INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
21–0784/2022/28–1–49–58
DOI: 10.17957/IJAB/15.1951
http://www.fspublishers.org

Full Length Article

Optimizing Primary Nutrient Requirements in Vegetable Cultivation using Response Surface Methodology for Lead Contaminated Soils

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Received 16 July 2021; Accept 04 July 2022; Published 31 July 2022

Abstract

The present study investigated the effect of lead (II) in soil on the growth rate and the morphology of four vegetables, such as spinach, lettuce, carrots, and potatoes. Results showed that carrots and potatoes could not grow in lead-contaminated soil at 1200 and 1000 mg/kg, respectively, whereas lettuce and spinach could grow even at 1500 mg/kg lead concentration. The amount of lead decreased in roots with order of carrots > potatoes > spinach > lettuce > stems and leaves of spinach > carrots > potatoes > lettuce. The results showed that N-K-application increased lead amount in biomass of these vegetables, whereas P-fertilizer and agricultural lime reduced the lead accumulation. These obtained results support farmers in the cultivation and soil improvement, leading to enhance soil health, food safety and quality.

Keywords: Lead accumulation; Cultivation mode; Fertilizers; Soil health; Food safety

Introduction

Currently, one of the serious ecological problems, the world facing is heavy metal pollution in agricultural soils. This problem is a consequence of industrialization, urbanization (Tan *et al.* 2016; Qian *et al.* 2017; Xu *et al.* 2020), mineral mining (Xiao *et al.* 2017), industrial wasting (Qing *et al.* 2015; Golui *et al.* 2019; Yang *et al.* 2019; Alam *et al.* 2020), abuse pesticides (Peña *et al.* 2020) and chemical fertilizers in agricultural cultivation (Ning *et al.* 2017; Edogbo *et al.* 2020; Wang *et al.* 2020). The farming on heavy metal contaminated soil will lead to the absorption and accumulation of these metals on agricultural products (Xu *et al.* 2006; Bi *et al.* 2010; Malandrino *et al.* 2011; Naz *et al.* 2013; Rodriguez *et al.* 2014; Edogbo *et al.* 2020). Therefore, the accumulation of heavy metals in agricultural products is becoming serious concern for many countries, including Vietnam, because of their toxicity, sustainability and bioaccumulating potential. Heavy metals can be accumulated in vital organs such as kidney, bone, and liver where it can pose threats to the health of human beings (Bosch *et al.* 2016; Lamas *et al.* 2016; Ma *et al.* 2016). Moreover, long-term exposure to these can cause physical, muscular and neurological degenerative processes, and even cancer (Álvarez-Mateos *et al.* 2019).

There have been many studies focused on the methods to decrease amount of heavy metals in soil to limit their accumulation in agricultural products. Several techniques, included physical separation, chemical washing, electro-remediation have been suggested to solve soil contamination by heavy metals (Akcil *et al.* 2015). However, these methods require high costs and disrupt the soil structure. Use of natural materials such as goethite, magnetite, and hematite reduce the activity of heavy metals in contaminated soil as iron oxides in these materials can bind strongly with heavy metal (Hartley *et al.* 2004; Liu *et al.* 2014; Suda and Makino 2016). As chemical properties of soil changes, heavy metals immobilized by iron oxides may be released back into the soil. Moreover, the presence of iron ion in soil also affects the growth of plants. Although applying some natural materials (e.g., red-mud and biochar) to the soil remediation work has certain environmental benefits (Lu *et al.* 2018; Xu *et al.* 2018), this practice takes much time and expenses to absolutely solve the problem of heavy metals in soil. Some studies have focused on identifying plants with a high potential for heavy metal accumulation to be used as phytoremediation (Álvarez-Mateos *et al.* 2019). Nevertheless, several factors such as growth rate of plants, phytotoxicity of metals, geochemistry of the contaminated soil and uptake ability of metals prevent the efficiency of such methods (Jin *et al.* 2019; Al-Thani and Yasseen 2020). These evidences show that the

To cite this paper: Thanh TLT, HN Van, VP Dinh, TL Van (2022). Optimizing primary nutrient requirements in vegetable cultivation using response surface methodology for lead contaminated soils. *Intl J Agric Biol* 28:49–58

