The Morphological and Physiological Appearance of Two Vegetable Plants Due to Lead Exposure

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ABSTRACT

This research aimed to determine the effect of different concentrations of Lead (Pb) on the morphology of kale and spinach plants. The process involved planting kale and spinach seeds in tubs and transferring them to polybags with planting media in the form of soil and sand at a ratio of 4:1 after strong roots were developed. It is important to note that the media were analyzed to ensure the Pb content in the soil was below the threshold before planting. Pb was later provided 1 week before planting in the form of $PbNO_3$ in the media at a dose of 1 and 2 g/polybag and mixed effectively to ensure even distribution, while the sample used as the control was not given any Pb. The transplanting process was conducted after the plants were 18 days old in the nursery and the initial observations at 9 DAT showed that the kale leaves were darker with a score of 3 than spinach with a score of 1, but the spinach leaves became darker in color with score 3 as the age of the plants increased. Moreover, the kale changed to a lighter color with a score of 2 from the 9th day of observation after transplanting, while spinach requires 15 DAT to become score 2 until the end of the observation. It should be pointed out that both plants showed morphological changes due to the existence of the Pb but their base leaves did not reflect any effect. The kale leaf tip became blunt, while the spinach leaf tip was not affected and both plants were discovered to have longer roots and more root hairs in the control compared to the treatments. Furthermore, the total chlorophyll of spinach in the control was higher than kale but observed to reduce as the concentration of Pb increased in the treatments. The morphology and physiology of spinach and kale plants changed due to the Pb exposure with the spinach was discovered to be more sensitive as indicated by more visible morphological damage to its leaves at the end of the observation. It is possible to use the morphology of spinach and kale to detect Pb-contaminated land.

Keywords: kale, lead, morphology, physiology, spinach.

INTRODUCTION

Heavy metal pollution on agricultural land is one of the most important ecological problems being experienced globally. According to the Environmental Protection Agency (EPA), Lead (Pb) is the most common heavy metal contaminant in the environment (Islam et al., 2007) due to its ability to induce many morphological, physiological, and biochemical changes in plants that disrupt their growth and yield.

The Pb toxicity causes a decrease in the percentage of seed germination (Shafiq et al., 2008), plant growth and yield (Kumar & Jayaraman, 2014), nutrient and mineral disturbances (Lamhamdi et al., 2013; and Nareshkumar et al., 2014), inhibits

photosynthesis (Tian et al., 2014), enzyme activity (Malar et al., 2016), as well as causes imbalance and changes in membrane permeability (Sharma & Dubey, 2005; Israr & Sahi, 2008). It is important to note that germination and root growth are the stages of plant development considered to be very sensitive to the contamination of this element.

It is, however, possible to neutralize the concentration of Pb in the soil using heavy metal-fixing or hyperaccumulator plants through a phytoremediation process (Tordoff et al., 2000; García et al., 2003). Kale (*Ipomea aquatica*) and spinach (*Spinacia oleracea*) have long been known to have important roles as hyperaccumulator plants (Naz et al., 2013; Alexander et al., 2006; Huang et al., 2009) but previous studies mostly

focused on the amount of Pb uptake in their shoots and roots. Therefore, the present research aimed to determine the level of changes in the morphology and physiology of spinach and kale plants due to the administration of Pb at different concentrations.

MATERIALS AND METHODS

Research place

The research was conducted in the greenhouse of the Faculty of Agriculture, Malang Islamic University, from October to December 2020 with a daily temperature of 20–29 °C, an altitude of 550 m above sea level, 112°06' – 112°07' South latitude, and 7°06' – 8°02' North latitude.

Materials

Kale and spinach seeds were sown in nursery tubs until they grew and had sufficiently strong roots to be transferred. The planting media used was in the form of soil and sand with a ratio of 4: 1 placed in 5 kg polybags and analyzed to ensure the Pb content in the soil was below the threshold before the planting process. Pb was provided later, 1 week before planting in the form of $PbNO_3$ in the media at a dose of 1 and 2 g/polybag and mixed effectively to ensure even distribution while the sample used as the control was not given any Pb. It is also important to note that Super Phosphat-36 and KCl fertilizers were applied 7 days before planting at 20 and 25 kg/ha doses, respectively, while 50 kg/ha of ZA fertilizer was provided during the process of transplanting. The plants were transplanted after reaching 18 days in the nursery.

