operation (e.g., TKN, TAN, NO_3^-, NO_2^-, Cu and Zn), followed by Tukey-Kramer post-test represent statistical differences with comparisons were made separately within each parameter at a significant level of 0.05. Principial component analysis was performed to identify the relationship between Cu and Zn levels in chicken manure at highest concentration in the experiment (Cu = 0–150 mg/kg, and Zn = 0–600 mg/kg) and nitrogen parameter concentration. Indicates that the relationship was positive relation and heavy metals did not cause negative effects on nitrogen concentration for plant growth and the relationship was shown in Figure 19.

Table 8 %Cu and Zn output distribution of lettuce-based bioponic system with 200 g dry weight of chicken manure.

Phase I: Lettuce-based bioponics								
	%Water		**%Lettuce**		**%CM_{after}**		**%Output distribution error**	
Supplementation condition (mg/kg)	Cu	Zn	Cu	Zn	Cu	Zn	Cu error	Zn error
Control	11.3 ± 1.3^{a}	$12.8 \pm 2.4^{a,b}$	15.5 ± 1.5^{a}	27.1 ± 14.7^{a}	73.2 ± 1.8^{b}	60.2 ± 14.0^{a}	51.69 ± 2.1^{b}	30.08 ± 7.1^{b}
Cu 50: Zn 200	55.0 ± 0.4^{a}	21.7 ± 0.1^{b}	7.6 ± 2.7^{a}	24.2 ± 8.0^{a}	$37.4 \pm 8.6^{a,b}$	54.2 ± 36.1^{a}	$26.61 \pm 1.9^{a,b}$	$40.49 \pm 3.3^{a,b}$
Cu 100: Zn 400	58.4 ± 0.4^{a}	6.7 ± 0.5^{b}	5.1 ± 2.9^{a}	29.3 ± 6.4^{a}	36.6 ± 1.5^{a}	64.0 ± 10.9^{a}	$31.28 \pm 7.3^{a,b}$	$59.61 \pm 7.1^{a,b}$
Cu 150: Zn 600	64.8 ± 0.8^{a}	32.9 ± 3.2^{a}	3.9 ± 2.7^{a}	21.4 ± 8.6^{a}	31.3 ± 4.4^{a}	45.7 ± 11.0^{a}	28.54 ± 0.9^{a}	53.29 ± 2.9^{a}

Values reported as mean ± standard deviation ANOVA. The superscripts a and b represent statistical differences by Tukey-Kramer post-hoc test ($p < 0.05$). Comparisons were made within each column.

Table 9 %Cu and Zn output distribution of pak choi-based bioponic system with 200 g dry weight of chicken manure.

Phase II: Pak choi-based bioponics								
	%Water		**%Pak choi**		**$\%CM_{after}$**		**%Output distribution error**	
Supplementation condition (mg/kg)	Cu	Zn	Cu	Zn	Cu	Zn	Cu error	Zn error
Control	61.6 ± 0.9^{a}	54.9 ± 0.3^{a}	5.0 ± 1.3^{a}	11.6 ± 2.4^{a}	33.4 ± 0.3^{a}	33.5 ± 6.9^{a}	3.04 ± 21.7^{b}	15.39 ± 2.4^{b}
Cu 50: Zn 200	67.6 ± 3.0^{a}	47.6 ± 0.1^{a}	3.8 ± 0.7^{a}	13.7 ± 0.1^{a}	28.6 ± 10.5^{a}	38.8 ± 32.5^{a}	$3.13 \pm 11.2^{a,b}$	$41.27 \pm 5.3^{a,b}$
Cu 100: Zn 400	60.7 ± 0.8^{a}	53.6 ± 0.3^{a}	4.1 ± 0.4^{a}	16.1 ± 0.5^{a}	35.2 ± 3.2^{a}	30.3 ± 24.5^{a}	32.73 ± 2.6^{a}	48.15 ± 0.1^{a}
Cu 150: Zn 600	73.6 ± 0.5^{a}	52.0 ± 0.2^{a}	2.5 ± 0.8^{a}	18.3 ± 3.2^{a}	23.8 ± 2.9^{a}	29.7 ± 3.2^{a}	8.15 ± 8.4^{a}	54.81 ± 0.8^{a}

Values reported as mean ± standard deviation ANOVA. The superscripts a and b represent statistical differences by Tukey-Kramer post-hoc test ($p < 0.05$). Comparisons were made within each column.

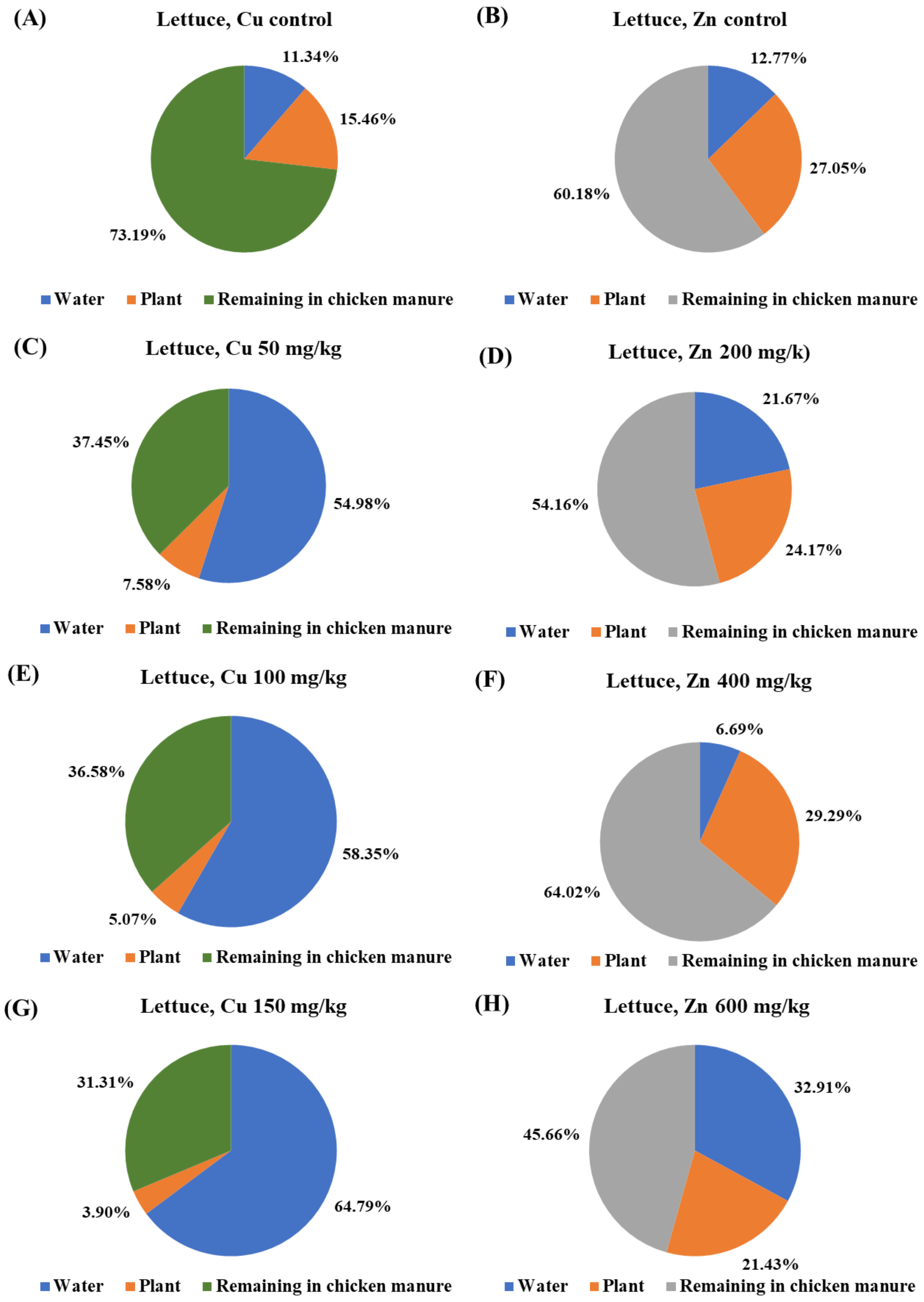


Figure 21 %Cu and Zn output distribution at different Cu (A, C, E and G) and Zn (B, D, F and H) supplementation of lettuce-based bioponic system.

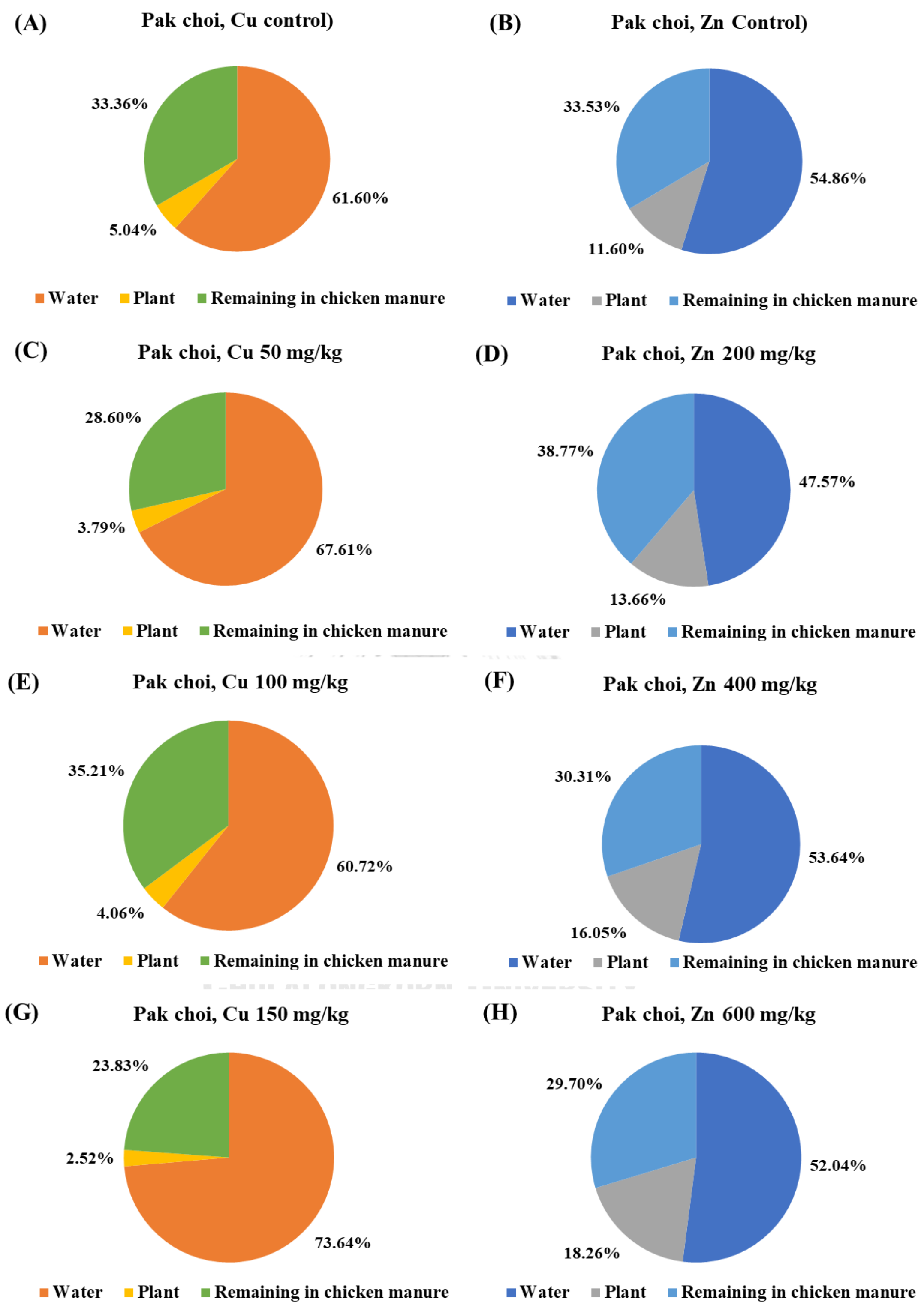


Figure 22 %Cu and Zn output distribution at different Cu (A, C, E and G) and Zn (B, D, F and H) supplementation of pak choi-based bioponic system.

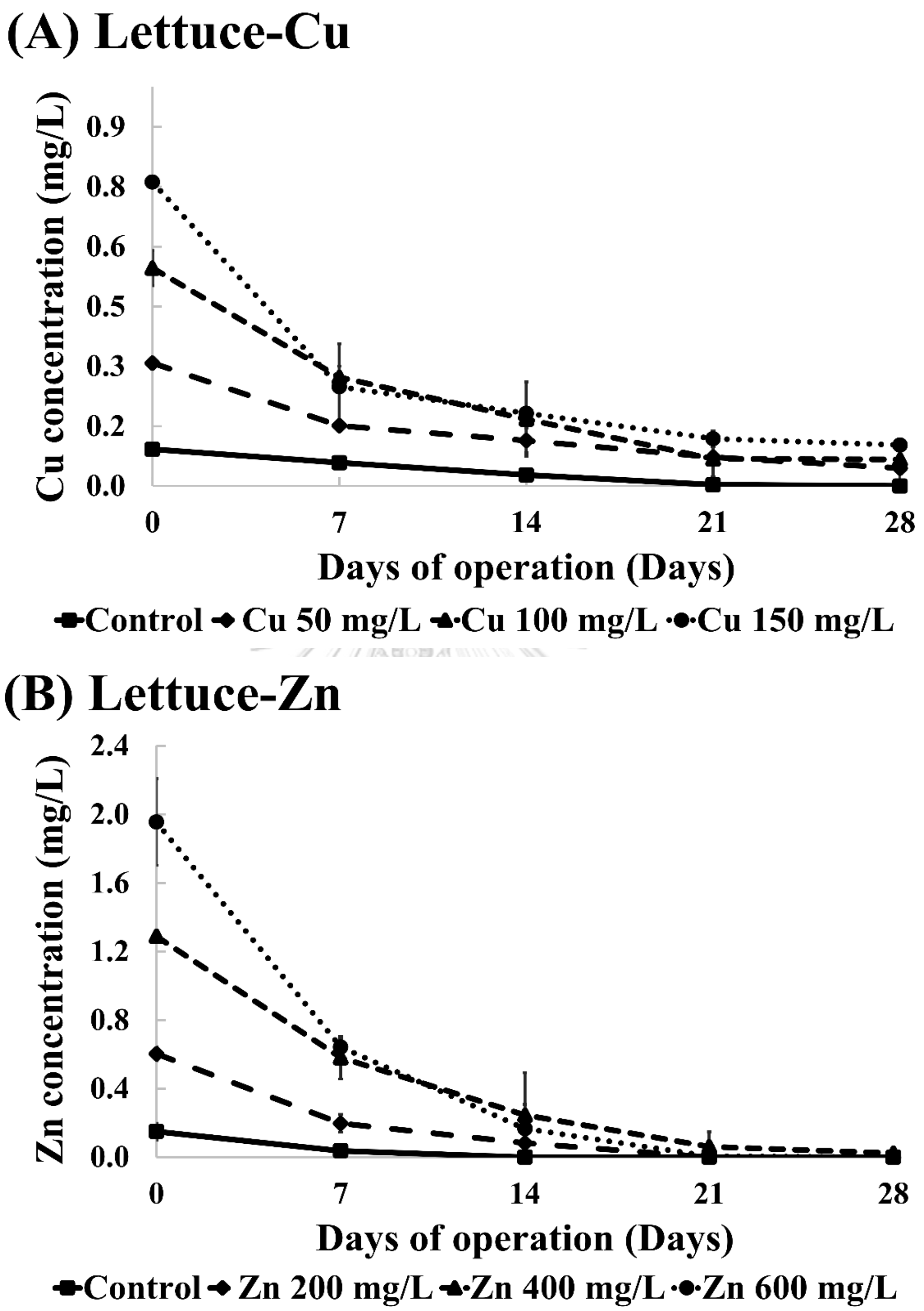
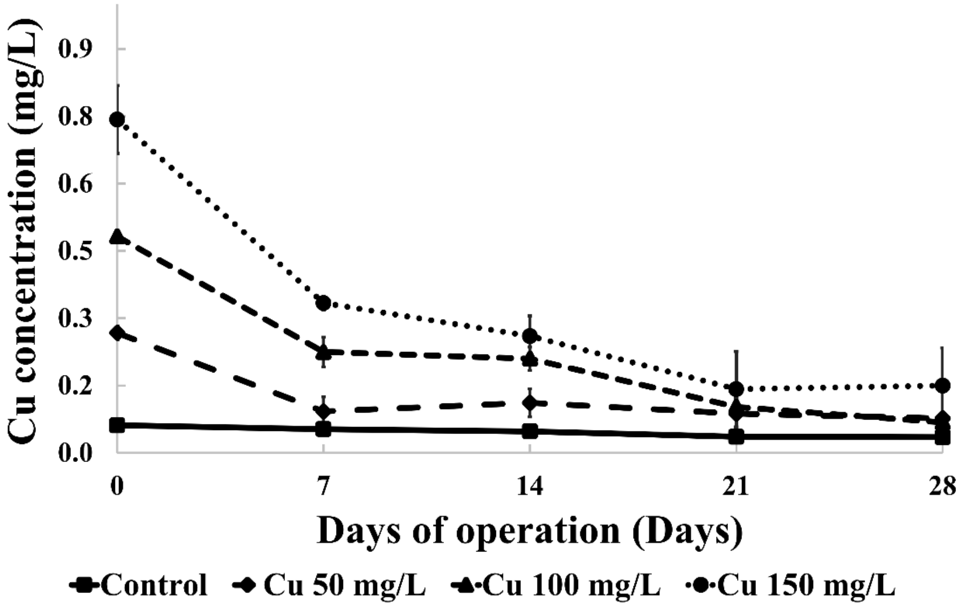


Figure 23 Cu and Zn concentration in bioponic recirculating water (A) represent Cu and (B) represent Zn of lettuce-based bioponics. Error bars represent standard deviation (n = 2).

