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Acacia auriculiformis Cunn. Ex Benth As Phytoextraction Agent: A Growth Response, Physiological Tolerance and Lead Removal Capability Evaluation

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Abstract

This study was conducted to determine *A. auriculiformis* capability to tolerate elevated Pb concentration. The uptake, distribution, and the capability of *A. auriculiformis* plant to remove lead (Pb) Pb from the soil was assessed, as well as the growth performance, and some physiological parameters of the plant. The results revealed that Pb toxicity has no effect on *A. auriculiformis* plant growth up to 1 g/kg of soil, with the maximum amount of Pb absorbed in soil treated with 1 g/kg of Pb. The bioconcentration factor (BCF), and translocation factor (TF) values were 0.78 and 3.55 respectively, indicating that *A. auriculiformis* is an ideal phytoremediator for soils containing 1 g/kg Pb. In conclusion, *A. auriculiformis* exposed to high Pb concentration (1 g/kg of soil) showed good growth and development, thereby a high tolerance capacity, so it is a suitable candidate for Pb phytoremediation over the short or medium term.

Keywords: Proline, Catalase, transpiration rate, net photosynthesis, bioconcentration factor, translocation factor

1. Introduction

The exploitation of various types of deposits in the underground lead (Pb) to the accumulation of Pb on the surface which has an effective effect on plant growth and development (Adhikari *et al.*, 2014). Extensive researches have been conducted on the early stages of plants' growth and how they can be affected by heavy metals in order to help distinguish species that are heavy metal-tolerant which is crucial for this field of study as with nowadays pollution, the phytoremediation of polluted sites from toxic heavy metals is unescapable (Ali *et al.,* 2013). The high concentration of Pb intervenes various plant physiological process and development such as photosynthesis, mineral nutrition, sugar transport, seedling growth and seed germination (Zerkout *et al.* 2018).

It has been reported that plants are able to manipulate the heavy metal from the soil by restoring it in their roots (Masvodza *et al.,* 2013; Majid *et al.*, 2012; Ochonogor and Atagana, 2014). Phytoremediation has become the way out to extract heavy metal properly without affecting the environment and potentially cost-effective (Illié *et al.*, 2015), especially the phytoextraction technique. Phytoextraction is an important aspect of phytoremediation; it consists especially of the extraction of heavy metals from soil and storing them in roots, shoots and leaves (Souza *et al.,* 2013). One disadvantage of using this technique is that it takes a longer time than other treatments, due to plant limitation, where in most cases high contaminant concentration can reduce the speed of plant growth (Meriem *et al.*, 2015; Ali *et al.*, 2013; Moosavi and Seghatoleslami, 2013). However, fast growing and high heavy metal tolerance plant has been used, which requires an intensive search to identify the potential plant. The choice of phytoremediation candidates is important. Some plants can be short-lived, too small to be significant as pool to contain heavy metals or serve as food for herbivores. Ideal phytoremediation agents should be a hardy plant, long-lived, fast growing, big and inedible.

Acacia plants fit the above description perfectly, they are adapted to a wide range of environments, both tropical and temperate, and this adaptability has made them popular for planting on degraded lands in Asia and elsewhere (Turnbull *et al.,* 1997). *Acacia* spp. have the potential to rehabilitate the soil through absorption and storage of heavy metals their leaves, shoots and roots which makes them the best phytoremediation candidates (Veronica *et al.*, 2011). *Ex-situ* studies using seedling have shown that *Acacia sp.* is able to tolerate and accumulate heavy metals in a different part of the plant (Majid *et al.*, 2012; Mahdavi *et al.,* 2014). *In situ* studies using *Acacia sp.* have also been reported, such as the use of *A. saligna* and *A polyacantha* at the gold mine area in Zimbabwe (Masvodza *et al.,* 2013). In Malaysia, several studies using *Acacia mangium* in ex-tin mine (Ahmad Zuhaidi and Jeyanny, 2018), gold mine (Ahmad Zuhaidi *et al.*, 2018) and sewage disposal site (Mohd *et al.*, 2013) had shown a positive response, especially in translocation of Aluminium (Pb), Ferum (Fe), Zinc, (Zn) Copper (Cu) and Cadmium (Cd). However, *A. auriculiformis* has not been

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tested or reported as phytoremediation agent. Therefore, it is necessary to study other *Acacia* plants that can resist higher concentrations and have similar phytoremediation properties.