Research design

The data were analyzed using a factorial randomized block design consisting of two factors with the first being the 2-level dose of Pb at 200 mg·kg⁻¹ soil (1 g/polybag) and 400 mg·kg⁻¹ soil (2 g/polybag) while the second was the indicator plant species including spinach and kale. Each treatment combination has three sample plants and all the treatments were repeated three times.

Observation variable

The plants were observed non-destructively from 3 days after transplanting (DAT) with an interval of 6 days. The total root length was determined using the intersection method of Newman (1966) simplified by Tennant (1975) which involved using R = 11/14×N×0.786 where R is the total root length (cm), N is the number of intersection points, and 0.876 is a constant for a line size of 1×1 cm. The morphology of the plants was evaluated using a color chart where 1 represents the light color and 4 for the dark color. The other parts analyzed include the surface, margin, tip, and bone arrangement of the leaf with the 2nd leaf from the shoot used for the analysis. Moreover, the total chlorophyll was evaluated using spectrophotometry while the Pb in leaves and roots was analyzed destructively at harvest using AAS.

Statistics analysis

All the observational data were analyzed using Analysis of Variance (ANOVA) and continued with the Honestly Significant Difference (HSD) test at the 5% level in case the results obtained from ANOVA are significantly different.

RESULTS

Kale leaf morphology

In the first observation at 3 DAT, the kale leaves did not show any symptoms of necrosis and there was no difference between treatments but some changes were observed in the leaf color from 9 DAT with the control showing a score of 3, while the treatments with 1 and 2 g Pb/polybag showed a score of 2 as indicated in Figure 1 and Table 1. This means the kale plants with 1 and 2 g Pb/polybag had a lighter leaf color than the control and no