(A) Pak choi-Cu



(B) Pak choi-Zn

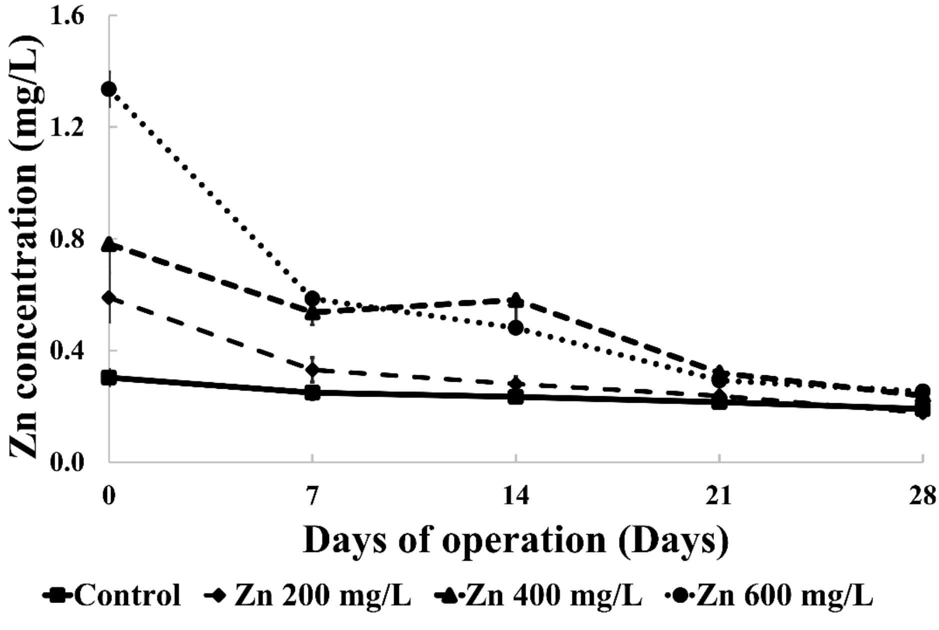


Figure 24 Cu and Zn concentration in bioponic recirculating water (A) represent Cu and (B) represent Zn of pak choi-based bioponics. Error bars represent standard deviation (n = 2).

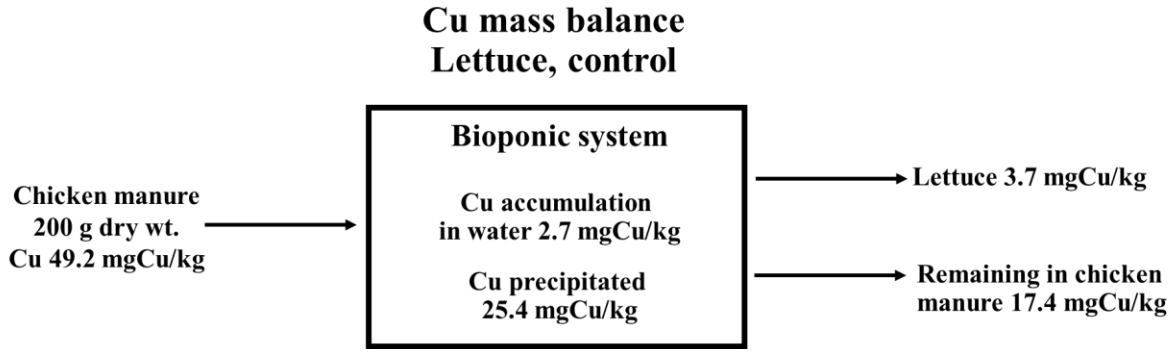


Figure 25 Cu mass balance of lettuce-based bioponics (phase I) at control condition.

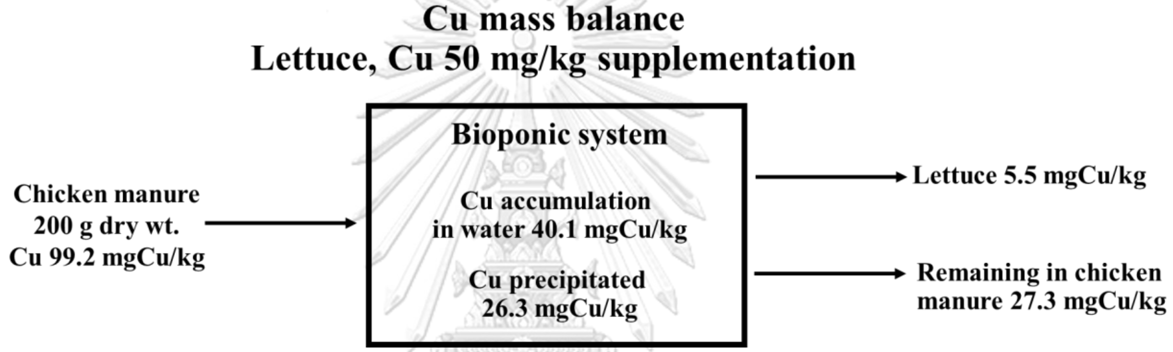


Figure 26 Cu mass balance of lettuce-based bioponics (phase I) at Cu 50 mg/kg supplementation condition.

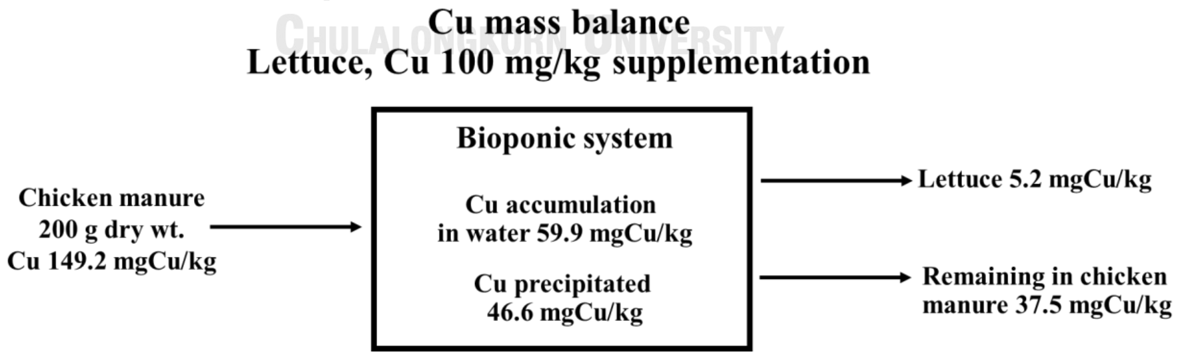


Figure 27 Cu mass balance of lettuce-based bioponics (phase I) at Cu 100 mg/kg supplementation condition.

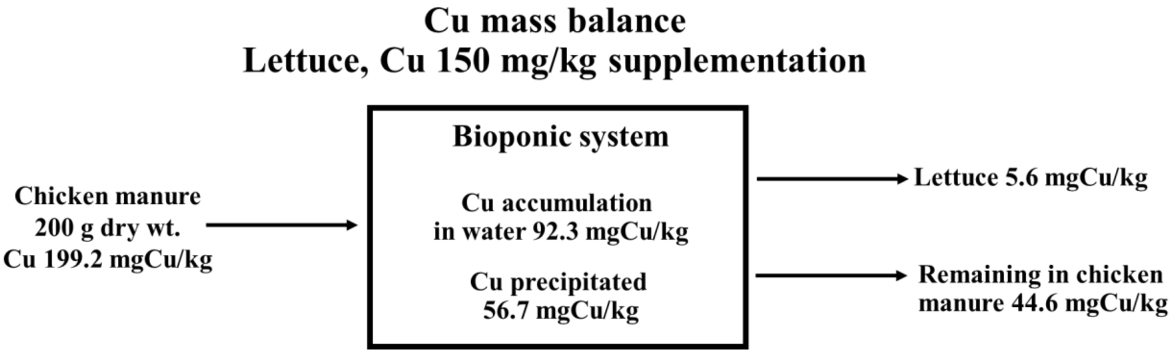


Figure 28 Cu mass balance of lettuce-based bioponics (phase I) at Cu 150 mg/kg supplementation condition.

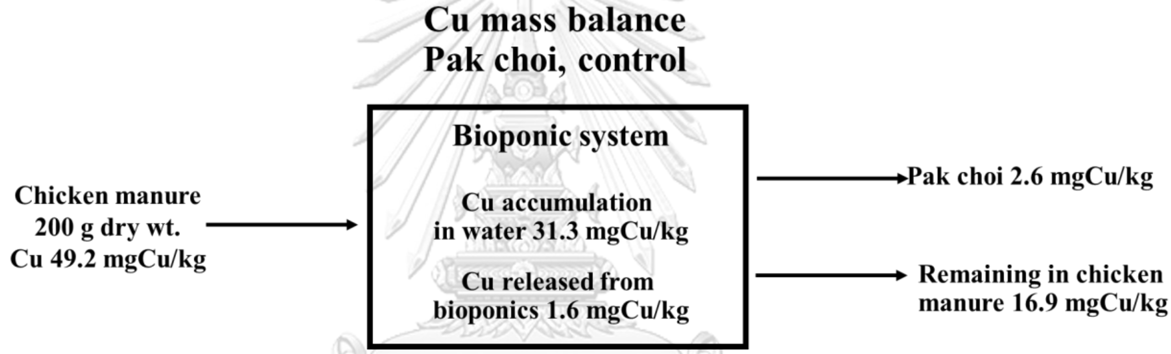


Figure 29 Cu mass balance of pak choi-based bioponics (phase II) at control condition.

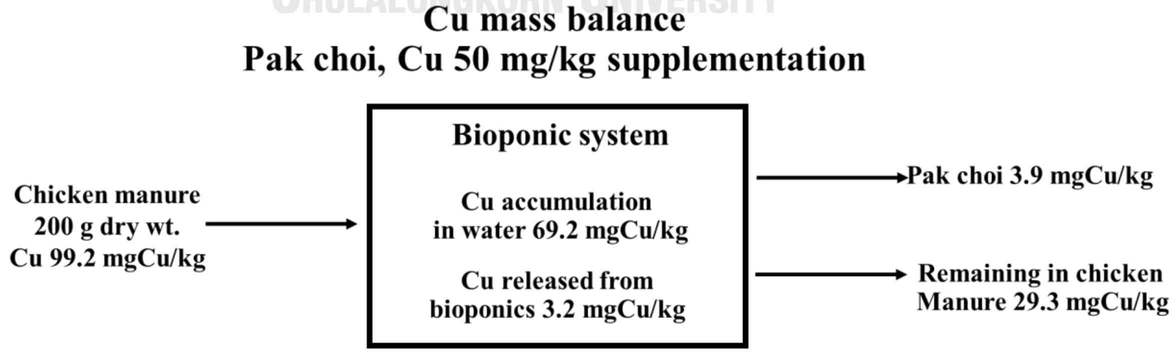


Figure 30 Cu mass balance of pak choi-based bioponics (phase II) at Cu 50 mg/kg supplementation condition.

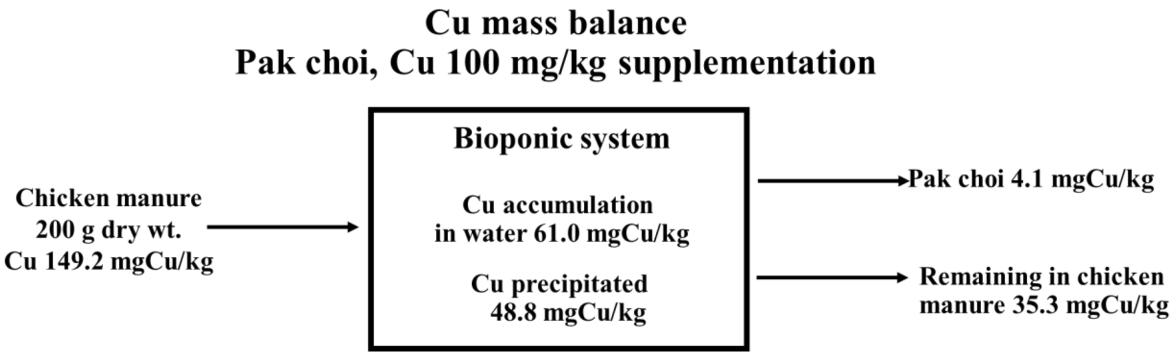


Figure 31 Cu mass balance of pak choi-based bioponics (phase II) at Cu 100 mg/kg supplementation condition.

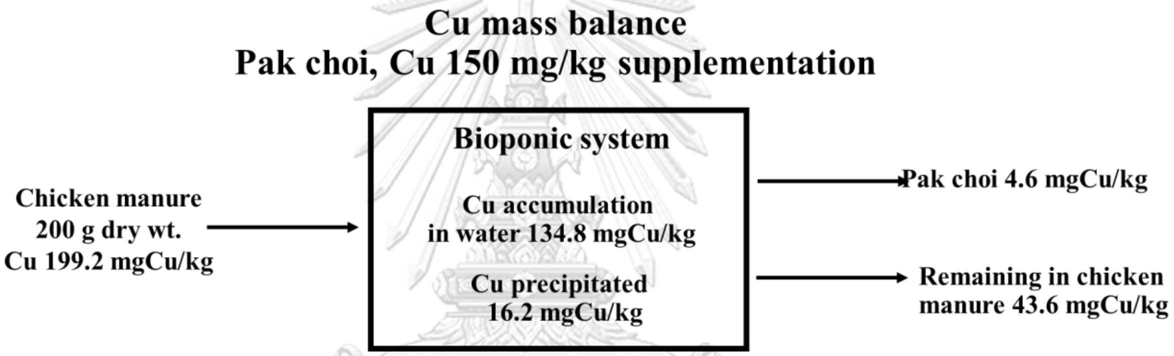


Figure 32 Cu mass balance of pak choi-based bioponics (phase II) at Cu 150 mg/kg supplementation condition.

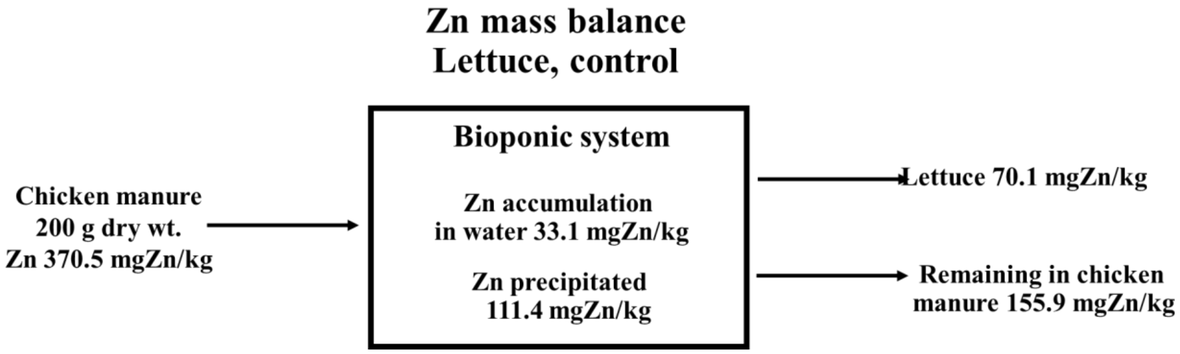


Figure 33 Zn mass balance of lettuce-based bioponics (phase I) at control condition.