Acacia auriculiformis can be a potential candidate in this matter as it produces high biomass yield and well adapts to degraded or poor soil conditions (Sofea *et al.,* 2017). It is a fast-growing multipurpose tree species in the Leguminosae family that can reach 30 m of height and 30 cm of diameter (Turnbull *et al.,* 1997). However, phytoremediation using *A. auriculiformis* species on Pb contaminated soils has never been reported. Based on the attributes mentioned, theoretically, *A. auriculiformis* should be able to tolerate, absorb and accumulate a large amount of Pb from the soil providing a perfect remediation technique to clean up Pb from the soil.

Therefore, the current study was conducted:

I. to examine the capability of A. auriculiformis plants to tolerate elevated Pb concentration,
II. to evaluate the uptake and distribution of Pb in different parts of the plant, and;
III. to determinate the capability of A. auriculiformis plant to remove Pb from the soil.

2. Materials and Methods

2.1. Growth parameters measurement

Germinated seeds were planted in plastic polybags containing a mixture of topsoil and sand, which were artificially spiked with different Pb concentrations (ranging from 0 to 3 g/kg soil) under ambient conditions (Srinivas *et al*., 2013). The pants were arranged in a completely randomised design, and normal watering was performed every 2 days. Physical growth parameters such as shoot height, leaves number, basal diameter, and root length were measured every 3 weeks for a period of 90 days.

2.2. Physiological parameters measurement

Several selected different physiological parameters were measured to determine the plant’s physiological response to Pb occurrence in soil. Net photosynthesis, total chlorophylls, internal CO_2 concentration, transpiration rate and water use efficiency (WUE) were measured directly from the plant leaves using a portable photosynthesis system LI-6400 according to Liu *et al.,* (2014) and Sinclair *et al.,* (1984).

Cell membrane integrity was estimated by measuring the electrolytic conductivity using a conductometer (Pike *et al.*, 1998) by comparing the electrolytic conductivity of fresh leaves submerge in stirred distilled water (EC1) and the electrolytic conductivity of the same sample after heated for 1 hour at 95 °C ± 0.5 °C (EC2). Membrane integrity (%) was based on the ratio between EC1 and EC2.

The relative water content (RWC) was determined based on method by Scippa *et al.,* (2004) following the formula:

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

Where FW is the leave fresh weight; TW is the turgescence weight (TW) and DW is the dry weight (DW).

2.3. Proline and Catalase enzyme activities

The proline or pyrrolidine 2-carboxylic acid was measured using Troll and Lindsay’s (1955) method modified by Magné and Larher (1992). This technique is based on the proline's ability to react in acid and hot environments with ninhydrin to give a pink-coloured compound soluble in organic solvents such as toluene. The optical density of the samples was determined by spectrophotometry at a length of 520 nm. The standard curve was constructed using the series of proline concentrations prepared from a stock solution of 10 µg/ml.

Catalase activity (CAT) was determined as previously described by Weydert and Cullen (2009). Catalase activity was measured by monitoring the decrease in absorbance at 240 nm resulting from the decomposition of hydrogen peroxide (H_2O_2). One unit of catalase activity was defined as the amount of enzyme necessary to decompose 1 µmol/min H_2O_2 in 60 s at 25 °C ± 0.5 °C. The quantity of CAT is calculated based on the molar extinction coefficient $\Sigma = 40\ mM^{-1}.cm^{-1}$.

2.4. Quantification of Pb in plant parts.

The direct aqua regia method by McGrath and Cunliffe (1995) was used for the analyses of Pb concentrations in the samples. The Pb concentration in the sample was determined by using an air/acetylene (2.5: 15.0 L/min) flame atomic absorption spectrophotometer (AAS) at wavelengths of 283.31 nm (Lakshmi *et al.,* 2015).

2.5. Quantification of macronutrients in Acacia auriculiformis plants

Root sample solution was prepared as recommended by Abuye *et al.,* (2003) and quantification of macronutrient in the solution was then analysed by using air/acetylene (2.5: 15.0 L/min) flame atomic absorption spectrophotometer (AAS) at wavelengths of 239.856 nm for *Calcium* (Ca), 202.588 nm for *Magnesium* (Mg), 404.414 nm for *Nitrogen* (N), 213.618 nm for (P), and 766 nm for *Potassium* (K) (Silvana *et al.,* 2010).