further changes were recorded up to 27 DAT. Moreover, the leaf surface was flat and not wavy for control and 1 g Pb/polybag throughout the experiment while the 2 g Pb/polybag became wavy from 21 DAT. It was also discovered that the leaf edge was flat from the beginning to the end for the control specimen while the edge for both treatments with Pb became wavy at 27 DAT. Furthermore, the leaf base was curved shape without differences for all the treatments throughout the experiment while the leaf tip was pointed in all treatments between 9–15 DAT but the control treatment had a pointed leaf tip up to the end of the observation period (Figure 2). It should be noted that the leaf tip for 1 g Pb/polybag treatment became slightly blunt at 27 DAT
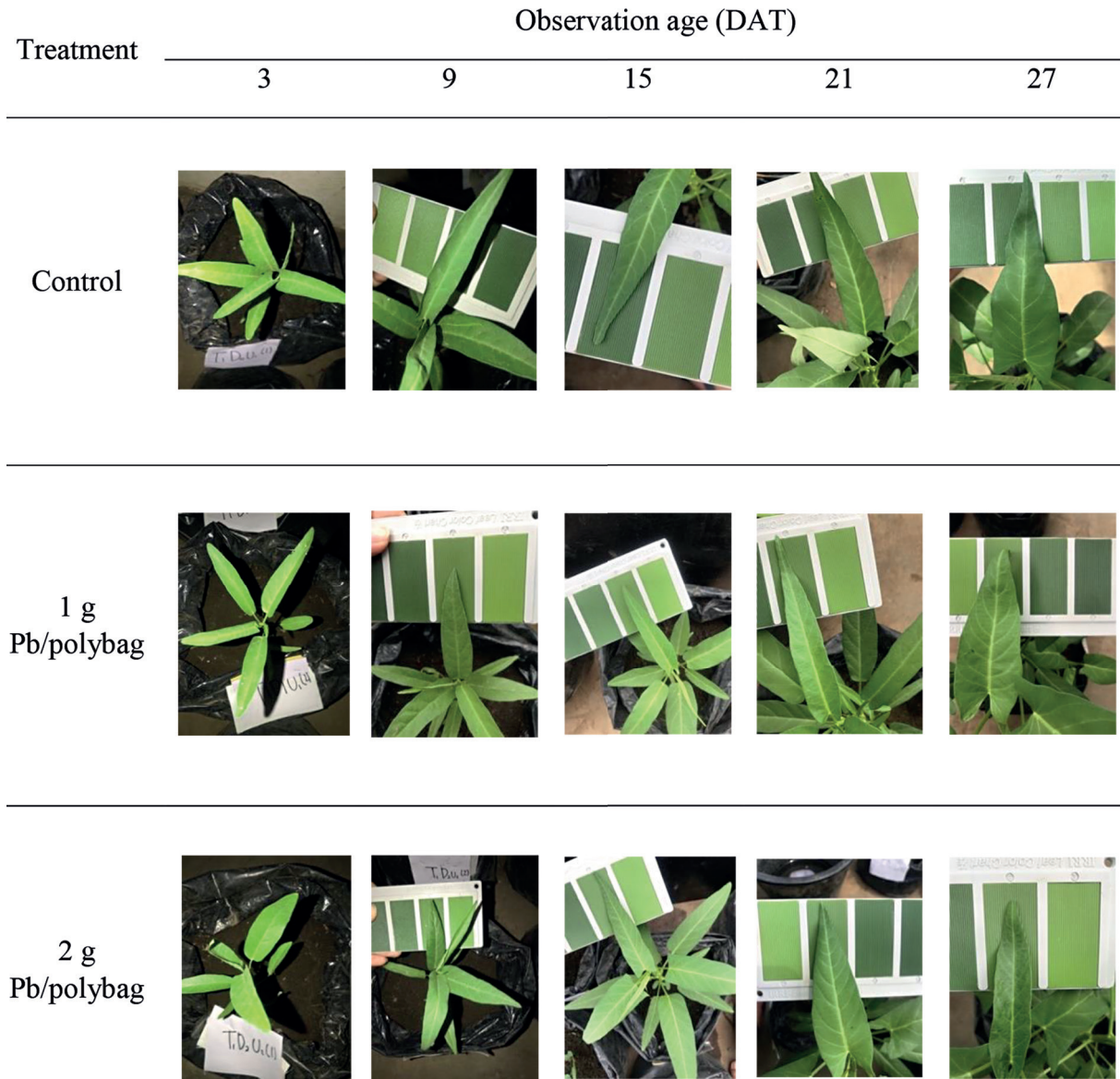
Treatment	Observation age (DAT)				
	3	9	15	21	27

Figure 1. The color score of kale leaves at various observation ages

Table 1. Morphology of kale leaves

Leaf color score				
Treatment	9 DAT	15 DAT	21 DAT	27 DAT
Control	3	3	3	3
1g Pb/polybag	2	2	2	2
2 g Pb/polybag	2	2	2	2
Leaf surface				
Treatment	9 DAT	15 DAT	21 DAT	27 DAT
Control	Flat	Flat	Flat	Flat
1g Pb/polybag	Flat	Flat	Flat	Flat
2 g Pb/polybag	Flat	Flat	Wavy	Wavy
Leaf edge				
Treatment	9 DAT	15 DAT	21 DAT	27 DAT
Control	Flat	Flat	Flat	Flat
1g Pb/polybag	Flat	Flat	Flat	Wavy
2 g Pb/polybag	Flat	Flat	Flat	Wavy

Table 1. Cont. Morphology of kale leaves

Leaf base				
Treatment	9 DAT	15 DAT	21 DAT	27 DAT
Control	Curved	Curved	Curved	Curved
1g Pb/polybag	Curved	Curved	Curved	Curved
2 g Pb/polybag	Curved	Curved	Curved	Curved
Leaf tip				
Treatment	9 DAT	15 DAT	21 DAT	27 DAT
Control	Pointed	Pointed	Pointed	Pointed
1g Pb/polybag	Pointed	Pointed	Pointed	A bit blunt
2 g Pb/polybag	Pointed	Pointed	Blunt	Blunt
Leaf bone arrangement				
Treatment	9 DAT	15 DAT	21 DAT	27 DAT
Control	Perfect	Perfect	Perfect	Perfect
1g Pb/polybag	Perfect	Perfect	Perfect	Imperfect
2 g Pb/polybag	Perfect	Perfect	Imperfect	Imperfect