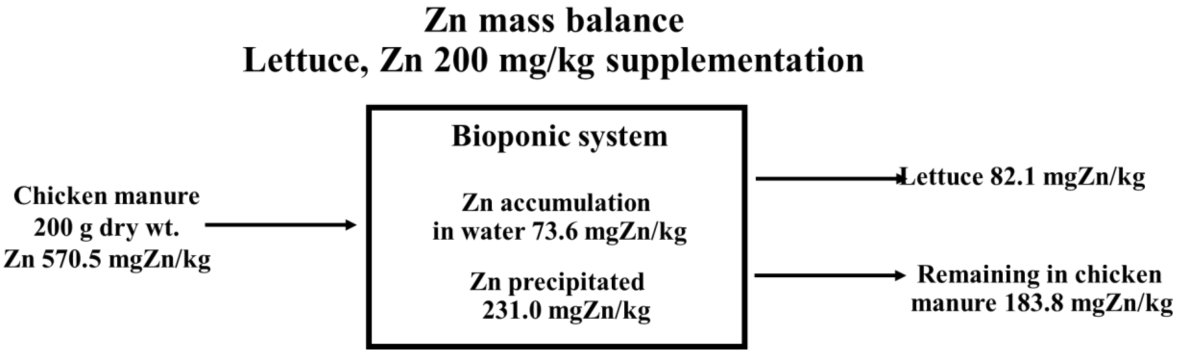


Figure 34 Zn mass balance of lettuce-based bioponics (phase I) at Cu 200 mg/kg supplementation condition.

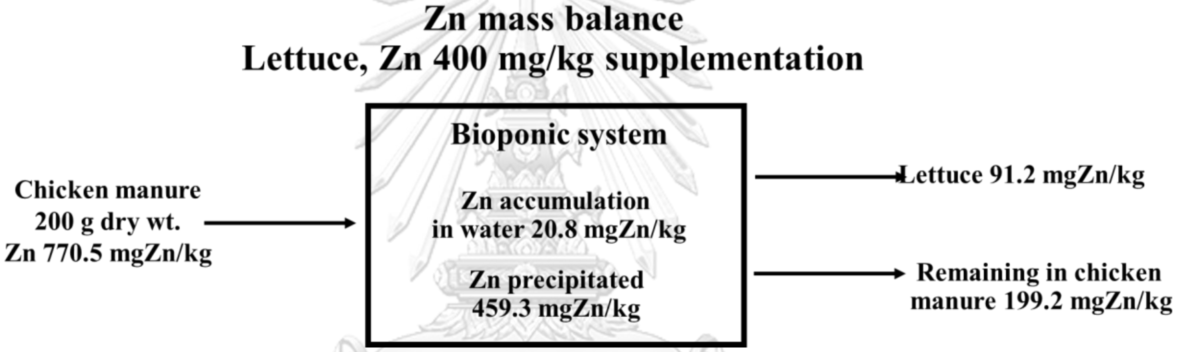


Figure 35 Zn mass balance of lettuce-based bioponics (phase I) at Cu 400 mg/kg supplementation condition.

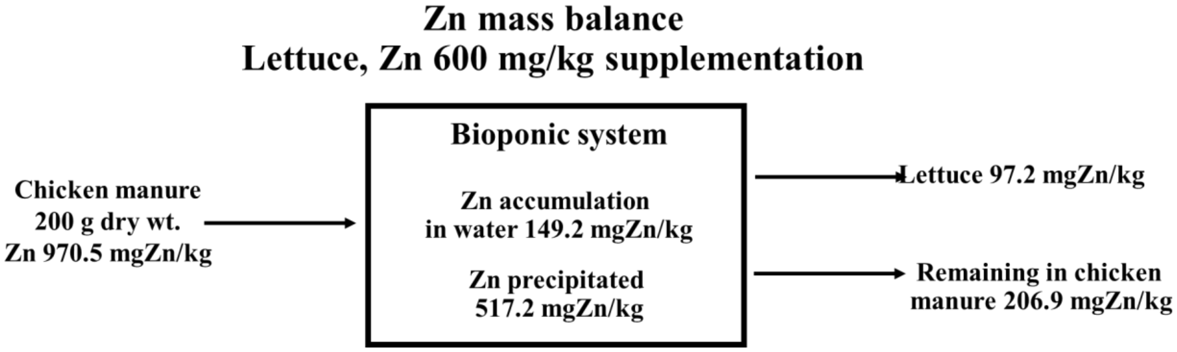


Figure 36 Zn mass balance of lettuce-based bioponics (phase I) at Cu 600 mg/kg supplementation condition.

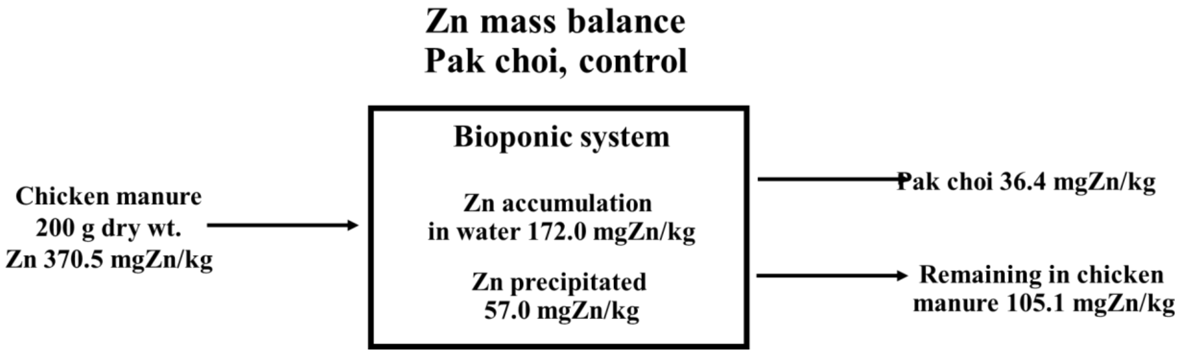


Figure 37 Zn mass balance of pak choi-based bioponics (phase II) at control condition.

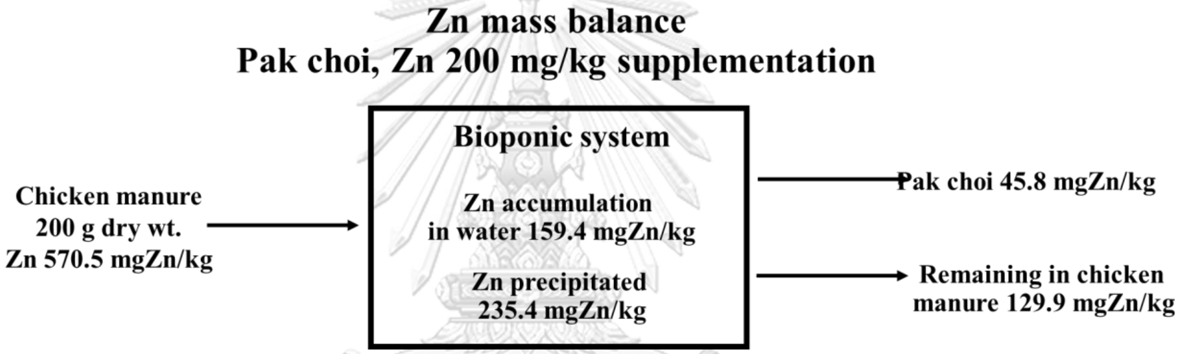


Figure 38 Zn mass balance of pak choi-based bioponics (phase II) at Cu 200 mg/kg supplementation condition.

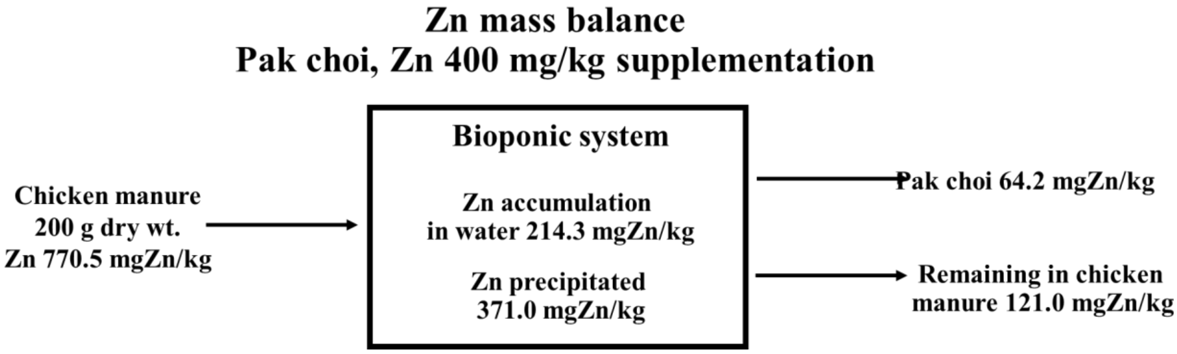


Figure 39 Zn mass balance of pak choi-based bioponics (phase II) at Cu 400 mg/kg supplementation condition.

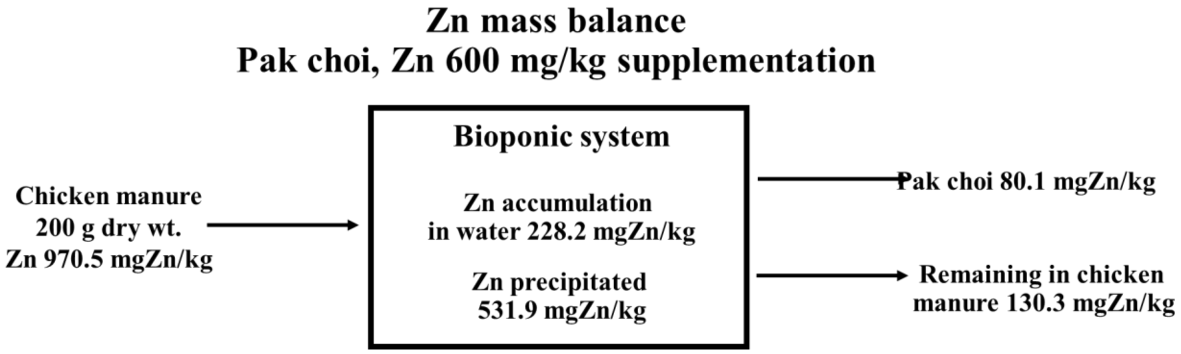


Figure 40 Zn mass balance of pak choi-based bioponics (phase II) at Cu 600 mg/kg supplementation condition.

4.3 Bioconcentration factor, translocation factor, and distribution coefficient of Cu and Zn in bioponics

The bioconcentration factor (BCF) is a measurable indicator of plant contamination that is generally used to evaluate the toxicity and accumulation of heavy metals transferred from water to plant biomass (Yanez et al., 2019). The BCF values of Cu and Zn in lettuce root were 28.86 and 199.94 L/kg, while the BCF values in pak choi root were 24.19 and 386.44 L/kg respectively. BCF in lettuce shoot was 6.69 L/kg for Cu and 42.61 L/kg for Zn, whereas BCF in pak choi shoot was 10.50 L/kg for Cu and 223.04 L/kg for Zn. The BCF values for Cu and Zn in plant roots and shoot are presented in Table 10. The BCF values of lettuce and pak choi in roots are higher than shoot of both heavy metals similar together to the previous study, average BCF values in root of Cu and Zn was 5.11 and 6.28 for respectively, and shoot was 1.82 and 11.03 respectively (Wang et al., 2013a) and (Cai and Song, 2019) also reported the BCF of Zn was 0.297 was higher than Cu was 0.059 in plants. Lettuce may have a low tolerance to heavy metal accumulation in plant tissues, as well as a low capacity and tolerance to grow in stressful environments (e.g., heavy metals) (Rascio and Navari-Izzo, 2011) causing lettuce less ability to uptake heavy metals than pak choi. Pak choi has a high potential for heavy metal uptake and accumulation in tissue. Furthermore, pak choi has the ability and tolerance to grow in extreme environmental conditions (Kamran et al., 2019). Due to pak choi root having a specific diverse microbial community (rhizobacteria) than lettuce, it is one major factor for enhancing the ability for nutrients and heavy metals uptake and bioaccumulation in plant biomass. This indicates that the ability of Cu and Zn of bioaccumulation and transfer from root to shoot of pak choi has greater potential than lettuce obviously. Thus, Cu and Zn were absorbed by lettuce and pak choi and accumulation into shoot with lower Cu and Zn concentrations than roots. Because Zn was used at a higher concentration than Cu in the experiment, lettuce and pak choi roots were directly exposed to bioponic water. This resulted in a higher Zn content in water than Cu, as well as ion exchangeable nutrients and heavy metals in a bioponic system. Which indicate plants have a migration ability to transfer Cu and Zn to shoot because Cu and Zn are classified category to be necessary nutrients required for plant growth also (Kouame Kouame Victor and Yapi Dope Armel Cyrille, 2016).

Translocation factor (TF) is an indicator of a plant's ability to transport heavy metals from roots to accumulate in shoot (Kharazi et al., 2021). The TF values for each Cu and Zn of lettuce and pak choi was shown in Table 10. The highest TF was related to Zn was 0.52 L/kg and Cu 0.42 L/kg in pak choi respectively, while the lowest TF values was 0.12 L/kg were found for Cu in lettuce cultivated. The result TF was obtained higher in pak choi than lettuce because pak choi could grow and tolerance in heavy metal contaminated environments and tend to accumulate high concentration levels of heavy metals in edible parts compared to lettuce (Gupta et al., 2021). The mobility transfer of Cu lower than Zn in pak choi and lettuce, and pak choi grown in different varied four conditions of the experiment have a high ability Zn uptake than Cu and transfer from root to shoot obviously.

The value of the partition coefficients (K_d) is the potential mobility of Cu and Zn dissolution from chicken manure into the water in the bioponics was shown in Table 10. K_d of Cu and Zn was found in lettuce cultivated was 544.39 L/kg and 452.19 L/kg respectively, are higher than Cu and Zn in pak choi cultivated was 443.61 L/kg and 263.12 L/kg respectively. In this study result, the K_d for Zn is more released and dissolution into the water than Cu. Because Zn is sensitive to fluctuations and mobility would increase under high pH conditions more rapidly than Cu (Diatta, 2010).

Table 10 The bioconcentration factor (BCF), translocation factor (TF) of plants and K_d of distribution coefficients of chicken manure.

Sample	BCF in root	BCF in shoot	TF	K_d
Lettuce-Cu	28.86	6.692	0.12	544.39
Lettuce-Zn	199.94	42.61	0.19	452.19
Pak choi-Cu	24.19	10.51	0.43	443.61
Pak choi-Zn	386.44	223.04	0.52	263.12

4.4 Microbial community in plant roots of bioponics and their roles in nitrogen transformations

High-throughput sequencing was used to analyze the density of the bacterial community in plant roots samples under all four supplementations with 200 g dry wt. of chicken manure per system. The relationship of microbial community in plant roots may promote plant growth for example, enhance nutrient uptake, improve drought tolerance, assistance in pathogen defense, and even contribute in environmental remediation for plant growth (Jones et al., 2019). In bioponics plant roots samples, microbial community play an important role in nutrient degradation and bioavailability for plant growth, with a diversity of bacterial communities in each sample consists of the dominant high top 20 phyla with different relative abundance. The bacterial community relative abundance of all roots samples at the phylum level is shown in Figure 41. The highest relative abundances of phylum were found in plant roots samples of the bioponics was *Planctomycetes* ranged from 17.68% to 25.69%. *Planctomycetes* are considered to play a significant role in global environmental cycles, contributing to the carbon, nitrogen, and sulfur cycles. Especially, in anaerobic ammonia oxidation in the process of converting ammonia and nitrite directly into dinitrogen without the usual conversion from nitrite to nitrate and nitrogen gas (Faria et al., 2018). Suggested that operated bioponics with optimal DO concentration in bioponics was recommended to prevent nitrogen loss from the bioponic system. One of the dominant bacterial phyla in root samples of bioponics belongs to *Proteobacteria* and *Firmicutes*, the density was found to ranged from 16.53% to 23.68% and 9.63% to 23.06% respectively. Thus, *Proteobacteria* and *Firmicutes* community in plant root are playing a key role in organic matter decomposition and nutrient transformations especially, nitrogen fixation and transformation by producing many kinds of glycosyl hydrolases, such as cellulases, and amylases in the bioponics, which can be used as a carbon resource by other bacteria (Wei et al., 2017).