treatment of heavy metals in agricultural soils is not simple, and requires a lot of time as well as cost. Therefore, in many countries, especcially in poor and underdeveloped countries, farmers are still cultivating in polluted areas (Rodriguez *et al.* 2014; Ávila *et al.* 2017; Alam *et al.* 2020). This situation is extremely worrying because the absorption of heavy metals from soil to plants is one of the main ways for heavy metals to infiltrate through the food chain, and to a certain level, these metals will cause risks to human health (Akinyele and Shokunbi 2015; Ávila *et al.* 2017; Qian *et al.* 2017). It seems that, so far, there not have been many reports related to the identification of the kinds of vegetables which have the ability to grow in metal polluted soil with limitative accumulation. There have been also no studies monitoring changes in morphology as well as productivity of vegetables grown in lead-contaminated soil. Moreover, as far as we know, there are very few studies which evaluated the effect of farming mode on the ability to absorb and accumulate of heavy metals from soil to plants (Zhu *et al.* 2010; Liu *et al.* 2016). These works only surveyed effect of each factor of cultivation process, such as the amount of nitrogen fertilizer and on the accumulation of heavy metals in plants. Zouheir Elouear et al (Elouear *et al.* 2016) evaluated the effects for the use of KCl on the growth and heavy metal accumulation of alfalfa (*Medicago sativa* L.) in a Cd, Pb and Zn mine soil but this kind of plants was used as a phytoextraction in contaminated soil.

Among heavy metals, lead is a highly toxic one which, plays no role in metabolic processes in the organism and can be toxic even in tiny amounts (Pourrut *et al.* 2011). Lead is a contaminant that can easily accumulate in soil and sediments. Lead accumulates in the surface soil layer, and its concentration decreases with the depth of the soil. Therefore, lead can easily be absorbed and accumulated by plants in different parts when cultivated on contaminated soil. When exposed to a certain extent, lead is toxic to animals, including humans, damaging the nervous system and causing brain disorders. High exposure to lead will result in blood disorders in animals. Like mercury, lead is a neurotoxin, accumulating in soft tissue and in bones, which is difficult to eliminate (Tan *et al.* 2016).

In an attempt to make good use of lead-polluted soil, present study paid attention to the growth responses of potatoes (*Solanum tuberosum* L.), carrots (*Daucus carota* L.). - representing for tuber vegetables - spinach (*Spinacia oleracea* L.), and lettuce (*Lactuca sativa* L.) - representing for leafy vegetables. These vegetables are planted widely in Vietnam and other areas in the world. Besides, the accumulation of lead from contaminated soil on the biomass of these vegetables were determined. Amounts of lead in different parts of examined plants were also investigated. On the other hand, the effect of lime and N-, P-, K-fertilizer on lead accumulation from soil to these vegetables were also investigated. The result of present work will help in choosing kinds of vegetables which can adapt to lead contaminated soil with limitative accumulation. Besides, the result obtained of this study provides the basic to identify the effects of some factors in cultivation mode to limit the accumulation of lead from soil to vegetables. The result of this investigation also suggests kind of vegetables and suitable cultivation mode to limit the content of lead in the edible parts of vegetables grown in lead contaminated soil.

Materials and Methods

Experimental treatments and designs

Experimental was conducted in Ward 8, Dalat city, Lam Dong Province (11°57’39.7’’N –108°26’28.3’’E, 103°36’ E–109°35’ E) in Vietnam. Lam Dong Province presents area with suitable climatic and soil conditions for the cultivation of carrots, potatoes, lettuce, and spinach. The present study was conducted from March to September, during 2019 vegetation season. The soil was collected from uncontaminated paddy fields (0–20 cm of topsoil) of areas specializing in vegetable cultivation. After air-dried and passed through a mesh sieve (2 mm mesh size), fifteen kilograms of soil was placed in each pot (40 cm in diameter and 35 cm in height). The properties of soil sample used in this study were analysed (Table 1).

Empirical model included two areas

Area 1: Study on accumulate levels of lead from soil to plants. In this area, vegetables were grown under cultivation mode currently used by farmers, except for the soil which was contaminated by lead at different levels of content, including: 100, 200, 400, 600, 800, 1000, 1200 and 1500 mg/kg. The soil was spiked with different lead doses by spraying of $Pb(NO_3)_2$ solution and then stabilized for three weeks before use. Total concentration of lead in the soils of all treatments after lead additions were determined, ensuring sting that the doses of lead added to the soils of all treatments were accurate. For comparison, experimental field had a controlled area where studied vegetables were grown under the same conditions as models for uncontaminated soil mentioned above.

Area 2: Investigation the effect of cultivation mode to lead accumulation from soil to plants. The soil was contaminated by lead at 100 mg/kg and the added amounts of agricultural lime ($CaCO_3$) as well as N-, P-, K- fertilizers were changed. The amounts of lime added to the soil and commensurate soil pH are presented in Table 2. Other soils were fertilized with phosphorus (P) as calcium phosphate [$Ca(H_2PO_4)_2$] or potassium (K) as potassium chloride (KCl), or nitrogen (N) as ammonium nitrate (NH_4NO_3). The amounts of each fertilizer added for each kind of vegetables were stated in Table 3. The amounts of each fertilizer for every studied vegetable were changed depending on the Technical process of each kind the planted vegetable (Department of Agriculture and Rural Development of Lam Dong Province 2013).