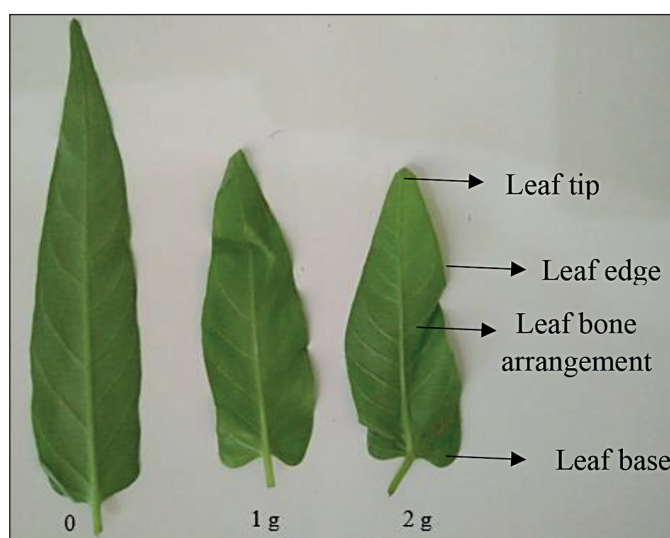**Figure 2.** Morphology of kale leaves at the end of the observation

Table 2. Morphology of spinach leaves

Leaf color score				
Treatment	9 Dat	15 Dat	21 Dat	27 Dat
Control	1	2	3	3
1g Pb/polybag	1	2	2	2
2 g Pb/polybag	1	2	2	2
Leaf surface				
Treatment	9 DAT	15 DAT	21 DAT	27 DAT
Control	Flat	Flat	Flat	Flat
1g Pb/polybag	Flat	Wavy	Wavy	Wavy
2 g Pb/polybag	Flat	Wavy	Wavy	Wavy
Leaf base				
Treatment	9 DAT	15 DAT	21 DAT	27 DAT
Control	Rounded	Rounded	Rounded	Rounded
1g Pb/polybag	Rounded	Rounded	Rounded	Rounded
2 g Pb/polybag	Rounded	Rounded	Rounded	Rounded