The prevalence of the bacterial community following with *Actinobacteria* relative abundance ranged from 7.37% to 14.52%. *Actinobacteria* have the potential to play important ecophysiological roles in plant residue decomposition and possess genes for nitrogen fixation and the production of antibiotics (Bao et al., 2021). However, the dominant relative abundance of the bacterial community has importance for enhanced nitrogen transformation and nutrients available for plant uptake applied with chicken manure fertilizer based-bioponics. Furthermore, *Nitrospira*e is an important and dominant nitrite-oxidizing bacteria in biological nutrient removal systems, especially when dissolved oxygen and substrate levels are low. Moreover, a previous study discovers *Nitrospira*e can performing complete ammonia oxidation (comammox) about the actual role of *Nitrospirae* in both nitrification steps (Mehrani et al., 2020). Although its less relative abundance of *Nitrosprirae* in plant root samples of bioponics was found ranged from 0.20% to 0.76%. The relative abundance of *Nitrospira* in root samples of bioponics is not different from previous research study was found 0.2% to 0.9% in bioponics (Wongkiew et al., 2021).

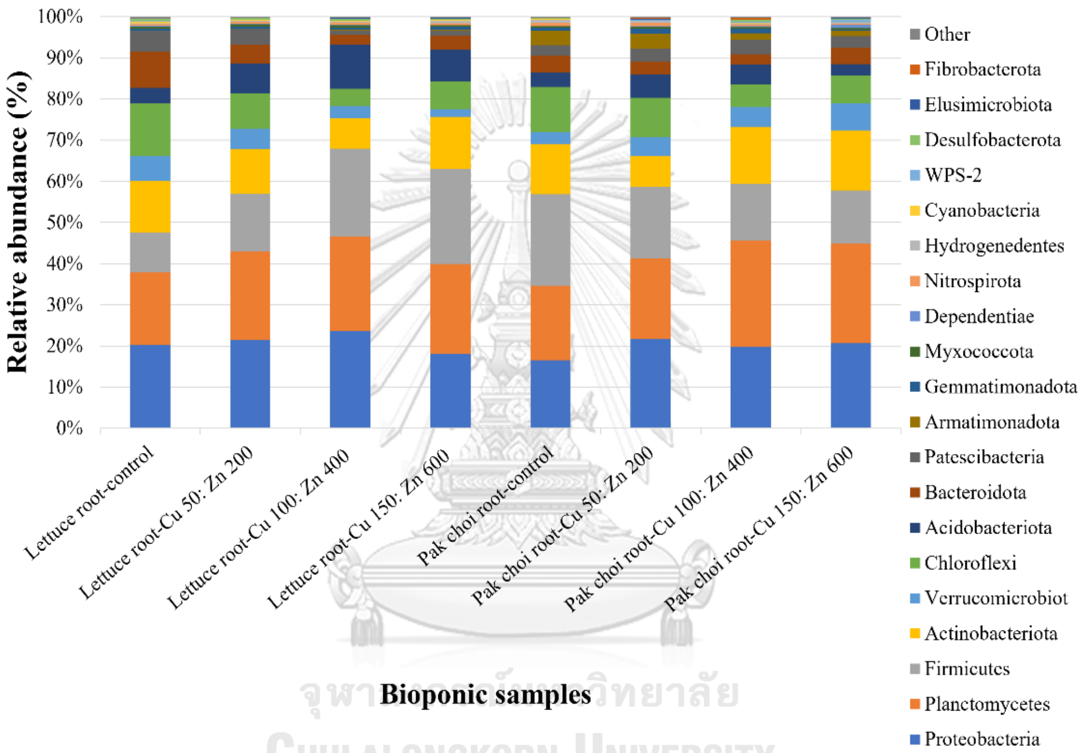


Figure 41 Relative abundance of bacteria community at the phyla in plants root at phylum level.

In this study, the bacterial community composition in plant root of bioponics was found several phyla have play a key role and relevant to nitrogen decomposition and transformation such as *Planctomycetes*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Nitrospirae*, and *Actinobacteria* (Chen et al., 2019). Long-term bioponics operation might result in a shift in microbial community structure depending on the major conditions that might be associated with pH and DO concentration. The pH has a major impact on the relative abundance, prevalence, and diversity of bacterial community structure in plant roots. Furthermore, pH is important in controlling the rates of microbial decomposition of organic matter. The pH of bioponics water was found between 7 to 9, causing individual phyla such as *Actinobacteria, Proteobacteria,* and *Planctomycetes* to be dominant in high relative abundance due to prevalence more than others because there commonly were found in alkaline conditions (Bartram et al., 2014; Yun et al., 2016). In bioponics, the DO concentration is the one factor that effected and influences prevalence, diversity, and bacterial community composition. DO concentrations of this study in bioponics water was found ranged from 4 to 5 mg/L, according to the previous study indicated that DO concentration was found range 4.5 to 5.5 mg/L is the highest rate for nitrogen transformation (Wang et al., 2018). It was suggested that in a bioponic system, the nitrification bacteria group needed an optimal DO concentration of 6-7 mg/L to improve nutrient conversion and performance of nitrogen transformation.

4.5 Microbial biomarkers in bioponics exposed to Cu and Zn

The major dominant top 30 at genera level of plant root in lettuce and pak choi-based bioponics cultivated included *Luteolibacter* (rhizobacteria; 0.24%–2.78%), *WD2101_soil_group* (phosphorus solubilizer; 0.34%–10.98%), *Pseudoxanthomonas* (denitrifier; 0.04%–13.01%) (Wongkiew et al., 2021), and *Blastopirellula* (strictly aerobe responsible in COD and ammonium removal; 0.09%–9.33%) (Chen et al., 2019), whereas major dominant uncultured genera were in the families of *Pirellulaceae* (ammonia oxidizer; 1.89% to 28.39%) (Yamashita et al., 2019), *Caldilineacea* (filamentous bacteria; 1.11%–13.51%), *Blastocatellaceae* (chemoorganotroph; 0.17%–9.39%) (Huber et al., 2017), and *Gemmataceae* (strictly aerobe and chemoorganotroph found in freshwater; 1.25%–10.33%), respectively (Dedysh et al., 2020) was shown in Figure 42. A heatmap of microbial community structure at genus level reveals significant genera (effect size > 0.6, $p < 0.05$) in roots under the Cu and Zn exposure. The heatmap shows that microbial profiles in plant roots with Cu and Zn supplementation were different from those without Cu and Zn supplementation, as shown by the cluster dendrogram was shown in Figure 43.

The dominant bacterial communities in plant roots at the family and genus levels indicate that these bacterial community groups are tolerant to Cu and Zn exposure (Miao et al., 2019). Moreover, these groups play important roles associated with organic nitrogen degradation and nitrogen transformation (e.g., ammonification, nitrification, and denitrification) in bioponics, which located around the surface of plant roots and support nutrients uptake by plants (Patel et al., 2021). Overall, key microbial community functioned well under the Cu and Zn supplementations although there some shifts in microbial community structure. A Long-term bioponics operation may result in a shift in microbial community structure depending on operating parameters such as pH and DO concentration. The extended error bar plot was further used to identify a bacterial community in phylum level that was significantly different in abundances in plants root of the experiment with effect size > 2.0, $p < 0.05$. When lettuce control and lettuce-exposed were compared, it was found that *Chloroflexi* in control had a significantly higher abundance proportion than exposed. While pak choi control and pak choi exposed was shown to have a higher abundance proportion of *Firmicutes* in control than exposed because during the period of the experiment is shown in Figure 44. At the phylum level, the relative abundances of *Chloroflexi* (nutrient-limited biomarker) in lettuce roots and *Firmicutes* (nutrient-rich biomarker) in pak choi roots at control condition were higher than those from Cu and Zn supplemented conditions. Indicating that *Chloroflex* and *Firmicutes*, these two phyla of bacterial community are represented bioindicators in the experiment applied with chicken manure of bioponic system.

Welch's t-test in STAMP was further used to identify significant microbial biomarkers (effect size > 2.0, p < 0.05) in bioponics under Cu and Zn exposure. Although Cu and Zn can provide beneficial effects on plant and microbial growth, high concentrations of Cu and Zn could pose either inhibitory or stimulating effects, resulting decrease/increase in relative abundance of some phylum (Eo and Park, 2016) of lettuce and pak choi was shown in Figure 44A and 44B respectively. The results suggested that the biomarkers from those phyla could well reflect the growth and NUE between the two plants, where pak choi had higher NUE than lettuce (Figure 6). Similarly, previous study was reported that several bacterial phylum dominant in soil such as *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, *Firmicutes*, *Verrucomicrobia*, and *Planctomycetes*, were dominant under high exposure to heavy metals (Song et al., 2018).

Cu and Zn supplementations of each conditions have an effect shifted the bacterial community structure in plant roots, and the significant biomarkers from those genera were dependent on plant types. At Cu and Zn supplementation in lettuce based bioponics, *Lachnospiraceae* (uncultured genus; fermentative chemoorganotrophs) (Stackebrandt, 2014), *WD2101_soil_group*, and *Ruminiclostridium* (anaerobes found in decayed plants) (Wu et al., 2021) were significantly higher than that at control was shown in Figure 45. This suggested that lettuce roots could be weaken under Cu and Zn exposure, leading to partial root decay, anaerobic environment, and decrease in plant growth and NUE. In contrast, in pak choi-based bioponics, *Mesorhizhobium* (plant growth promoter) (Verma et al., 2013) was significantly dominant at Cu and Zn supplemented condition, and *Lachnospiraceae* (uncultured genus), and *Ruminiclostridium* were dominant at control condition was shown in Figure 46. The relationship of these key important biomarkers (depending on plant type) can promote plant growth through a variety of mechanisms, for example, symbiotic mutualists can promote nutrient availability for plant uptake and can enhance Cu and Zn uptake to plant tissues (Liu, 2020). The results suggested that Cu and Zn could promote the growth of beneficial bacteria in pak choi roots; however, Cu and Zn bioaccumulation in plant roots and shoot could cause an inhibitory effect on growth. Although these genera were tolerable to Cu and Zn exposure and play key roles associated with nitrogen transformation and plant growth promotion in bioponics (Wongkiew et al., 2021), key consideration should focus to the inhibitory levels of Cu and Zn and the adverse level on plant growth. Furthermore, non-essential element heavy metals besides Cu and Zn should be evaluated for their harmful effects on bacterial community in bioponic systems.

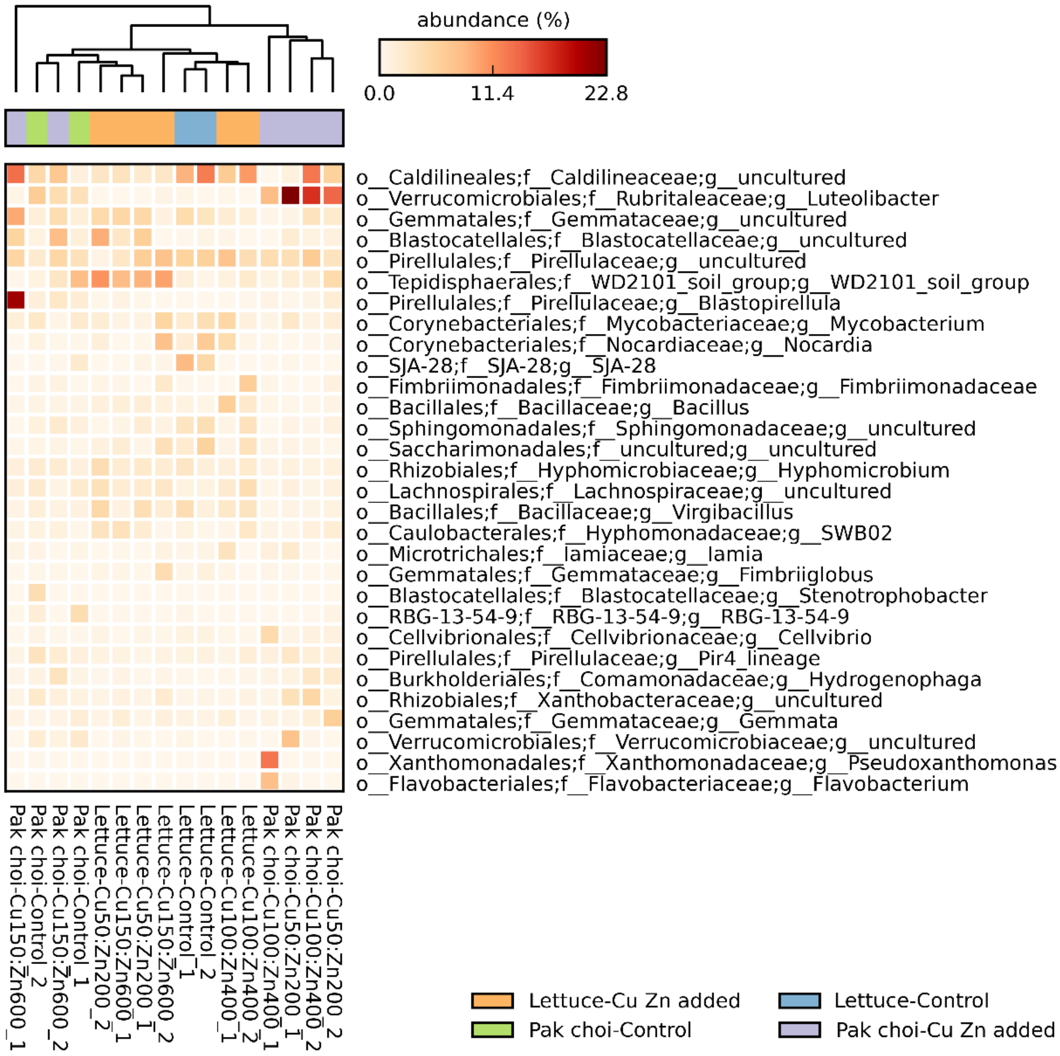


Figure 42 Relative abundance of bacteria at the genus levels in plant roots. Top 30 abundant bacterial genera were shown in this heatmap.

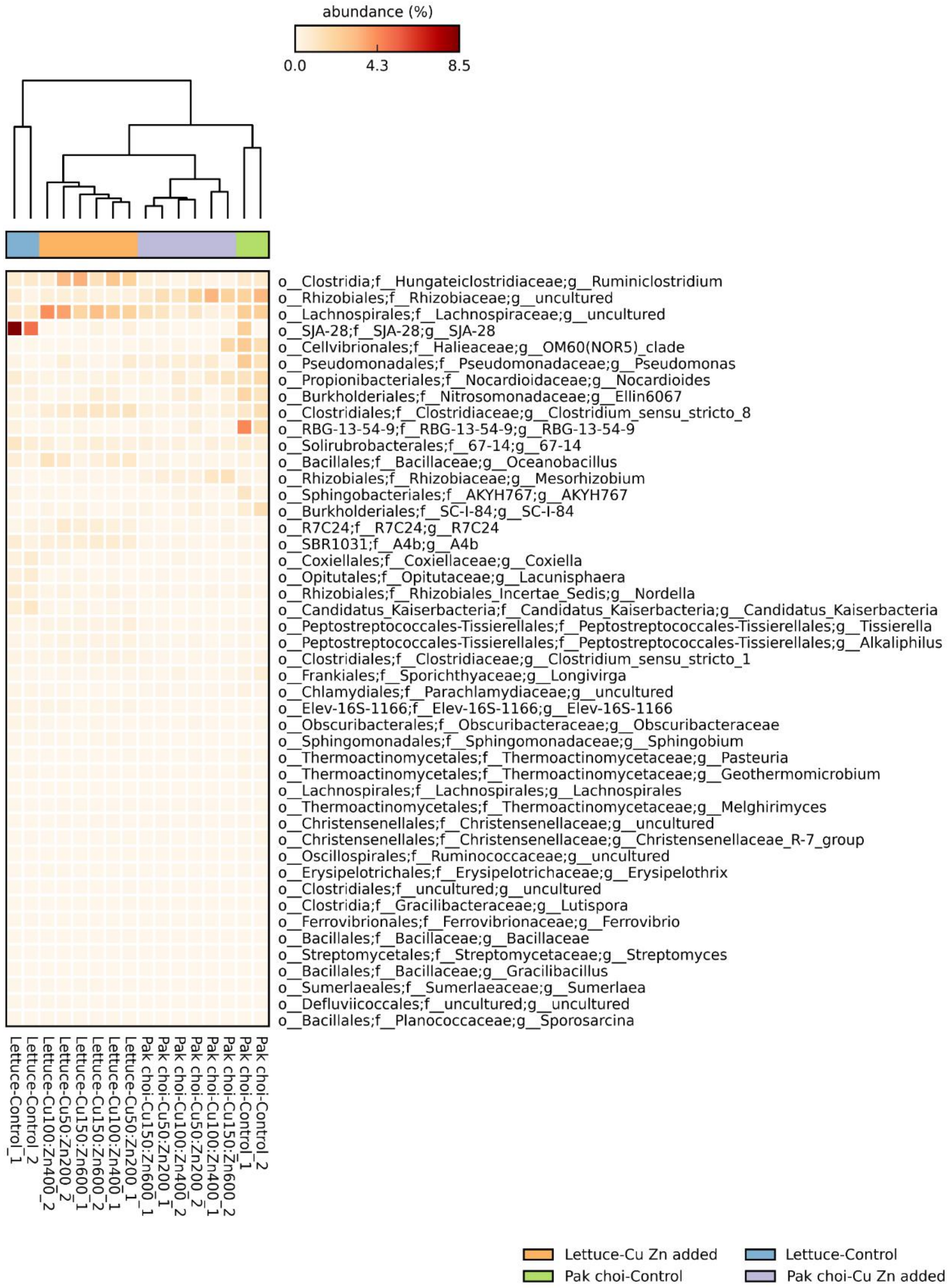


Figure 43 Relative abundance of bacteria at genus levels in plant roots. Bacterial genera with effect size > 0.6, $p < 0.05$ were shown in this heatmap.