Table 1: Properties of soil sample

	Unit	Content
pH		5.97 ± 0.01
Total organic matter	(g/kg)	8.62 ± 0.7
Total of nitrogen	(g/kg)	0.28 ± 0.04
Total of phosphorus	(g/kg)	0.62 ± 0.01
Total of potassium	(g/kg)	16.22 ± 0.12
Lead		not detected

Values are means of three replicates followed by ±standard error of means (n = 3)

Table 2: The amount of lime added in the soils and corresponding pH

Treatment	Amount of lead (mg/kg soil)	Amount of lime (g/15kg soil)	pH
Control	100	0	5.97
T1	100	7.5	6.42
T2	100	13.8	7.05
T3	100	19.2	7.52

Table 3: The amount of N, P, K fertilizer added to the soil

Samples	Spinach			Lettuce			Carrots			Potatoes		
	N*	P*	K*	N*	P*	K*	N*	P*	K*	N*	P*	K*
Control	2.30	10.30	2.5	3.00	4.50	0.90	4.90	14.10	6.00	4.10	5.60	1.40
T4	1.15	10.30	2.5	1.50	4.50	0.90	2.45	14.10	6.00	2.05	5.60	1.40
T5	3.45	10.30	2.5	4.50	4.50	0.90	7.35	14.10	6.00	6.15	5.60	1.40
T6	4.60	10.30	2.5	6.00	4.50	0.90	9.80	14.10	6.00	8.20	5.60	1.40
T7	2.30	5.15	2.5	3.00	2.25	0.90	4.90	7.05	6.00	4.10	2.80	1.40
T8	2.30	15.45	2.5	3.00	6.75	0.90	4.90	21.15	6.00	4.10	8.40	1.40
T9	2.30	20.60	2.5	3.00	9.00	0.90	4.90	28.20	6.00	4.10	11.2	1.40
T10	2.30	10.30	1.25	3.00	4.50	0.45	4.90	14.10	3.00	4.10	5.60	0.70
T11	2.30	10.30	3.75	3.00	4.50	1.35	4.90	14.10	9.00	4.10	5.60	2.10
T12	2.30	10.30	5.00	3.00	4.50	1.80	4.90	14.10	12.00	4.10	5.60	2.80

(*: mg/kg soil)

Specifically, the amount of each kind of N-, P- and K-fertilizer applied for each type of the vegetables was decreased a half, increased a half, and increased double as much as those suggested by Technical process of this vegetable planting. The control experiments were carried out by adding the amount of N-, P-, K-fertilizers the same as those suggested by above technical processes.

Planting vegetables

Lettuce and spinach seeds were submerged in water for about 48 h at room temperature (20–25°C), prior to germinating under moist condition at 32°C for 30 h. These germinated seeds were then grown in uncontaminated soil within 15 days before being transplanting into the pots (five plants per pot).

Carrot seeds were soaked in warm water at 40°C for 8 h before being kept for six days until the seeds germinated. These germinated seeds were then shown with a density of 10 seeds per pot.

Potatoes seedlings were collected at nursery, then planted in each treatment with a density of 2 plants per pot.

These vegetables were grown under cultivation mode as defined by Department of Agriculture and Rural Development, Lam Dong Province, Vietnam. Plants were watered every two days or as needed to maintain field capacity. Each pot was given a plastic tray below to collect leachate returned to the pots. Mature plants (after 45 days for lettuce and spinach, 100 days for carrots and potatoes) were harvested at the same time.

Soil and plants analysis

After preparing soil samples, the soil pH was measured with a glass electrode by distilling water slurry (1:2.5, w/v) (Margesin and Schinner, 2005). For determining the content of elements in soil, soil sample was dissolved by a mixture of HNO_3, HCl and HF in a microwave digester (iLINK MARS 6 CEM). The total and bio-available contents of nitrogen (N), phosphorus (P) and potassium (K) were determined by the AOAC Official Method 955.04, 958.01 and 965.09. The content of lead in soil was performed using an Atomic Absorption Spectrophotometer (AAS, SHIMADZU AA-7000).

At the end of the growth period, the plants were carefully removed from the soil. The stems, leaves, and roots were separated, cleaned, and washed adequately, then they were dried at 60°C in the drying oven (MEMMERT UF55) to their constant weight. These dried samples were homogenized separately in a porcelain mortar.

Acid digestion with vegetable parts (500 mg each) was performed in a microwave digester using 10 mL of 65% HNO_3, cooled, filtered and made up to 25 mL using deionized water. Lead determined in the respective samples

Fig. 1: Effects of lead on the morphology of studied vegetables

was done by Atomic Absorption Spectrophotometer. Blank tests indicated that the level of contamination induced by the acid digestion procedure was negligible.

Statistical analyses

All experiments were triplicated (n = 3) and the experimental data were presented in average ± standard deviation (SD). The analytical procedure was validated based on the internationally certified plant standard reference material (Citrus Leaves 1572). The Relative Standard Deviation (RSD) of each analysis was found within ± 2.4% of the certified values. The one-way analysis of variance (ANOVA) was utilized to evaluate the significant differences between the lead content in particular vegetables and contaminated soil. Statistical significance was evaluated via the student's *t*-test with the *P*-value < 0.005.