Table 2. Cont. Morphology of spinach leaves

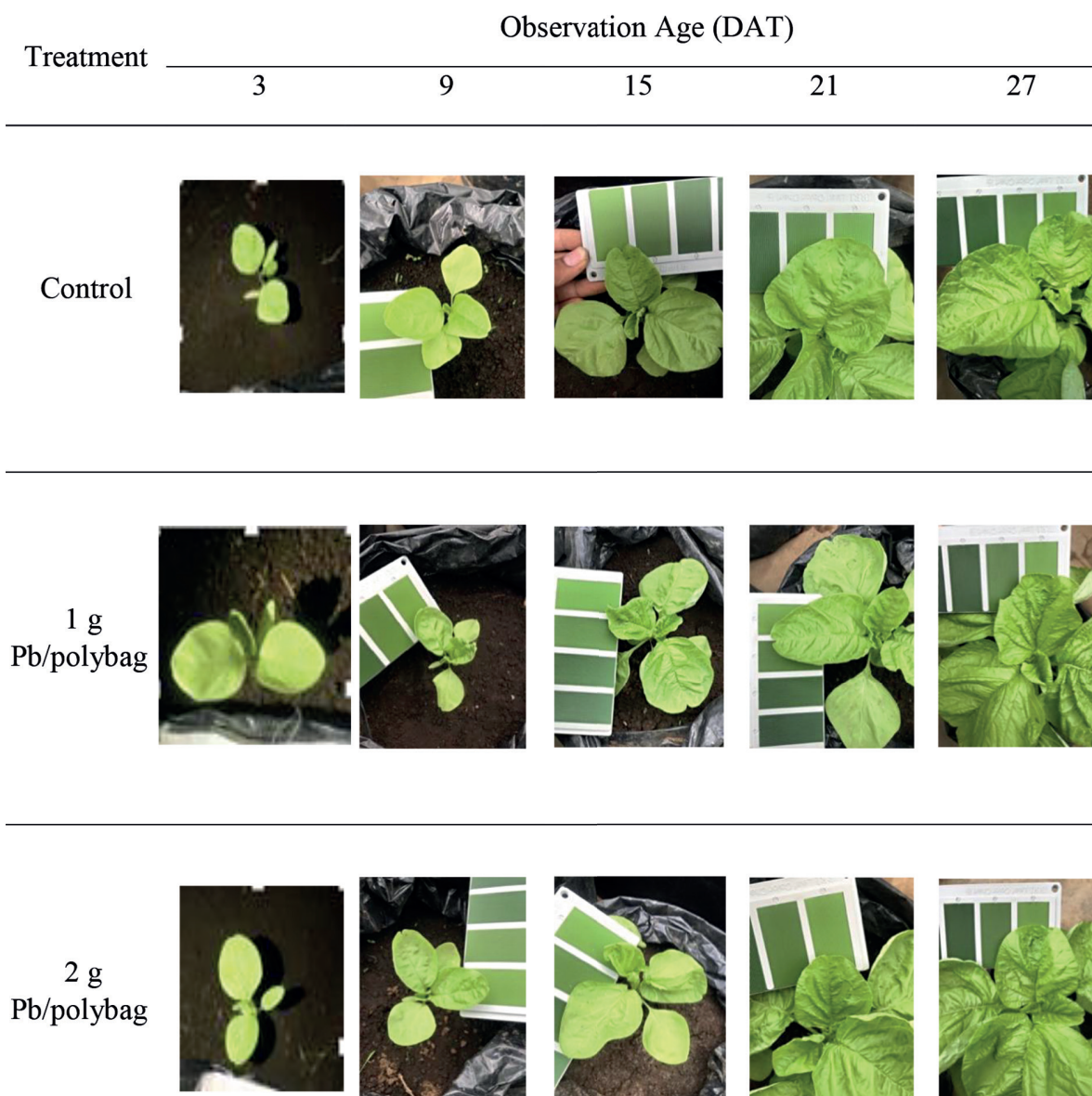|				
Leaf tip				
Treatment	9 DAT	15 DAT	21 DAT	27 DAT
Control	Oval	Oval	Oval	Oval
1g Pb/polybag	Oval	Oval	Oval	Oval
2 g Pb/polybag	Oval	Oval	Oval	Oval
Leaf bone arrangement				
Treatment	9 DAT	15 DAT	21 DAT	27 DAT
Control	Perfect	Perfect	Perfect	Perfect
1g Pb/polybag	Perfect	Perfect	Imperfect	Imperfect
2 g Pb/polybag	Perfect	Perfect	Imperfect	Imperfect
Leaf edge				
Treatment	9 DAT	15 DAT	21 DAT	27 DAT
Control	Flat	Flat	Flat	Flat
1g Pb/polybag	Flat	Flat	Flat	Wavy
2 g Pb/polybag	Flat	Flat	Wavy	Wavy

Figure 3. The color score of spinach leaves at various observation ages

and the leaf tip of the 2 g Pb/polybag treatment became blunt at 21 DAT. It was also discovered that the leaf bone arrangement for the control was perfect up to the end of the observation but observed to be broken (imperfect) at 27 DAT for 1 g Pb/polybag and from 21 DAT for 2 g Pb/polybag.