2.6. Determination of Pb removal factor

To examine the ability of the plant to accumulate Pb with respect to its concentration in the soil and plant potential to transfer metals from the roots to shoots and leaves, the bioconcentration factor (BCF) and the translocation factor (TF) were calculated based on the following equations 1 and 2, respectively (Majid *et al.,* 2012):

$$BCF = \frac{[\text{Pb concentration in plant tissue}]}{[\text{Initial concentration of Pb in soil}]} \quad \text{(Equation 1)}$$

$$TF = \frac{\text{Pb concentration (stems +leaves)}}{\text{Pb concentration (roots)}} \quad \text{(Equation 2)}$$

3. Results

3.1. The effect of Pb on A. auriculiformis growth parameters

Figure 1 shows the effect of Pb concentrations on the growth parameters of *A. auriculiformis* plants after twelve weeks of growth. The growth parameters of *A. auriculiformis* seedlings in different treatments varied significantly compared to the control during the whole period of growth. There was no significant difference between the control treatment and the plants treated with 1 g/kg Pb. However, plants grown in 2 and 3 g/kg Pb-treated soil had more yellow and wilted leaves with smaller buds in comparison to the control.

After 3 weeks, plants grown in 1 g/kg Pb-treated soil showed no significant difference compared to the control for all the measured parameters. Increasing the Pb concentration up 2 g/kg Pb-treated soil had led to a decrease in the shoot height, basal diameter, leaves number and root length by 52%, 16%, 31% and 65%, respectively compared to the control (Figure 2).

Figure 1. The effect of different Pb concentrations on shoot height, basal diameter, leaves numbers and root length of *A. auriculiformis* plants after twelve weeks of growth.