Results

Effect of lead on the morphology and growth rate of some vegetables

The changes in the morphologies of four kinds of vegetables (spinach, lettuce, carrots, and potatoes) planted in soil with different concentrations of Pb showed no toxicity symptoms in the spinach and the lettuce at the concentration of lead less than 800 mg/kg. The growth parameters including height of plants, number and dimension of leaves, average yield showed a negligible change. However, there is a stunted growth in soil contaminated by lead higher than 1200 mg/kg for the spinach and 800 mg/kg for the lettuce. Carrots can normally grow in soil contaminated by the excess of lead from 100 to 400 mg/kg. Meanwhile, the growth inhibition together with a progressive decrease in the number and dimension of leaves, the low weight of tubers is observed at above 600 mg/kg of the Pb(II) concentration. In particular, the dead plants were recorded at the lead doses of 1200 mg/kg or above. In potatoes, the rate of growth reduced significantly when the Pb(II) concentrations of 600 and 800 mg/kg and plants could not grow in soil with the Pb(II) content of 1000 mg/kg and above (Fig. 1).

The results showed that Pb was highly toxic to the growth of the carrot roots. The diameter, length, weight, and yields of carrot roots decrease dramatically with the increase in the Pb level in the soil. Indeed, the change in carrot root in shape is recorded at the Pb(II) concentration of 200 mg/kg and above. When the Pb concentrations of 200–400 mg/kg, although the carrot growth is not significantly

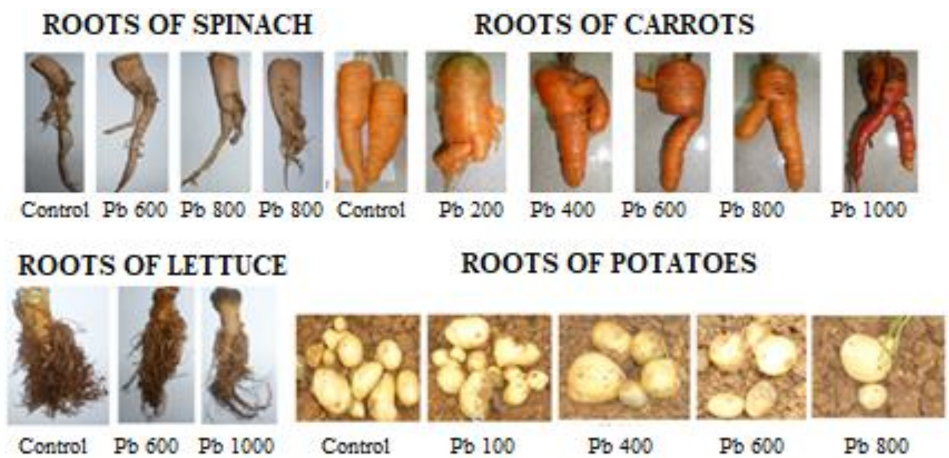

Fig. 2: Changes in the morphological form of spinach, carrots, lettuce, and potatoes roots when grew on lead-contaminated soils

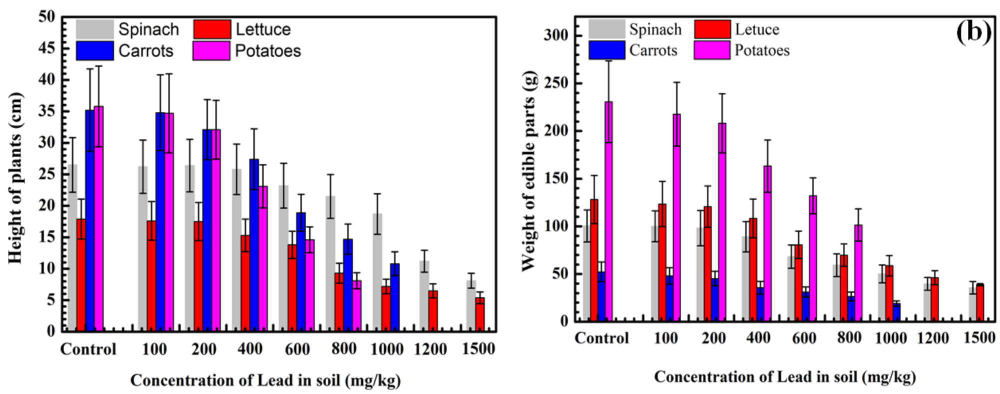

Fig. 3: Effect of lead on the growth characteristics of inspected vegetables (a) height of plants, (b) fresh weight of edible parts per plant (error bar = SD, n = 3)

influenced, the root shape is altered and the roots yield is decreased by 20% or more. The root shape and weight are significantly affected under the Pb levels of 600–1000 mg/kg. In potatoes, a decrease in the number and dimension of roots was observed in all the Pb(II) concentration from 400 to 800 mg/kg (Fig. 2).