Spinach leaf morphology

The spinach leaves were observed to have a lighter color than kale and did not show any symptoms of necrosis 3 DAT. It was also discovered there was no difference between the treatments up to the age of 9 DAT with a score of 1 but the changes became noticeable at 15 DAT with each treatment recorded to have a score of 2. The lead color of the control changed to a score of 3 while 1 and 2 g Pb/polybag treatments maintained 2 as indicated in Figure 3 and Table 2. This implies the spinach plants with Pb had a lighter leaf color than the control and this did not change up to 27 DAT. Furthermore, the leaf surface was flat and not wavy for the control through the observation period but the 1 and 2 g Pb/polybag treatments had wavy leaves from 15 DAT. The findings also showed that the leaf base was

rounded while the leaf tip was oval for all treatments at different observation periods. Moreover, the leaf bone arrangement was perfect for the control to the end of the observation period but observed to be broken or become imperfect for 1 and 2 g Pb/polybag treatments at the age of 21 DAT while the leaf edge was flat from 9 to 27 DAT for the control but became wavy at 27 DAT for 1 g Pb/polybag and 21 DAT for 2 g Pb/polybag (Figure 4).

Morphology of kale and spinach roots

The observation of the root morphology showed that kale and spinach had longer roots and more root hairs in the control than 1 and 2 g Pb/polybag treatments as indicated in Figures 5, 6, and 7.

The total root length was observed to be reduced at a higher Pb dose as indicated by the 2.13% and 3.28% for kale as well as 18.75% and 24.67% for spinach compared to the control.

Chlorophyll content

The chlorophyll content was discovered to have been reduced by 3.98% and 21.22% for kale

Figure 4. Morphology of spinach leaves at the end of the observation

Figure 5. Morphology of the kale roots at the end of the observation

Figure 6. Morphology of the spinach roots at the end of the observation
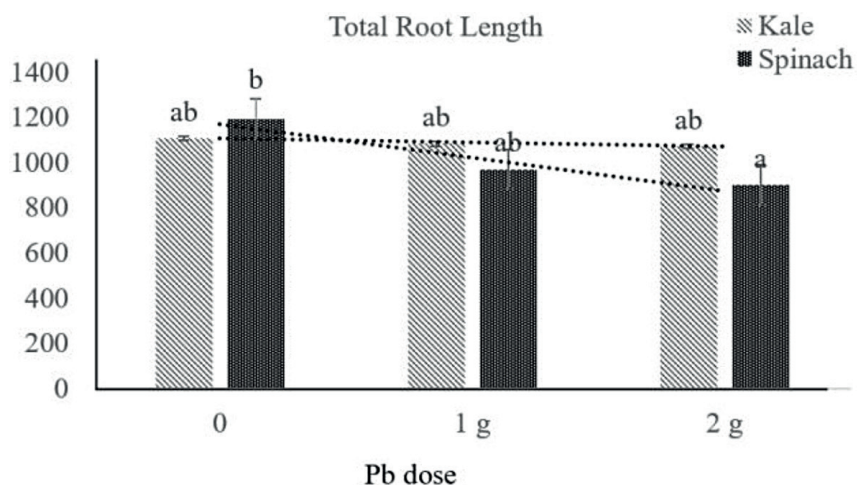

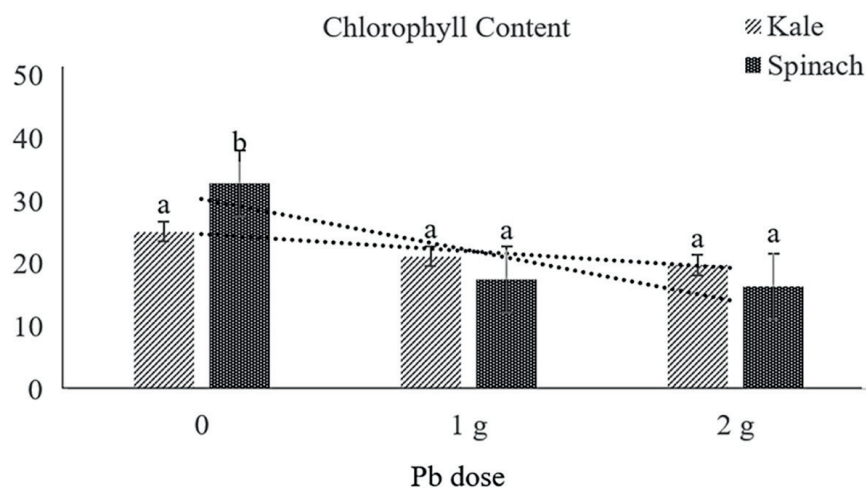**Figure 7.** Decrease in total root length due to increase in Pb dose

Figure 8. Decrease in the chlorophyll content due to increase in Pb dose

and 15.21% and 50.08% for spinach as presented in Figure 8.