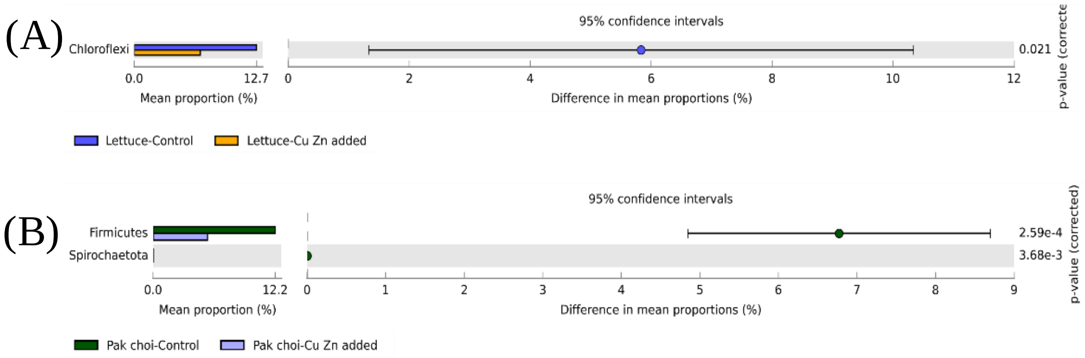


Figure 44 Differential abundances of microbial communities at the phylum levels in lettuce roots (A) and pak choi roots (B) from bioponics with and without Cu and Zn supplementation based on Welch's t-test (effect size > 2.0, p < 0.05).

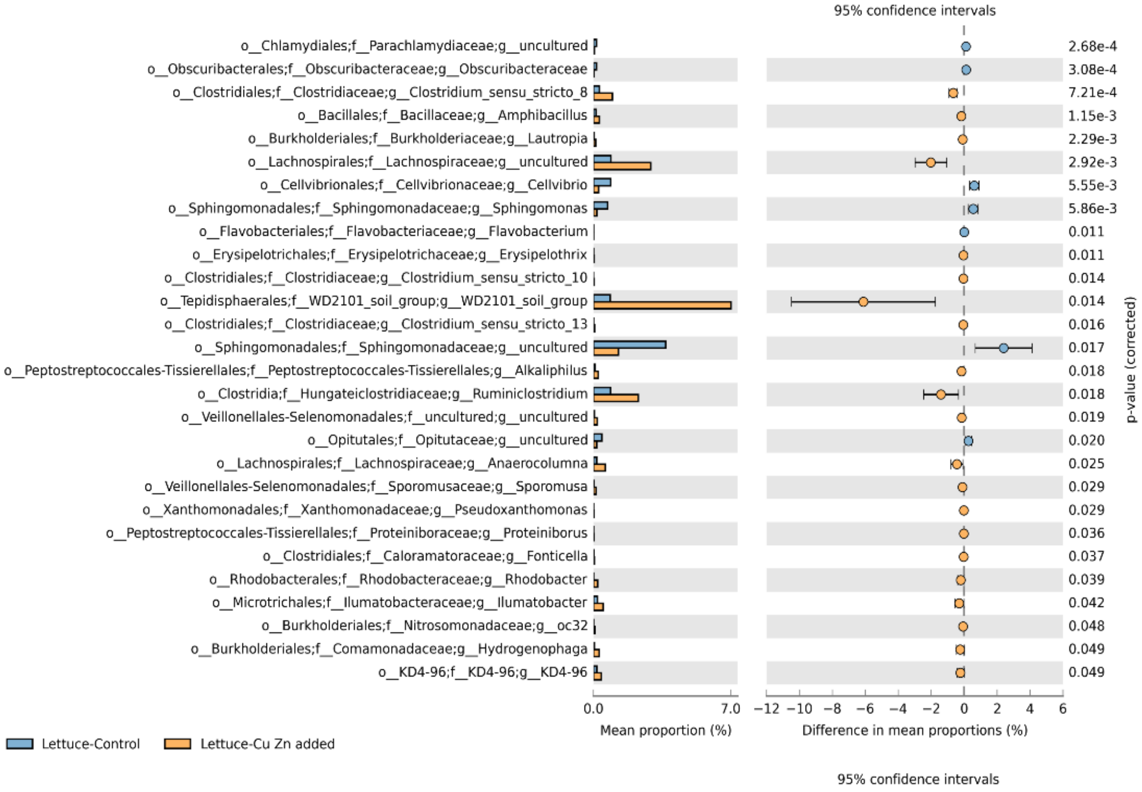


Figure 45 Differential abundances of microbial communities at the genus levels in lettuce roots from bioponics with and without Cu and Zn supplementation based on Welch's t-test (effect size > 2.0, p < 0.05).

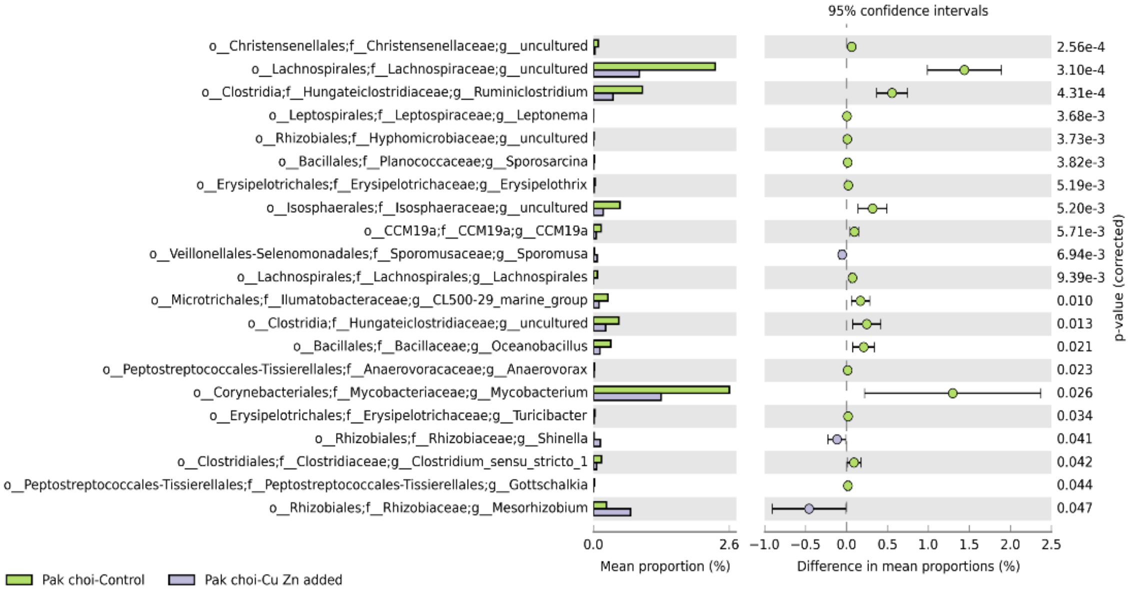


Figure 46 Differential abundances of microbial communities at the genus levels in pak choi roots and from bioponics with and without Cu and Zn supplementation based on Welch's t-test (effect size > 2.0, $p < 0.05$).

4.6 Noncarcinogenic risk assessment of vegetable consumption

The noncarcinogenic risk assessment results show the HQ for individual Cu or Zn and HI for summation of Cu and Zn, which indicates the possible risk of single and total heavy metal exposure through vegetable consumption, respectively. To calculate ADD_{Ing} through ingestion route was shown in Table 11 and conversion factor was used to conversion plants weight from dry weight to wet weight. ADD_{Ing} was used to represent Cu and Zn uptake rates and health risks from consuming lettuce and pak choi from the bioponics in this study was shown in Table 4 and 5 respectively. The ADD_{Ing} values through consumption of lettuce in adults and children were 0.001–0.002 for Cu and 0.019–0.036 for Zn, and those of pak choi were 0.001–0.004 for Cu and 0.029–0.110 for Zn. This study found that the ADD_{Ing} of exposures in children and adults to Cu and Zn were in range of other studies. ADD_{Ing} in this study were comparable with intake dose of vegetables from irrigated wastewater, in which the ADD_{Ing} of Cu and Zn in were 0.005–0.076 and 0.006–0.009, respectively (Ali et al., 2021). Human has a mechanism for detoxification of toxic elements such as heavy metals and toxic organic compounds (Si and Lang, 2018). The human body has a metallothionein gene that can be bound with heavy metals (e.g., Cu, Zn, and Pb) for decreasing the toxicity of heavy metals into the human body through vegetable consumption.

In this study, children had higher HQs of Cu and Zn than adults from consuming vegetables produced by lettuce and pak choi-based bioponics. The worse condition was found at the Cu 150: Zn 600 mg/kg condition for both plants was shown in Table 12. The highest HI values in children and adults were 0.195 and 0.142 in lettuce-based bioponics, while those in children and adults were 0.453 and 0.330 in pak choi-based bioponics. Although HQ and HI were affected by age, consumption rate, exposure time and frequency, and body weight (Ametepey et al., 2018), the results show all HI values (including upper boundary at 95% confident interval) were below risk levels ($HI < 1$). The results indicate the levels of Cu (150 mg/kg) and Zn (600 mg/kg) supplementation in chicken manure based-bioponics will not cause a long-term non-carcinogenic risk in children and adult over their lifetime from consuming bioponics grown lettuce and pak choi at the contamination levels. This was similar to previous studies health risk assessment of heavy metals (i.e., Cu and Zn) contaminated in soil through consumption of vegetables, where no adverse health effects from the consumption of vegetables in children and adults were noted (Cherfi et al., 2015). However, another study reported that chicken manure located nearby highly heavy metal contaminated areas (e.g., industrial zones and mining area) could be contaminated with other toxic elements (Pb, As, Mn, Hg, Cr, Cd) from soils and feeds and increase in health risk of vegetable consumption, which should be also considered in bioponics (Cerne et al., 2021). Further study should be evaluated non-carcinogenic risk and carcinogenic risk of other heavy metals such as Pb, Hg, Cr, and Cd in vegetables that could be contaminated in other organic waste substrates before certainly using in bioponic systems. Since bioponics can be applied with reclaimed wastewater and irrigation water, health risk assessment of all possible heavy metals as well as xenobiotics in vegetables must be considered for both carcinogenic and noncarcinogenic health risk assessment (Intriago et al., 2018).

Table 11 Average daily intake (ADD_{Ing}) of children and adults at different Cu and Zn concentrations in lettuce and pak choi-based bioponics.

Supplementation condition (mg/kg)	**Phase I: Lettuce-based bioponics**			
	ADD_{Ing}			
	Children		**Adult**	
	Cu	Zn	Cu	Zn
Control	0.002* (0.001)	0.027 (0.007)	0.001 (0.001)	0.020 (0.005)
Cu 50: Zn 200	0.002 (0.001)	0.026 (0.008)	0.002 (0.001)	0.019 (0.006)
Cu 100: Zn 400	0.002 (0.001)	0.030 (0.001)	0.002 (0.001)	0.021 (0.001)
Cu 150: Zn 600	0.003 (0.001)	0.036 (0.0001)	0.002 (0.001)	0.027 (0.001)
Supplementation condition (mg/kg)	**Phase II: Pak choi-based bioponics**			
Control	0.001 (0.001)	0.039 (0.010)	0.001 (0.001)	0.029 (0.007)
Cu 50: Zn 200	0.002 (0.002)	0.063 (0.024)	0.002 (0.001)	0.046 (0.018)
Cu 100: Zn 400	0.004 (0.004)	0.103 (0.042)	0.003 (0.003)	0.075 (0.031)
Cu 150: Zn 600	0.004 (0.003)	0.110 (0.043)	0.003 (0.002)	0.080 (0.031)

Symbol * represents mean, and values in parentheses represent the confidence interval level at 95% (CI 95%)

Table 12 Hazard quotient (HQ_{Ing}) and hazard index (HI) of children and adults at different Cu and Zn concentrations in lettuce and pak choi-based bioponics.

Supplementation condition (mg/kg)	**HQ_{Ing} Children** Cu	**HQ_{Ing} Children** Zn	**HQ_{Ing} Adult** Cu	**HQ_{Ing} Adult** Zn	**HI Children**	**HI Adults**
	Phase I: Lettuce-based bioponics					
Control	0.057 (0.001)	0.090 (0.025)	0.041 (0.001)	0.066 (0.018)	0.147 (0.024)	0.107 (0.018)
Cu 50: Zn 200	0.061 (0.003)	0.088 (0.026)	0.044 (0.002)	0.064 (0.019)	0.149 (0.028)	0.120 (0.021)
Cu 100: Zn 400	0.058 (0.034)	0.101 (0.005)	0.043 (0.025)	0.73 (0.004)	0.159 (0.039)	0.116 (0.028)
Cu 150: Zn 600	0.073 (0.014)	0.121 (0.005)	0.053 (0.010)	0.88 (0.004)	0.195 (0.010)	0.142 (0.007)
Supplementation condition (mg/kg)	**Phase II: Pak choi-based bioponics**					
Control	0.032 (0.009)	0.132 (0.033)	0.023 (0.006)	0.096 (0.024)	0.164 (0.042)	0.120 (0.030)
Cu 50: Zn 200	0.058 (0.039)	0.209 (0.082)	0.042 (0.028)	0.152 (0.059)	0.267 (0.120)	0.194 (0.088)
Cu 100: Zn 400	0.094 (0.092)	0.344 (0.140)	0.068 (0.067)	0.251 (0.102)	0.438 (0.233)	0.319 (0.169)
Cu 150: Zn 600	0.080 (0.083)	0.366 (0.144)	0.064 (0.061)	0.256 (0.105)	0.453 (0.227)	0.330 (0.166)

Symbol * represents mean, and values in parentheses represent the confidence interval level at 95% (CI 95%)

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study was conducted finding to 1) evaluate nitrogen transformation, nitrogen recovery, and plant growth in chicken manure-based bioponics at Cu (50–150 mg/kg) and Zn (200–600 mg/kg) supplementation. 2) investigate the effects of Cu and Zn supplementations on plant bioaccumulation, root microbial community, and dietary health risk. This study result found in this present study can conclusion important finding as following

1. Nitrogen concentration in bioponic system of phase I, phase II was measured as TKN, TAN, NO_2^- and NO_3^- and all nitrogen parameters two phases of the experiment have sufficient nitrogen for plants grown in bioponic system.

2. The nitrogen recovery from chicken manure 200 g dry wt./system was effective for plant production based bioponics. The yield of lettuce was range from 144.2 ± 19.6 to 194.8 ± 22.7 g wet wt. with %NUE was range from 2.4 ± 3.3 to 13.8 ± 3.5 and pak choi yield ranged from 163.29 ± 20.1 to 253.83 ± 14.4 with %NUE was range from 35.8 ± 29.7 to 71.2 ± 3.1 respectively.

3. In control condition was higher effective of plants yield and NUE than Cu and Zn supplementation conditions respectively. This indicates that Cu and Zn contaminated in chicken manure at low to high concentrations did not affect nitrogen concentration and plant growth in bioponics.

4. The ability of Zn was accumulated higher in edible part of lettuce and pak choi than Cu concentration obviously.

5. The major dominant phyla of bacterial communities in plants root was found with different relative abundance included *Proteobacteria*, *Planctomycetes*, *Firmicutes* and, *Actinobacteria*, indicates that Cu and Zn supplementations slightly affect to these dominant phyla of bacterial communities.