There was a considerable decrease in the height of plants and the weight of edible parts when the concentrations of Pb (II) increased from 100 mg/kg to 1500 mg/kg. For instance, carrots and potatoes will be dead if the Pb (II) levels in soil is higher than 800 mg/kg and 1000 mg/kg, respectively. The height and the edible parts' weight of the spinach decrease by 69.4 and 64.6% from 26.5 cm and 100.5 g, respectively, while a decline by about 70% occurs on the lettuce when the Pb(II) concentrations are changed from 100 mg/kg to 1500 mg/kg (Fig. 3).

The accumulation of lead in the biomass of studied vegetables

When lead content in soil ranged from 100 to 1500 mg/kg, the amount of this metal in the biomass of four studied vegetables ranged between 0.05 and 6.79 mg/kg. Lead accumulation in these vegetables were as the following the order: carrots > potatoes > spinach > lettuce. For example, the lead amount was 0.85 and 6.79 mg/kg in the roots of carrots, 0.20 and 1.79 mg/kg in edible parts of lettuce when the amount of lead in the polluted soil was 100 mg/kg and 1000 mg/kg, respectively. Besides, lead tended to be accumulated in the roots of all studied vegetables. Generally reporting, the amount of lead decreased in the order of roots of carrots > potatoes > spinach > lettuce > stems and leaves of spinach > carrots > potatoes > lettuce (Table 4).

For leafy vegetables, the average lead content in spinach roots was 2.53 times higher than in stems and leaves. Meanwhile, the lead content of lettuce roots is 2.40 times higher than in stems and leaves. For carrots, the lead content in roots is 2.67 times higher than in stems and leaves. This rate in potatoes is 2.65.

Besides the growth, the data of lead amount in the biomass of selected vegetables obtained in experimental field were recorded to give a clear answer to the question of which plants can be used as food under those farming circumstances. The results obtained showed that lead was a

Table 4: The amount of lead in inspected vegetables

Amount of lead in soil (mg/kg)	Amount of lead in the biomass of vegetables (mg/kg)							
	Spinach		Lettuce		Carrots		Potatoes	
	Stems & Leaves	Roots	Stems & Leaves	Roots	Stems & Leaves	Roots	Stems & Leaves	Roots
Control	0.07 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.05 ± 0.01	0.19 ± 0.02
100	0.23 ± 0.02	0.72 ± 0.06	0.20 ± 0.02	0.71 ± 0.06	0.25 ± 0.03	0.85 ± 0.09	0.28 ± 0.03	1.02 ± 0.09
200	0.33 ± 0.02	1.11 ± 0.10	0.26 ± 0.03	0.83 ± 0.07	0.96 ± 0.11	2.38 ± 0.24	0.46 ± 0.05	1.79 ± 0.18
300	0.89 ± 0.07	1.79 ± 0.16	0.68 ± 0.07	1.26 ± 0.11	1.37 ± 0.14	3.15 ± 0.30	1.57 ± 0.16	2.75 ± 0.25
400	1.19 ± 0.11	2.75 ± 0.25	0.73 ± 0.06	1.97 ± 0.21	1.90 ± 0.20	4.53 ± 0.43	1.67 ± 0.15	3.85 ± 0.40
600	1.31 ± 0.12	2.77 ± 0.25	1.17 ± 0.20	2.05 ± 0.22	2.15 ± 0.20	5.29 ± 0.50	2.00 ± 0.21	4.79 ± 0.46
800	1.79 ± 0.16	3.98 ± 0.40	1.62 ± 0.17	2.41 ± 0.22	2.48 ± 0.25	5.84 ± 0.60	2.72 ± 0.25	5.17 ± 0.50
1000	1.98 ± 0.20	4.97 ± 0.49	1.79 ± 0.19	3.89 ± 0.40	2.72 ± 0.26	6.79 ± 0.65	Dead	Dead
1200	2.16 ± 0.21	5.14 ± 0.49	1.93 ± 0.20	4.72 ± 0.49	Dead	Dead	Dead	Dead
1500	2.29 ± 0.21	5.52 ± 0.51	2.07 ± 0.21	4.99 ± 0.50	Dead	Dead	Dead	Dead