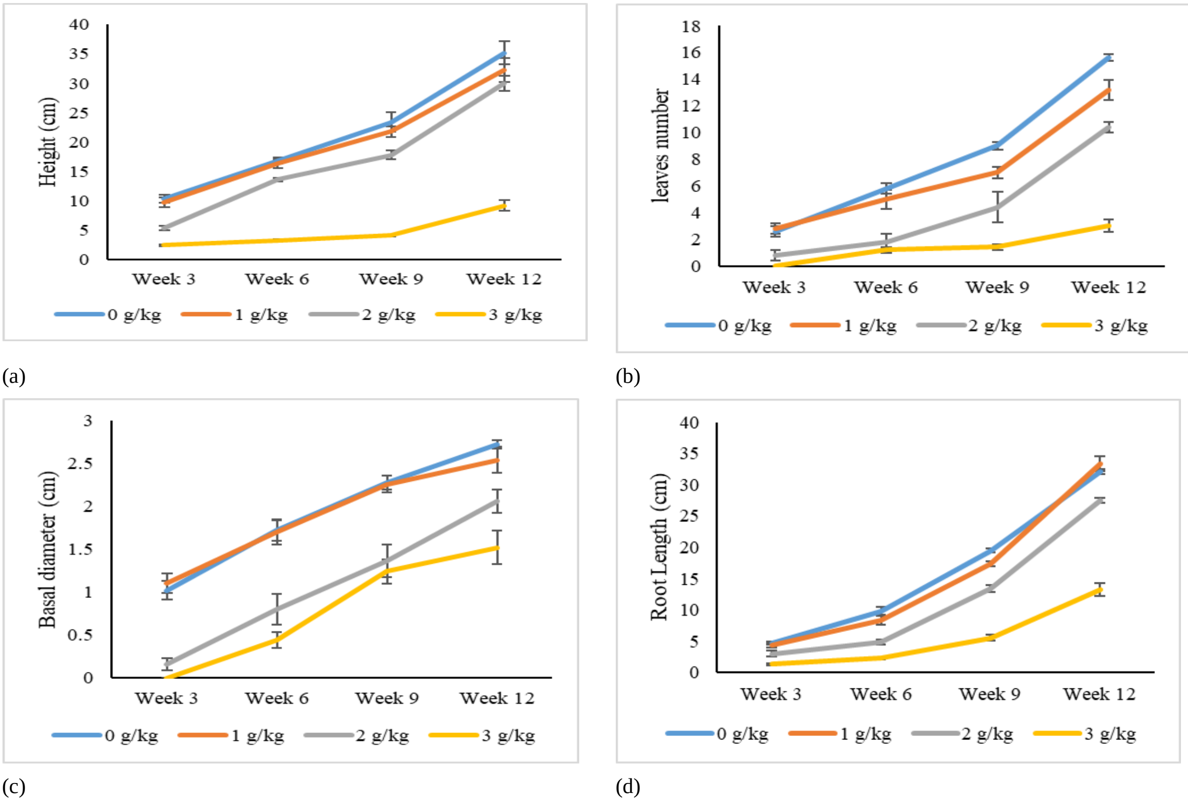


Figure 2. The effect of Pb on (a) the shoot height; (b) the leaves number; (c) the basal diameter and (d) the root length of *A. auriculiformis* plants.

Lead had no effect on *A. auriculiformis* plant growth up to a concentration of 1 g/kg Pb-treated soil, as there was no significant difference in the growth performance of *A. auriculiformis* seedlings between the control treatment and plants grown in 1 g/kg Pb-treated soil.

3.2. The effect of Pb on A. auriculiformis physiological parameters

The effect of different Pb concentrations on some physiological parameters such as the net photosynthesis, total chlorophylls, transpiration rate, the internal CO_2 concentration, water use efficiency (WUE) and the relative water content (RWC) in *A. auriculiformis* is shown in Table 1. The Pb stress at 1 g/kg and 2 g/kg Pb-treated soil showed a reduction on net photosynthesis, total chlorophylls, transpiration rate and the internal CO_2 concentration in *A. auriculiformis* compared to the control. The plant showed Pb stress at 1 g/kg Pb-treated soil with decreased in WUE by 12% and the RWC by 15% significantly compared to the control. However, *A. auriculiformis* showed no significant difference between 1 g/kg and 3 g/kg Pb-treated soil for WUE and between 2 g/kg and 3 g/kg Pb-treated soil for RWC, thus indicating that the effect of Pb on *A. auriculiformis* physiological properties may vary depending on the concentration.

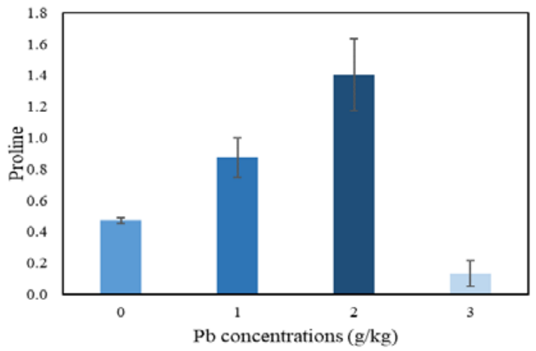
Table 1. The effect of Pb on selected physiological properties in *A. auriculiformis* leaves after 90 days planted in soil supplemented with Pb

Pb (g/kg)	Net Photosynthesis (Mmol/M^2/S)	Total Chlorophylls	Internal CO_2 Concentration (mol/mol)	Transpiration Rate (Mmol/M^2/S)	WUE	RWC
0	5.60 ± 0.34^a	54.66 ± 1.6^a	217.83 ± 4.6^a	2.66 ± 0.17^a	2.40 ± 0.08^a	68.95 ± 1.5^a
1	3.59 ± 0.19^b	43.48 ± 0.2^b	169.83 ± 6.6^b	1.55 ± 0.11^b	2.11 ± 0.25^b	58.39 ± 1.8^b
2	2.60 ± 0.18^c	41.83 ± 0.3^b	169.17 ± 4.5^b	1.33 ± 0.08^b	2.00 ± 0.24^b	55.67 ± 2.8^c
3	1.71 ± 0.15^d	35.81 ± 0.8^c	146.67 ± 2.6^c	1.11 ± 0.46^c	1.55 ± 0.15^c	55.83 ± 0.8^c