6. The biomarker at the phylum level, the relative abundances of *Chloroflexi* (nutrient-limited biomarker) in lettuce roots and *Firmicutes* (nutrient-rich biomarker) in pak choi roots. At the genus level were dependent on plant types in lettuce based bioponics was *Lachnospiraceae* , *WD2101_soil_group*, and *Ruminiclostridium*, while pak choi based bioponics was *Mesorhizhobium*.

7. The overall ADD_{Ing} values of Zn was higher than Cu in both of children and adults through consumption of lettuce and pak choi-based bioponics, the highest ADD_{Ing} was present in the highest Cu and Zn supplementation condition.

8. HQ and HI values of individual and overall Cu and Zn through lettuce and pak choi consumption based bioponics was lower than acceptable permission limit of 1 (HQ and HI<1) indicates that no adverse health effects of children and adults who consumed lettuce and pak choi-based bioponics.

5.2 Recommendations for future work

In this study, according to the result, following the recommendations to improve more understanding and concerns in the future or further study of plants production based bioponic system.

1. Sample size of all samples (e.g., root and shoot of plants, water) should be increasing accurate of statistical analysis (n = 3) of the sample.
2. The bioponic system should be operating with control of the pH and DO concentration in the optimal range to prevent nutrient loss from the system.
3. Non-essential element heavy metals (e.g., Pb, Cd, Cr) should investigate in plant tissues and health risk assessment using contaminated chicken manure in bioponics system.
4. Heavy metals have the potential to bioaccumulate in the microbial community; therefore, additional research should be conducted to investigate the effect and bioaccumulation of heavy metals on the microbial community (biomarker) relationship with nutrient release concentration in bioponic system.

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APPENDIX

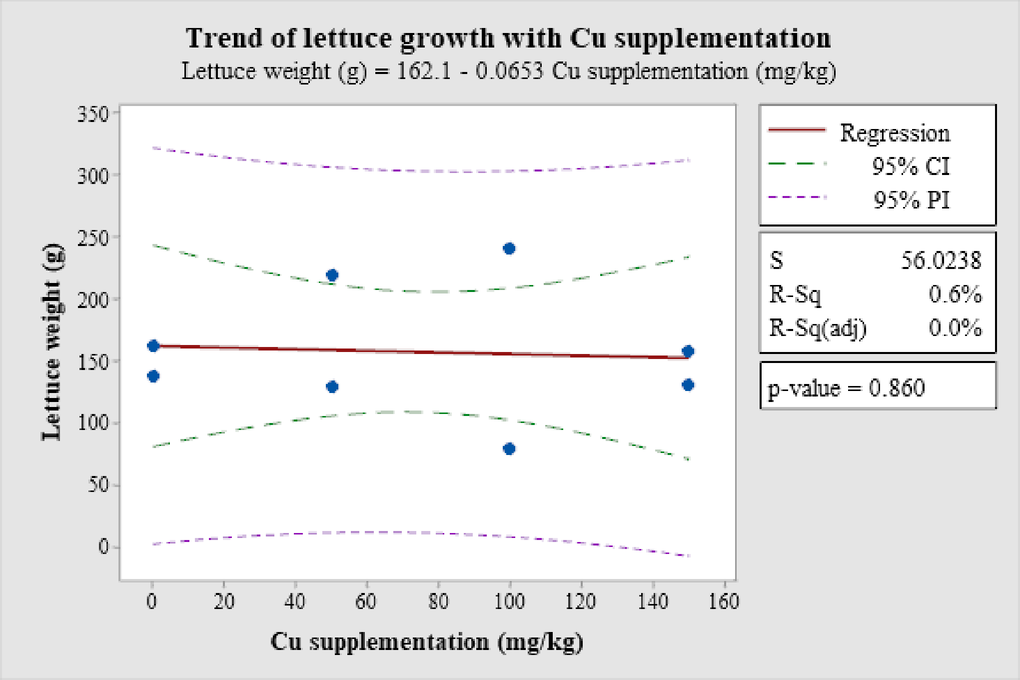

Figure 1 Slope of regression trend of lettuce growth with Cu supplementation (0, 50, 100, 150 mg/kg) of the experiment in phase I.

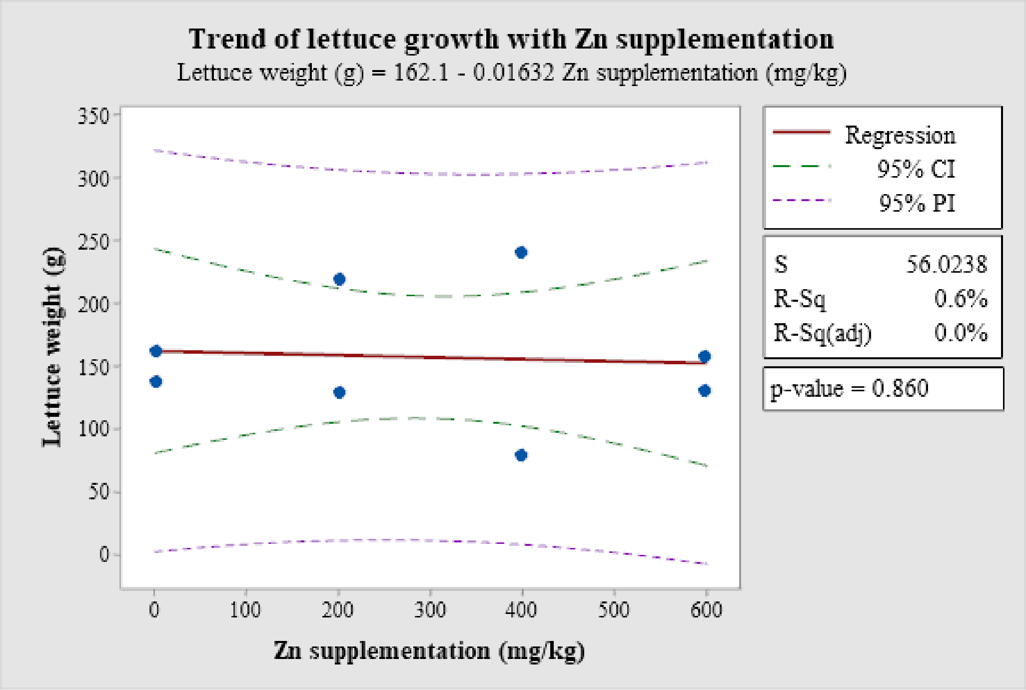

Figure 2 Slope of regression trend of lettuce growth with Zn supplementation (0, 200, 400, 600 mg/kg) of the experiment in phase I.

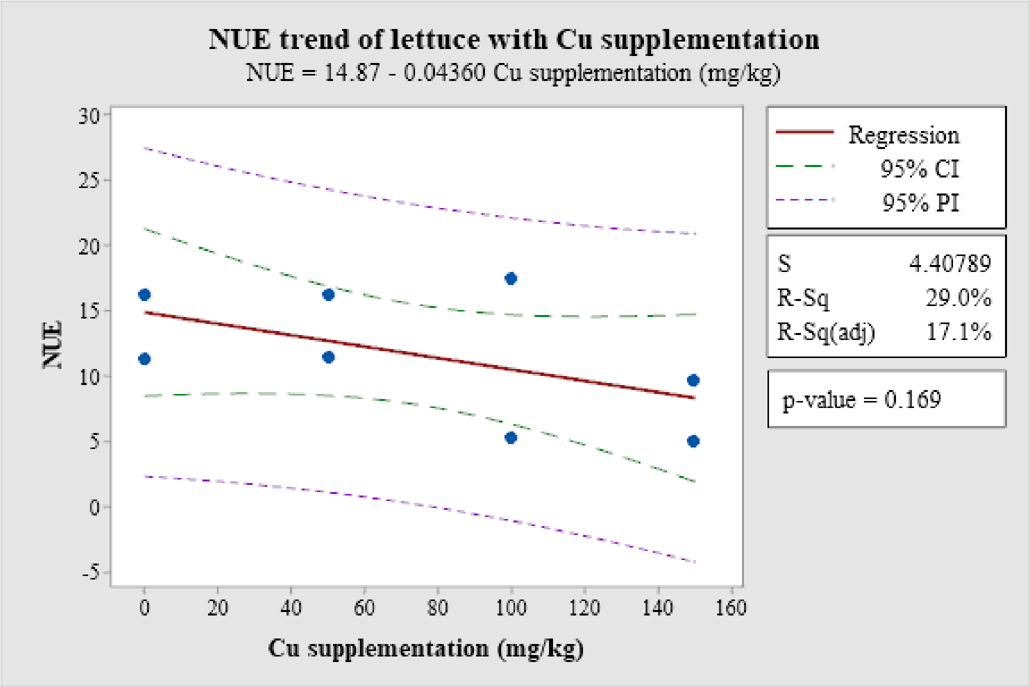

Figure 3 Slope of regression NUE trend of lettuce with Cu supplementation (0, 50, 100, 150 mg/kg) of the experiment in phase I.

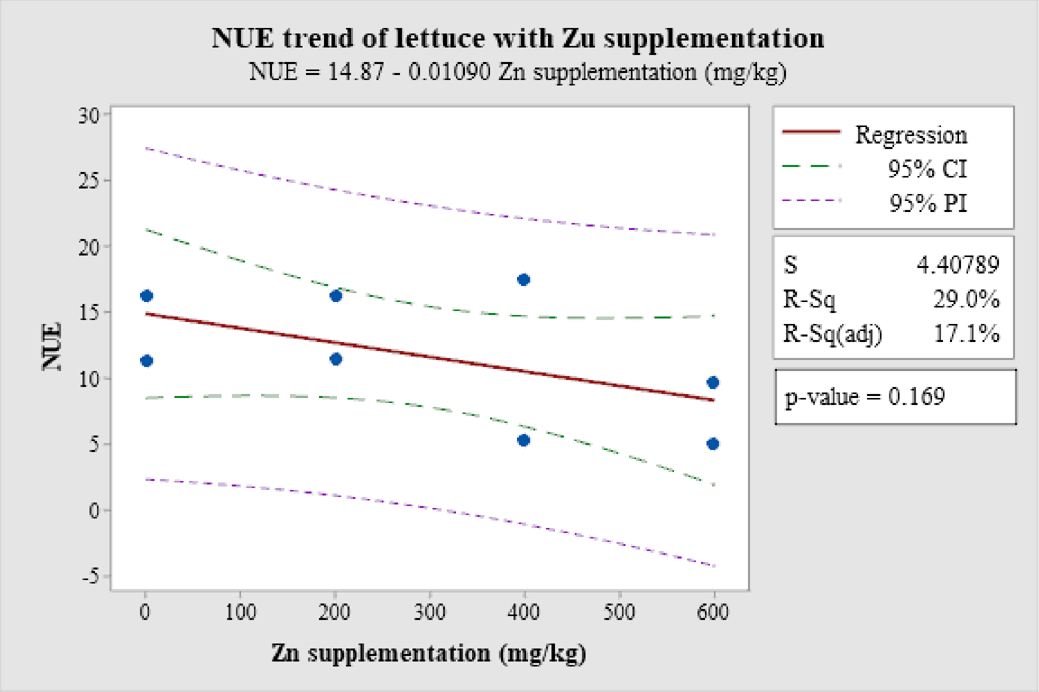

Figure 4 Slope of regression NUE trend of lettuce with Zn supplementation (0, 200, 400, 600 mg/kg) of the experiment in phase I.

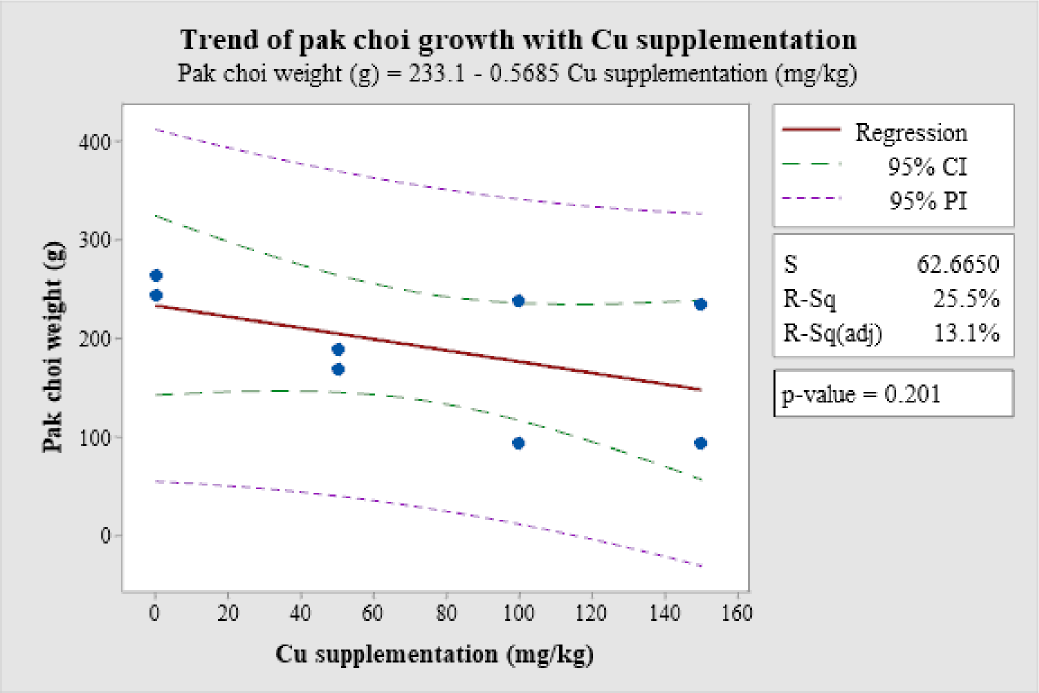

Figure 5 Slope of regression trend of pak choi growth with Cu supplementation (0, 50, 100, 150 mg/kg) of the experiment in phase II.

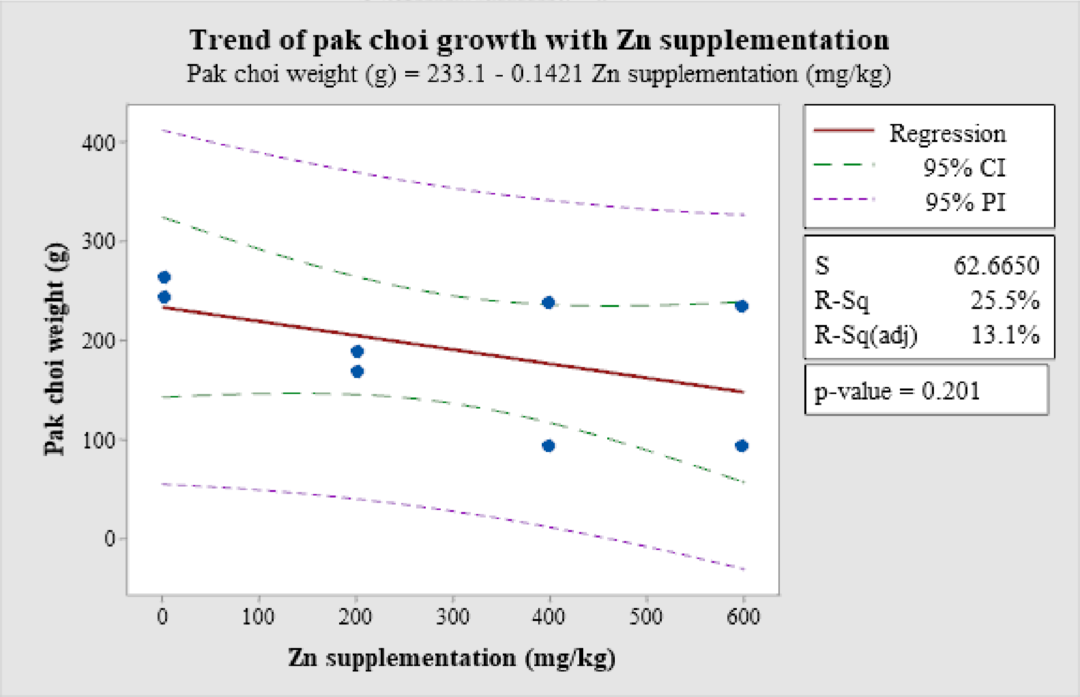

Figure 6 Slope of regression trend of pak choi growth with Zn supplementation (0, 200, 400, 600 mg/kg) of the experiment in phase II.