Pb content in shoots and roots

The Pb content in the shoots and roots of kale and spinach had the same pattern in which a higher content of Pb dose in the media led to an increase in the amount of the Pb absorbed in the shoots and roots. This was indicated by 6.86 times increase in the Pb content in the shoots at a dose of 1 g Pb/polybag and 7.51 times at 2 g Pb/polybag compared to the control for the kale while the roots had an increase of 3.22 and 7.33 times respectively. The values for spinach were recorded to be 8.39 and 9.30 times in the shoots
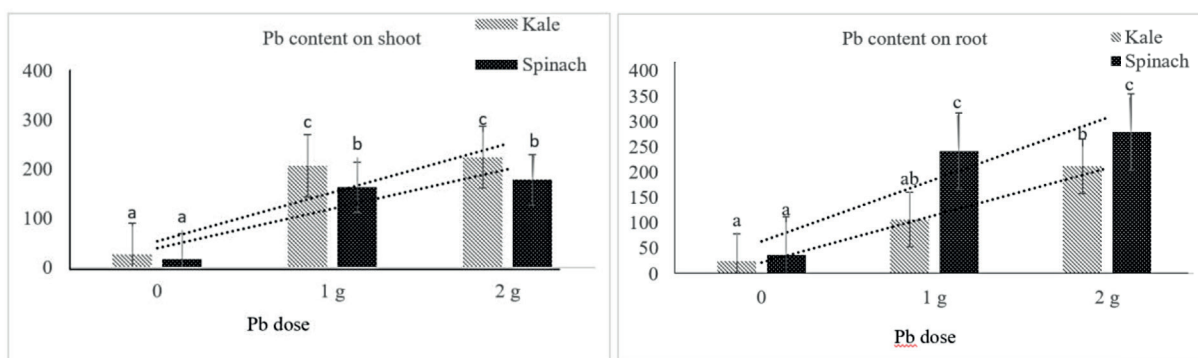
Figure 9. Increased Pb content in shoots and roots due to an increase in Pb dose

and 5.55 and 6.59 times in the roots at doses of 1 and 2 g Pb/polybag respectively compared to the control as indicated in Figure 9.

DISCUSSION

Lead (Pb) is a heavy metal considered to be highly toxic to the environment due to its ability to change physiological processes and ultrastructural aspects of plants (Sharma & Dubey, 2005). The sensitivity of plants to a particular heavy metal has been reported to generally depend on the metal concentration, exposure duration, plant variety and species, plant age, as well as plant tissue (Gao et al., 2010). It was also discovered that the decrease in plant growth is usually associated with the harmful effects of Pb on physiological processes and disturbances in the mineral nutritional status of plants or the Pb-induced inhibition of cell division (Sharma & Dubey, 2005). Figures 1–6 show that the Pb exposure significantly affects the leaf and root morphology of kale and spinach and this is probably due to its ability to cause imbalanced water status, impaired nutrient absorption, and inhibited cell development (Sharma & Dubey, 2005; Schwarz & Grosch, 2003). This is in line with the findings of Islam et al. (2007) on *Elsholtzia argyi* and Tian et al., (2014) that plant mesophyll and root tip cells were significantly damaged due to the toxicity of Pb. Zhang et al. (2003) also showed that chloroplasts are highly susceptible to heavy metal stress such as Pb which has the ability to completely damage the vacuoles, cell walls, and all organelles (Tian et al., 2014). Moreover, the entrance of Pb into a cell to bind with enzymes acting as catalysts usually leads to the disruption of chemical reactions with subsequent damage to the epidermal, spongy, and palisade tissues which are characterized by

necrosis and chlorosis. This was further believed to be the reason for the wavy surface and edges of the kale and spinach leaves.