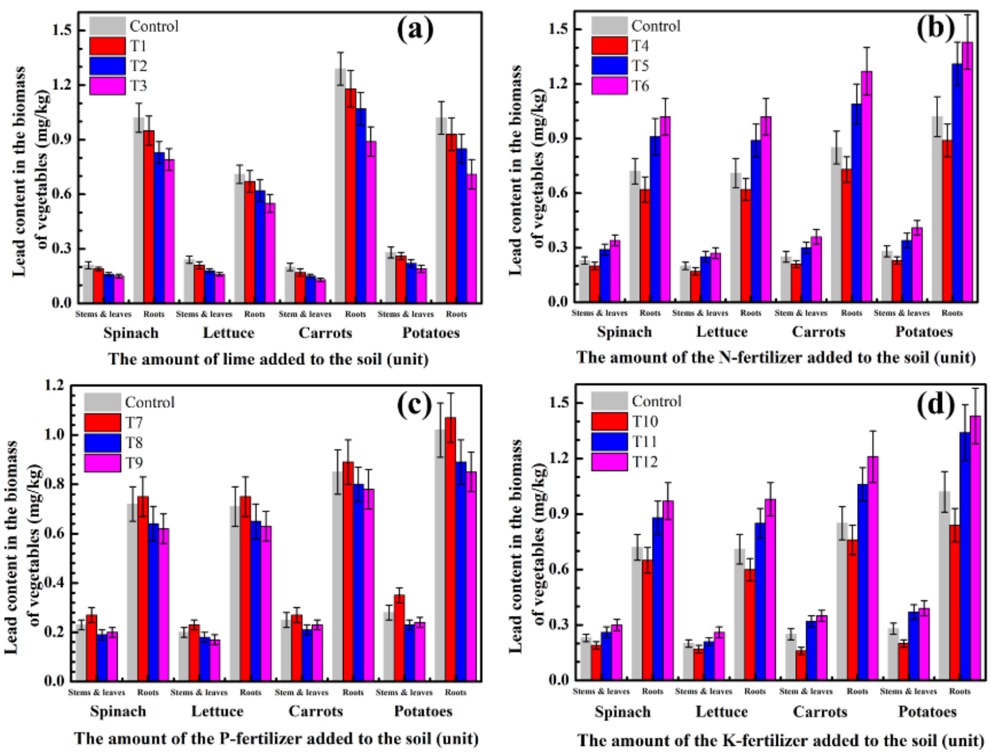

Fig. 4: Effects of agricultural lime (a) and N, P, K fertilizers (b, c, d) on lead accumulation of studied vegetables (error bar = SD, n = 3)

cumulative metal. When we increased lead amount in the soil, its levels hoarding in the biomass of examined vegetables were increased (Table 4).

The results showed that, when lead content in soil ranged from 100 to 1500 mg/kg, the amount of this metal in the biomass of four studied vegetables ranged between 0.05 and 6.79 mg/kg.

Effects of cultivation mode on the accumulation of lead from soil to vegetables

Effect of lime: The results showed that the level of absorption and accumulation of lead from the soil on the biomass of vegetables decreased when the pH of the soil increased from 6.0 to 7.5. The average lead content in every part of examined vegetables decreased by 7–15% when the pH in soil increased from 5.97 to 6.42, reduced by 13–25% when pH soil rose from 5.97 to 7.05 and decreased by 23–35% when pH increased from 5.97 to 7.52 (Fig. 4a).

The Effects of N-, P-, K-fertilizers on the Pb(II) accumulation

Inorganic fertilizers, such as N, P, K, are often used for a cultivation process due to their nutrient levels. However, the use of large amounts of fertilizers to achieve maximum yields can affect the chemical properties of agricultural soil

Table 5: Maximum and minimum levels of selected factors

Factors	Symbol	Unit	Min (-1)	Central point	Max (+1)
Mass of N fertilizer	X_1	mg/kg soil	1.15	2.875	4.6
Mass of P fertilizer	X_2	mg/kg soil	5.15	12.875	20.6
Mass of K fertilizer	X_3	mg/kg soil	1.25	3.125	5