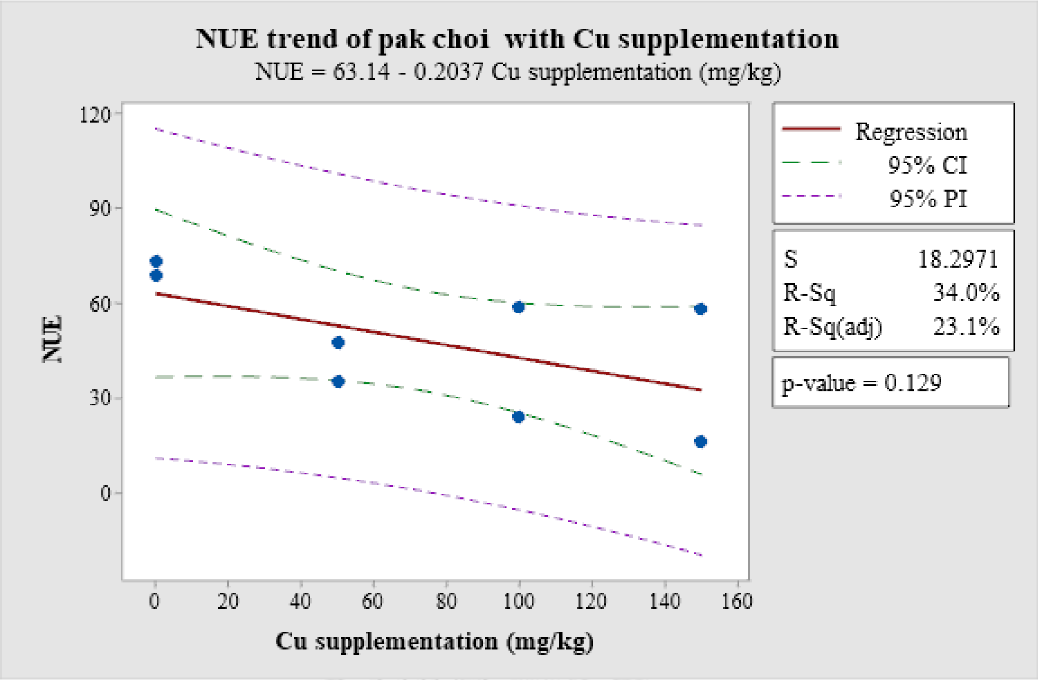

Figure 7 Slope of regression NUE trend of pak choi with Cu supplementation (0, 50, 100, 150 mg/kg) of the experiment in phase I.

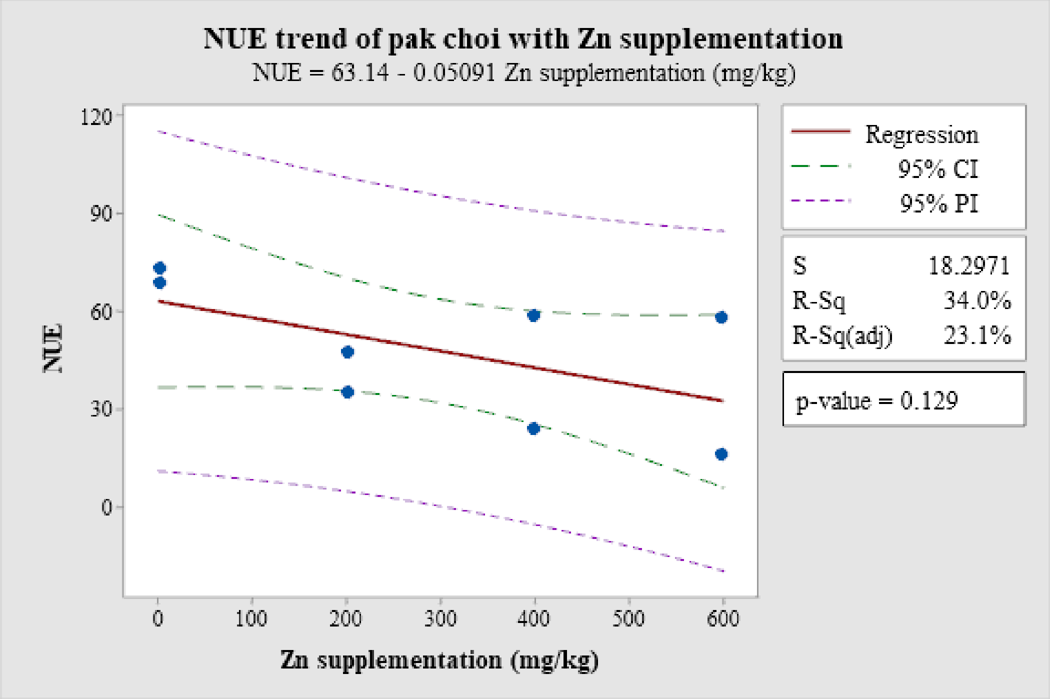

Figure 8 Slope of regression NUE trend of pak choi with Zn supplementation (0, 200, 400, 600 mg/kg) of the experiment in phase I.

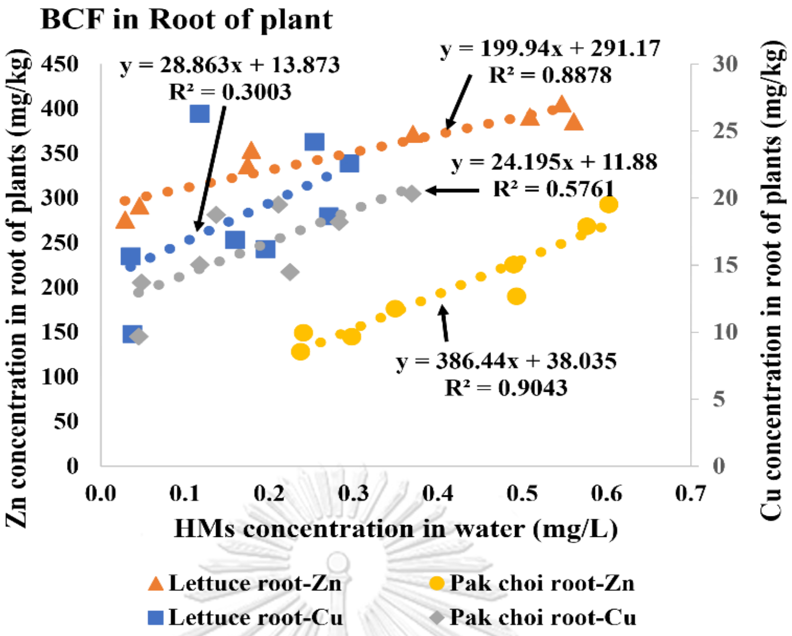

Figure 9 The bioconcentration factor values (BCF) of Cu and Zn in root of the lettuce and pak choi.

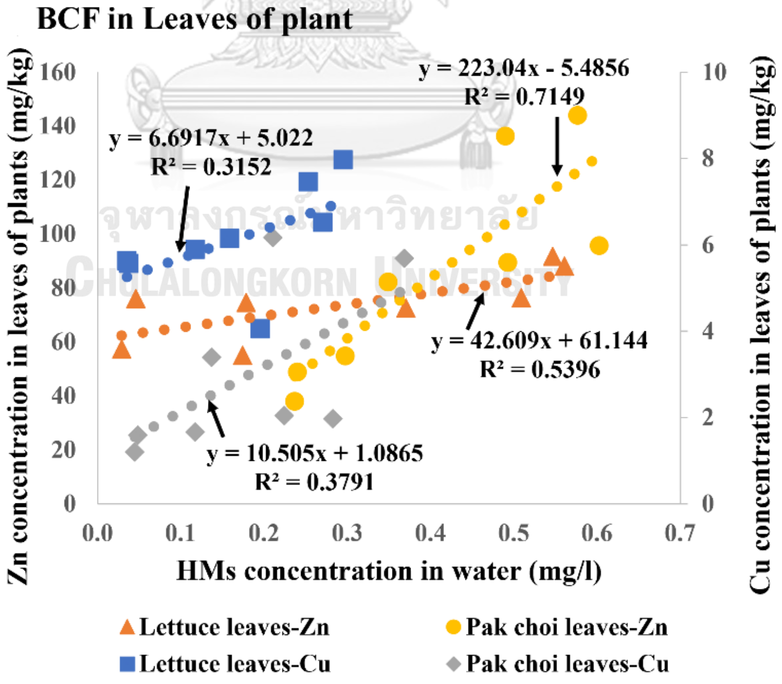

Figure 10 The bioaccumulation values (BCF) of Cu and Zn in shoot of the lettuce and pak choi.

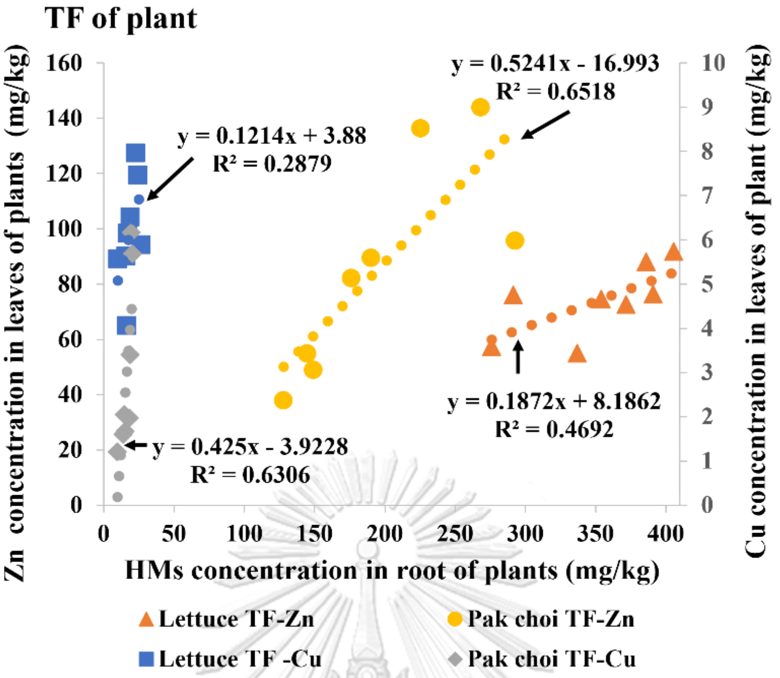

Figure 11 The translocation values (TF) in lettuce and pak choi of the bioponics.

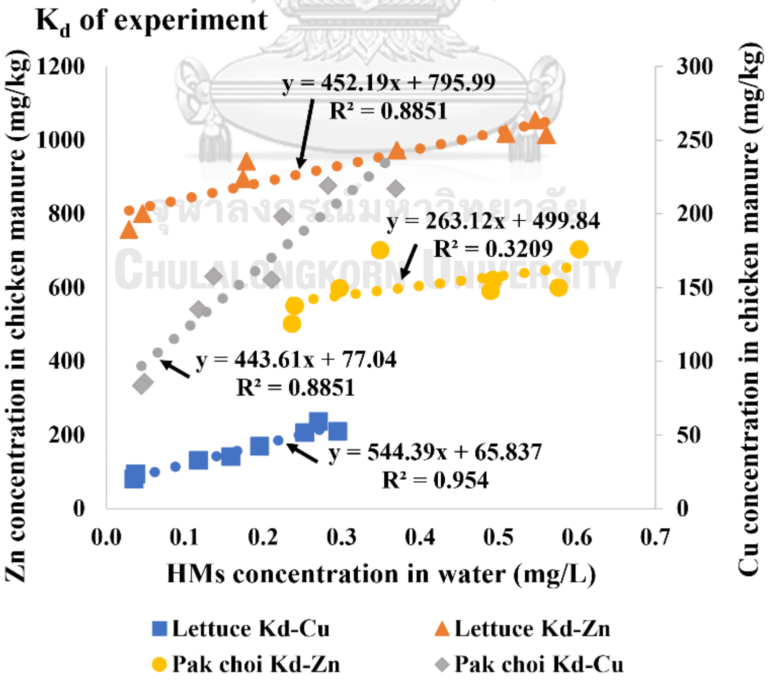

Figure 12 The partition coefficient (K_d) in lettuce and pak choi of the bioponics.

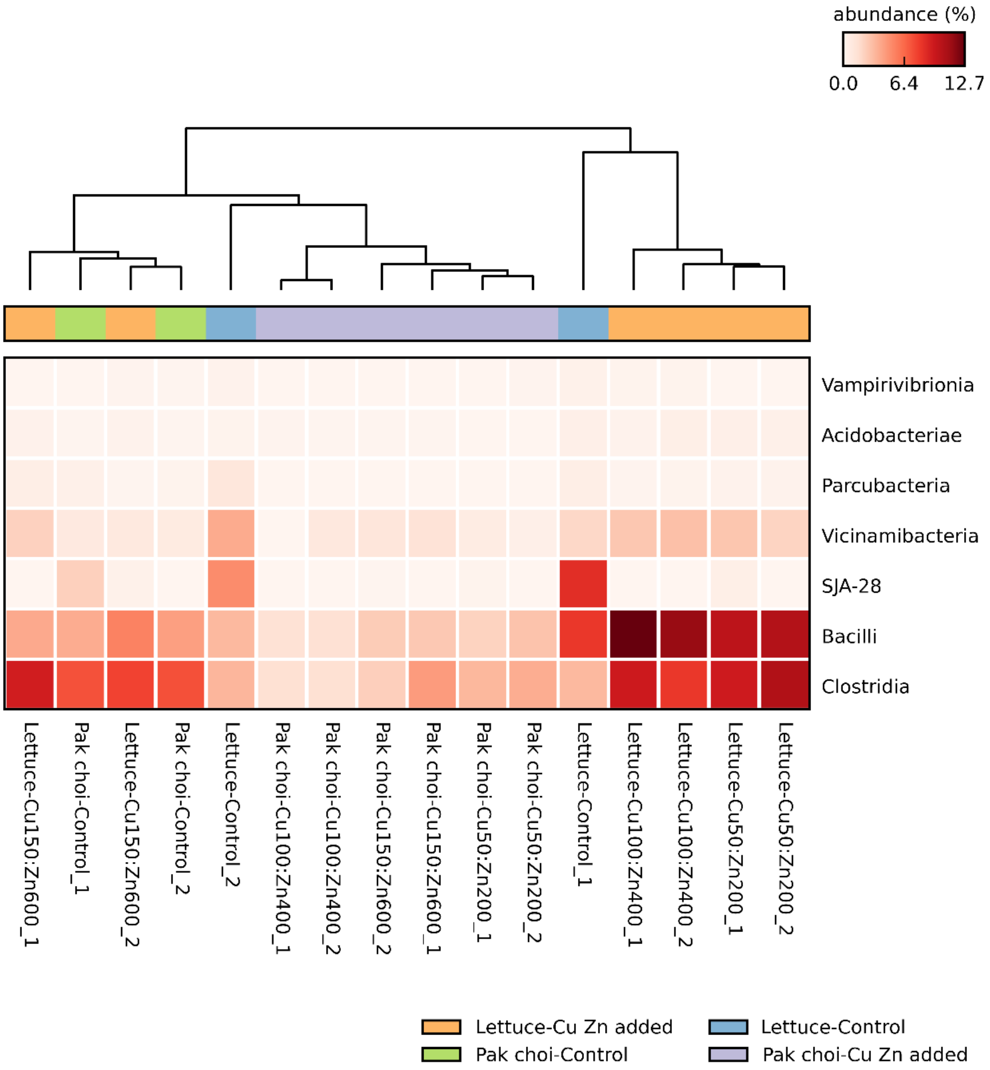

Figure 13 Relative abundance of bacteria the class level in plant roots. Bacterial class with effect size > 0.6 were shown in this heatmap.