Leaf chlorosis is one physiological symptom of Pb in plants and is normally caused by the inhibition of photosynthetic pigment synthesis (Sharma & Dubey, 2005). According to Myśliwa et al. (2002), δ-aminolevulinic acid dehydratase involved in the chlorophyll biosynthetic pathway is very sensitive to the presence of heavy metals. This was further confirmed by the significant decrease recorded in total chlorophyll and carotenoids in the leaves of treatments compared to controls (Sędzik et al., 2015; Tian et al., 2014) and this is possibly due to the breakdown of protein complexes, chloroplasts, and photosynthetic apparatus as well as the inhibition of the photosynthetic electron transport chain under heavy metal stress. Chlorophyll decomposition can also be caused by an increase in chlorophyllase activity under heavy metal stress (Hegedüs et al., 2001). Several previous studies have indicated the effect of heavy metals on the stomatal conductance, gas exchange, and chlorophyll content with subsequent influence on the net photosynthetic rate (Balakhnina et al., 2005). It was also found that the Pb-induced stress causes the loss of starch granules in chloroplasts and soluble thylakoids membranes for mesophyll cells (Tian et al., 2014). This is in line with the findings of this research that Pb makes the leaf color to be lighter for both kale and spinach as indicated in Figures 1 and 3 and also decreased the chlorophyll content as its concentration was increasing as presented in Figure 8.

Most of the Pb in roots is localized in the insoluble cell wall and nuclear fractions and this is related to the detoxification mechanism (Piechalak et al., 2002). The cell mechanisms with the ability to minimize the potential for toxicity are

rapidly activated after the exposure of the plants to Pb. This is in line with the observation of the roots of several species including *Pisum sativum* (Malecka et al., 2009) and *Allium sativum* (Jiang & Liu, 2010) which showed that the cell wall is the first barrier against the Pb stress due to its ability to immobilize and accumulate some or even most of the metal ion. Meanwhile, the capacity of the cell wall to bind divalent metal cations mainly depends on the number of polysaccharides with several carboxyl groups (Inoue et al., 2013). This is supported by the findings of this study that the root length of both kale and spinach reduced as the Pb dose increased which was indicated in Figures 5 and 6.

The Pb content in shoots and roots increased with the concentration of the Pb in the media and the kale shoots were observed to have higher content than spinach while the reverse is the case for the roots and this implies the kale shoots have a higher ability to absorb Pb from the soil than spinach. Meanwhile, the symptoms of leaf morphological damage were more visible in the spinach leaves as shown in Figures 2 and 4 and this is suspected to be due to its high sensitivity to Pb.

The Pb uptake in shoots and roots shows the ability of the plants not to change after the induction of the metal. Pb was bound by cell membranes, mitochondria, and chloroplasts after entering the plant, thereby, causing physical damage. Moreover, the plant cuticle which is the main barrier between the inner and outer environment of the leaf can serve as a diagnostic marker to the Pb exposure and the changes in the epicuticular wax have also been reported as one of the most important symptoms (Verma & Singh, 2006). It should be reiterated that metal absorption is determined by plant species, metal concentration in the media, and contact or exposure time. Furthermore, genetic factors and plant species as well as the type of plant tissue and treatment given to the soil medium are very decisive in the absorption of metals in the root zone and roots or shoots at varying levels (Podlipna et al., 2005). It is also influenced by the contact time between metals such that a longer contact time is expected to increase the absorption to a maximum point at a certain time before it decreases again (Lelifajri, 2010). This was observed in the difference in the abilities of the kale and spinach used in this research to absorb Pb in shoots and roots as indicated in Figure 9.

CONCLUSION

The morphology and physiology of the spinach and kale plants changed due to the Pb exposure but the spinach was found to be more sensitive, as indicated by the more visible morphological damage to its leaves at the end of the observation. This simply signifies it is possible to use the morphology of spinach and kale in detecting a Pb-contaminated land.

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