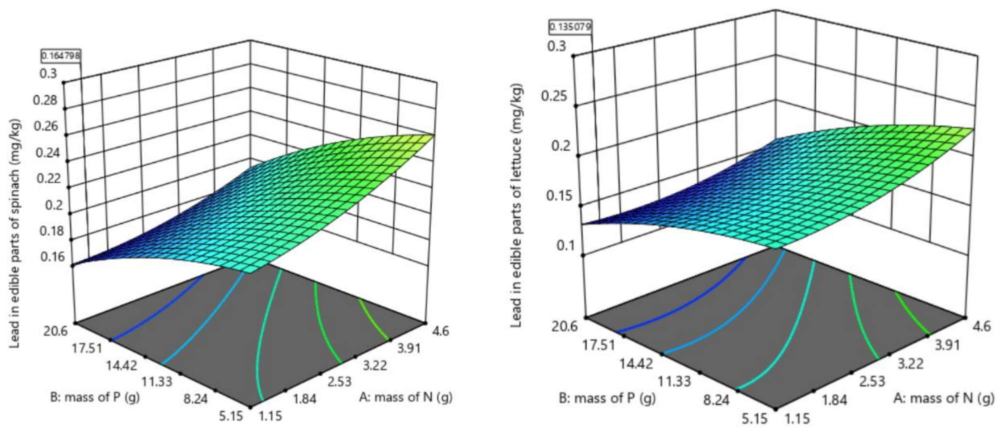

Fig. 5: Response surfaces estimated from factorial design

and the quality of crop products. In addition, the presence of this kind of fertilizer in the soil can affect the Pb(II) accumulation of some vegetables. **Fig. 4b, c, d** show the effects of N-, P-, K-fertilizers on the Pb(II) accumulation of four plants, namely carrots, potatoes, spinach, and lettuce. Clearly, the contents of accumulated lead in all the biomass were increased as the amount of N applied to the plants increased by 3 times. For instance, the accumulated lead content in the stem & leaves of spinach, lettuce, carrot, and potato increased order by 30.9, 55.6, 61.5 and 50.0%, whereas the lead content in the roots of the four kinds above rose by 52.0, 52.9, 30.4 and 55.8%, respectively. Similarly, the presence of K in the soil will promote the absorption and transport of lead from the soil to the plants. When increasing the amount of N fertilizer by 3 times during the cultivation process, the level of lead accumulation in the stem & leaves and roots increased by 32.4 and 37.6% for spinach, 66.7 and 47.2% for lettuce, 54.5 and 27.4% for carrot, 52.9 and 38.8% for potato, respectively. In contrast, the absorption of lead from the soil to the biomass of the studied vegetables was inhibited when the P fertilizer in the soil was increased by triple. For example, the amount of accumulated lead in the stem & leaves, and roots was ordered decreased by 39.6 and 33.1% for spinach, 40.7 and 38.8% for lettuce, 43.5 and 31.5% for carrot, and 51.5 and 42.8% for potato.

Design of experiments for estimation of optimum conditions on the cultivation mode

The above results showed that lettuce and spinach can adapt with lead contaminated soil better than carrots and potatoes. Besides, mass of inorganic fertilizers, i.e., N-, P-, K-fertilizer significantly affected the accumulation of lead from soil to vegetables. Thus, a two-level full factorial design with five replicates of central point was carried out to determine the mass of N-, P-, and K-fertilizers to limit the content of lead accumulated in the edible parts of spinach and lettuce. The independent variables selected are shown in Table 5 along with their min, medium and max levels, which were determined from preliminary trials.

The responses of this matrix included the amount of lead accumulated in the edible parts of spinach (Y_1) and lettuce (Y_2). The factorial design demonstrated that the variables in the studied required a final optimization. The response surface considering the equation is shown in Fig. 5. The relation among these factors and lead content in the edible parts of spinach and lettuce was described in these equations:

For spinach:

$$Y_1 = 0.2360 + 0.0187X_1 - 0.0188X_2 + 0.03X_3 - 0.005X_1X_2 - 0.0025X_1X_3 + 0.0125X_2X_3 + 0.007X_1^2 - 4.75X_2^2 - 3.08X_3^2.$$

For lettuce:

$$Y_2 = 0.208 + 0.0237X_1 - 0.0163X_2 + 0.04X_3 - 0.005X_1X_2 + 0.0077X_1X_3 + 0.0175X_2X_3 + 0.011X_1^2 - 0.009X_2^2 - 0.0015X_3^2;$$

where X_1: Mass of N fertilizer (mg/kg soil); X_2: Mass of P fertilizer (mg/kg soil); X_3: Mass of K fertilizer (mg/kg soil).

According to this result, the optimal conditions on the cultivation mode to limit the amount of lead accumulated in the edible parts of spinach and lettuce were determined as follows: 1.329 mg of N fertilizer, 19.950 mg of P fertilizer and 1.562 mg of K fertilizer per 1 kg of soil. At the optimal value of these factors, the content of lead accumulated in the edible parts of spinach and lettuce estimated by were 0.165 and 0.135 mg/kg, respectively ($p < 0.0001$).

Discussion

The results showed that the content of lead in the biomass of vegetables depends on the nature of each vegetable. Lead accumulation in these vegetables were as the following the order: carrots > potatoes > spinach > lettuce. The highest lead amount was found in roots of carrots (1.29 ÷ 6.79 mg/kg), while edible parts of lettuce showed the lowest lead level (0.20 ÷ 2.07 mg/kg), suggesting a resistance towards lead accumulation. Besides, lead accumulated in the roots of all studied vegetables. The amount of lead decreased in the order of roots of carrots > potatoes > spinach > lettuce > stems and leaves of spinach > carrots > potatoes > lettuce.

For leafy vegetables, the average lead content in spinach roots were 2.53 times higher than in stems and leaves. While the lead content of lettuce roots were 2.40 times higher than in stems and leaves. For carrots, the lead content in roots is 2.67 times higher than those in stems and leaves. This rate in potatoes is 2.65. Basu *et al.* (2013) showed that lead accumulation in the crops follows the order: carrots > beet > cabbage > brinjal > cauliflower > spinach > tomato > chilly. Whereas, its concentration in various parts of plants showed: root > stems > leaves > other edible parts. This demonstrated the characteristic tendency of lead to be accumulated in the roots rather than in other parts of plants.