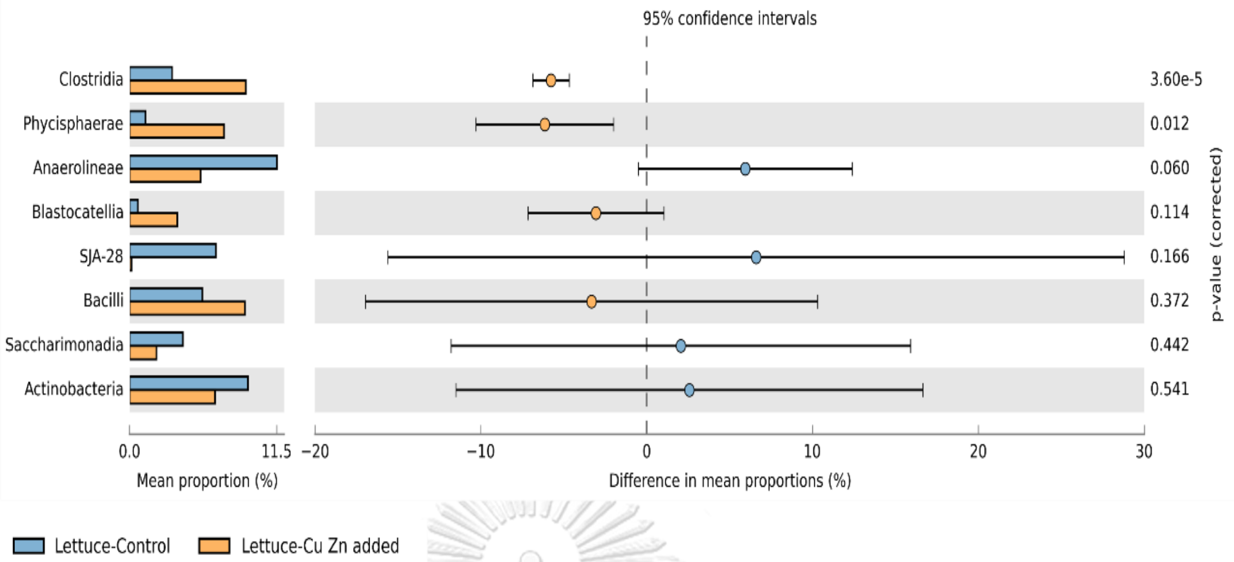

Figure 14 Differential abundance of microbial communities at the class level in lettuce roots from bioponics with and without Cu and Zn supplementation based on Welch's t-test (effect size > 2.0, p < 0.05).

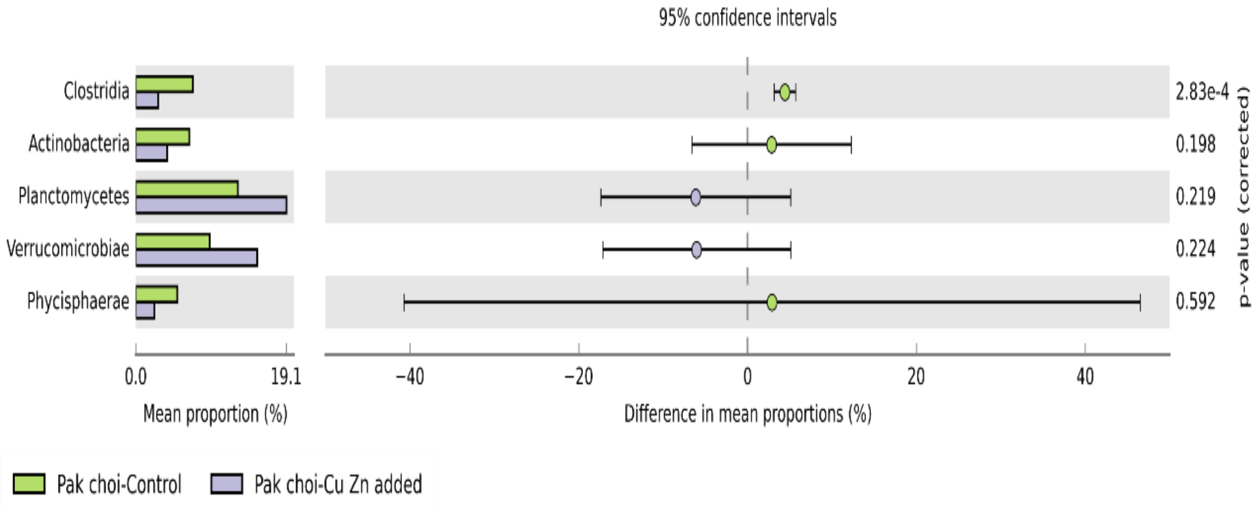

Figure 15 Differential abundance of microbial communities at the class level in pak choi roots from bioponics with and without Cu and Zn supplementation based on Welch's t-test (effect size > 2.0, p < 0.05)

Figure 16 Relative abundance of bacteria the order level in plant roots. Bacterial order with effect size > 0.6 were shown in this heatmap.

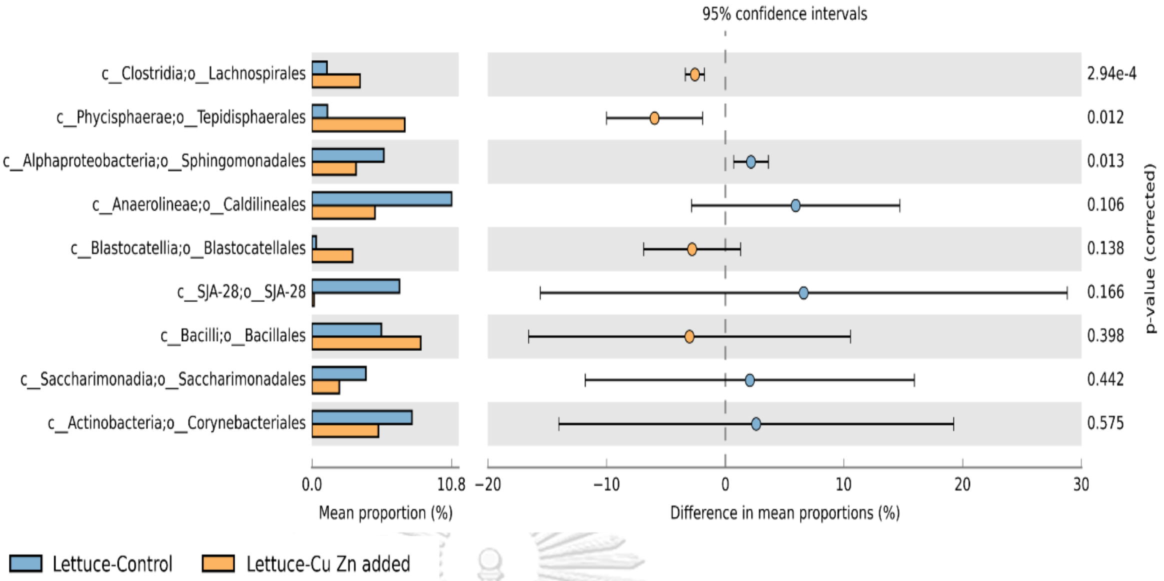

Figure 17 Differential abundance of microbial communities at the order level in lettuce roots from bioponics with and without Cu and Zn supplementation based on Welch’s t-test (effect size > 2.0, p < 0.05).

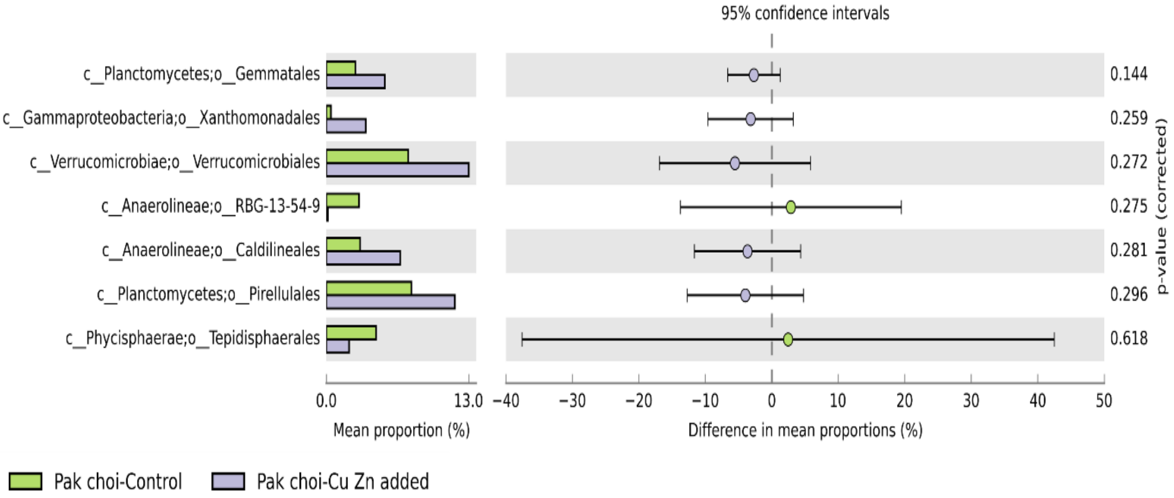

Figure 18 Differential abundance of microbial communities at the order level in pak choi roots from bioponics with and without Cu and Zn supplementation based on Welch’s t-test (effect size > 2.0, p < 0.05).

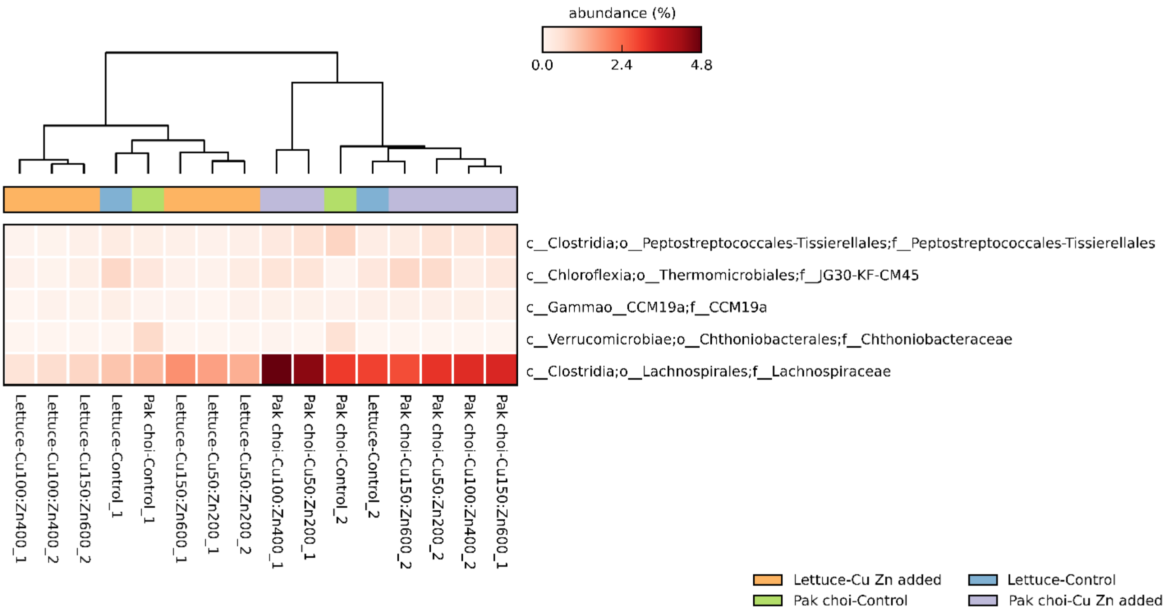

Figure 19 Relative abundance of bacteria the family level in plant roots. Bacterial family with effect size > 0.6 were shown in this heatmap.

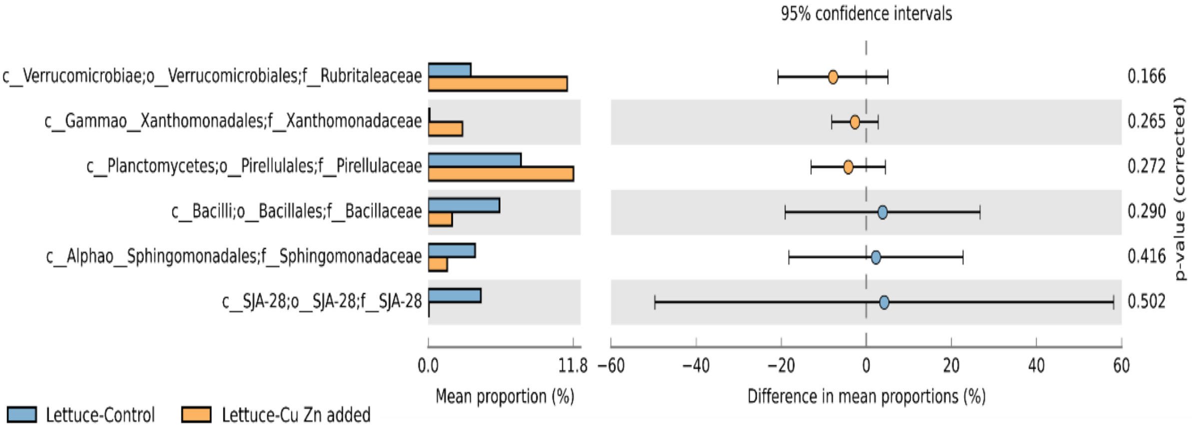

Figure 20 Differential abundance of microbial communities at the family level in lettuce roots from bioponics with and without Cu and Zn supplementation based on Welch’s t-test (effect size > 2.0, p < 0.05).

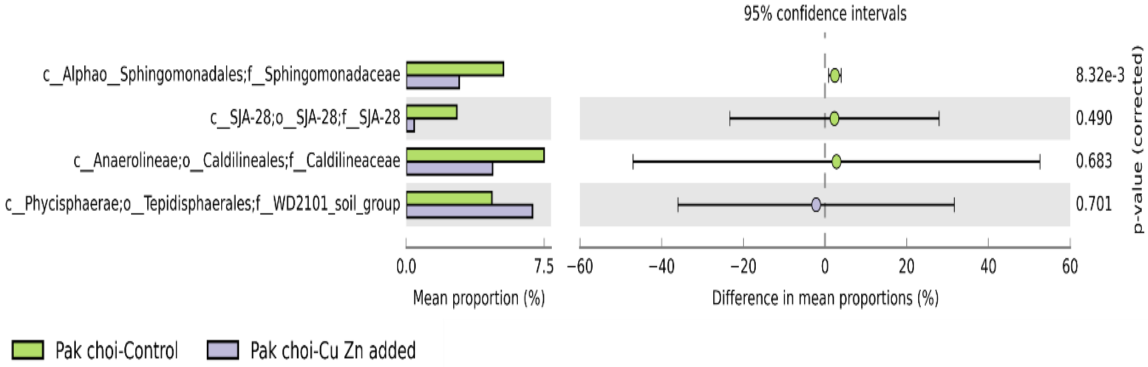

Figure 21 Differential abundance of microbial communities at the family level in pak choi roots from bioponics with and without Cu and Zn supplementation based on Welch's t-test (effect size > 2.0, p < 0.05).

Table 1 Microbial diversity indices from plant roots from lettuce-based bioponic systems under control and Cu-Zn supplemented conditions operated at chicken manure loadings of 200 grams/harvesting cycle.

	Lettuce-based bioponic systems (mg/kg)							
Conditions	**Control**		**Cu 50: Zn 200**		**Cu 100: Zn 400**		**Cu 150: Zn 600**	
Sample names	**Control _1**	**Control _2**	**Cu 50: Zn 200 _1**	**Cu 50: Zn 200 _2**	**Cu 100: Zn 400 _1**	**Cu 100: Zn 400 _2**	**Cu 150: Zn 600 _1**	**Cu 150: Zn 600 _2**
Taxa_S	284	275	296	283	270	302	304	288
Individuals	158951	158951	158951	158951	158951	158951	158951	158951
Simpson_1-D	0.9708	0.9614	0.9742	0.9655	0.9742	0.972	0.9785	0.9659
Shannon_H	4.344	4.078	4.411	4.216	4.323	4.362	4.511	4.166
Evenness_e^H/S	0.2712	0.2147	0.2782	0.2393	0.2793	0.2596	0.2993	0.2238
Chao-1	284	275	296	283	270	302.0	304	288
ACE	284	275	296	283	270	302.2	304	288

Table 2 Microbial diversity indices from plant roots from pak choi-based bioponic systems under control and Cu-Zn supplemented conditions operated at chicken manure loadings of 200 grams/harvesting cycle.

	Pak choi-based bioponic systems (mg/kg)							
Conditions	**Control**		**Cu 50: Zn200**		**Cu 100: Zn400**		**Cu 150: Zn600**	
Sample names	**Control _1**	**Control _2**	**Cu 50: Zn 200 _1**	**Cu 50: Zn 200 _2**	**Cu 100: Zn 400 _1**	**Cu 100: Zn 400 _2**	**Cu 150: Zn 600 _1**	**Cu 150: Zn 600 _2**
Taxa_S	321	317	290	313	256	318	305	319
Individuals	158951	158951	158951	158951	158951	158951	158951	158951
Simpson_1-D	0.9802	0.9813	0.9329	0.9637	0.9578	0.9409	0.9204	0.9732
Shannon_H	4.569	4.583	3.937	4.273	3.934	3.887	3.624	4.307
Evenness_e^H/S	0.3004	0.3084	0.1768	0.2292	0.1997	0.1534	0.1229	0.2327
Chao-1	321	317	290	313	256	318	305	319
ACE	321	317	290	313	256	318	305	319

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