By WHO standards (FAO/WHO, Codex Alimentarius Commission. (2001), 0.3 mg/kg is the maximum allowable limit of lead in edible parts of vegetables, beyond which health is affected. According to this regulation, all edible parts of potatoes and carrots exceeded the allowable limit when cultivated on soil contaminated by lead at 100 mg/kg in present study. For spinach and lettuce, when grown on contaminated soil by lead below 100 mg/kg and 200mg/kg, respectively, neither drop the crop yields nor pose any risk to consumers' health.

According to QCVN 03-MT:2015/BTNMT, Vietnam Ministry of Natural Resources & Environment 2015 the maximum allowable concentration of lead for Vietnamese agricultural soil is 70mg/kg. However, based on the results of this study, Pb content in edible parts of spinach grown in soil contaminated by Pb at 100 mg/kg did not exceed the allowable limit. Lettuce could grow in Pb soil 200 mg/kg but lead contents in stems and leaves were still lower than its permissible level.

The contents of accumulated lead in all the biomass increased as the amount of N applied to the plants increased. For instance, the levels of lead in edible parts of selected vegetables are risen from 25.0 to 28.4% and from 35.0 to 49.4% when increasing in the amount of nitrogen by a half and double, respectively. This can be explained by the soil acidification by adding of N-fertilizer into the soil (Tian and Niu 2015; Ghimire *et al.* 2017). The increase in the amount of nitrogen will promote nitrification, plant assimilation, and volatilization (as ammonia) of ammonium, resulting in the release of protons to the soil (Reuss and Johnson 2012). Additionally, the N fertilizer can stimulate the growth rate of plants due to an uptake of cations and the release of equivalent amounts of protons to the rhizosphere (Van Breemen *et al.* 1983). These leads to the increase in the content of dissolved lead in soil, which enhances the Pb(II) accumulation.

Similarly, an increase in the amount of K in soil promotes the uptake and transport of lead from the soil to biomass. Fig. 4c presents that when doubling the amount of K fertilizer, the lead content in stems plus leaves and roots of leafy vegetables increases by 30.4 and 34.7% for spinach, 30.0 and 38.0% for lettuce; meanwhile, the amount of this metal in roots of carrots and potatoes raised by 42.4 and 40.2%, respectively. Obviously, potassium promotes nutrient metabolism between plants and soil, leading to increasing the uptake of metal ions. In addition, this phenomenon may be related to the amount of chloride anion by using fertilizer as KCl salt due to the formation of soluble complexes of the participated $PbCl_2$ at the Cl^- high concentrations. The obtained results are consistent with some previous reports (Zhao *et al.* 2003; Elouear *et al.* 2016).

On the other hand, the uptake of lead from the soil on the biomass of vegetables decreases when the phosphorus are applied to the soil. With an (1.5–2 fold) increase in the amount of P fertilizer, the average content of lead accumulated in all parts of these vegetables reduces to 12.5% compared to control. Meanwhile, reducing the amount of phosphorus fertilizer raised the average lead content by 25.12%. It is understandable that by adding phosphorus fertilizer to the soil, Pb^{2+} reacts with phosphate anion to produce precipitated pyromorphite. Therefore, P-containing fertilizers reduce the solubility of lead, resulting in a decrease in the lead accumulation in plant biomass. However, Weber JS et al. (Weber *et al.* 2015) proved that the P fertilizer contained not only nutrient elements but also non-nutrient ones such as Cd or Hg, etc. The presence of these metals in phosphate fertilizer has caused the phenomenon of competition in the transport process of lead from soil to plants, reducing the accumulation of lead in plants. Thus, cultivation mode should add phosphorus at a suitable dose to avoid soil to be contaminated by other heavy metals (Table 4 and Fig. 4d).

Conclusion

Results showed that when the lead content in soil ranged from 100 to 1500 mg/kg, the accumulation of lead in the biomass of these vegetables ranged between 0.05 and 6.79 mg/kg. The amount of agricultural lime, N-, P- and K-fertilizer had certain effects on the accumulation of lead from soil to plants. The optimal conditions on the cultivation mode to limit the amount of lead accumulated in the edible parts of vegetables which can adapt to heavy contaminated soil with limitative accumulation - spinach and lettuce - were also determined.

Acknowledgments

This research was supported by a Grant-in-Aid for Scientific Research No. 08TĐ from Dalat University, Vietnam.

Author Contributions

TLTT and HNV planned the experiments, TLTT, V-PD and TLV interpreted the results, TLTT and HNV histological observation, TLTT and V-PD statistically analyzed the data and made illustration, TLTT, V-PD and TLV writing original draft and editing. All authors revised and completed the final version.

Conflicts of Interest

Authors declare no conflict of interest.

Data Availability

Data are available from the first author on reasonable request.

Ethics Approval

Not applicable in this paper

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