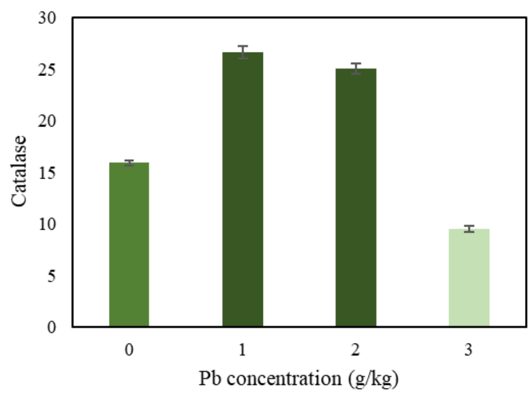
3.3. Proline and Catalase response towards Pb

The results related to Pb effect on proline content in *A. auriculiformis* plant are shown in Figure 3a. The highest proline content was recorded in *A. auriculiformis* plant treated with 2 g/kg Pb-treated soil (1.40 mg/ml) followed by 1 g/kg Pb-treated soil (0.87 mg/ml). Increasing Pb concentration up to 3 g/kg of soil had significantly decreased proline content to 0.13 mg/ml. Proline accumulation in *A. auriculiformis* plants treated with 1 g/kg and 2 g/kg Pb-treated soil explained the high water content relatively close to the control (Table 4.1).

The effect of Pb on the catalase (CAT) activity in *A. auriculiformis* plant (Figure 3b), indicating that the Pb caused a significant overproduction of the CAT enzyme in *A. auriculiformis* plants grown in 1 g/kg Pb-treated soil, which was the highest (24.45 µmol.min^{-1}.mg^{-1} protein), followed by plants grown in 2 g/kg Pb-treated soil (22.35 µmol.min^{-1}.mg^{-1} protein). The lowest production of CAT enzyme was observed in plants grown in 3 g/kg Pb-treated soil (13.71 µmol.min^{-1}.mg^{-1} protein).



(a)

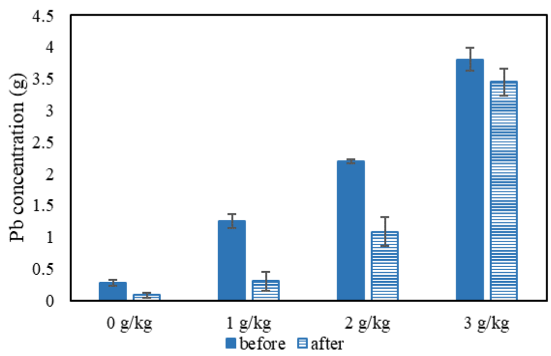


(b)

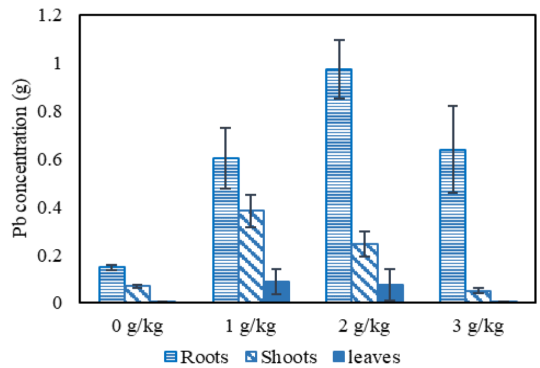
Figure 3. The effect of Pb on the (a) Proline and (b) Catalase activities in *A. auriculiformis* leaves.

3.4. Pb storage capability in different part of plant

Lead concentrations in the soil before and after planting *A. auriculiformis* were significantly influenced by Pb concentration (Figure 4a). Comparison of Pb concentration in soil between before and after *A. auriculiformis* planting showed that the lowest Pb reduction in soil was 0.28 g by control treatment, while the highest Pb concentration reduction was noted in the plants grown in 2 g/kg Pb-treated soil followed by 1 and 3 g/kg Pb-treated soil at 1.12 g, 0.95 g and 0.36 g, respectively Lead was detected in all plant parts of *A. auriculiformis*, but its distribution was not equal across parts as illustrated in Figure 4b. Roots contained the highest Pb concentration compared to other parts, followed by shoots and leaves. In roots, plants grown in 2 g/kg Pb-treated soil showed the highest Pb accumulation (0.97 g) followed by plants grown in 1 g/kg and 3 g/kg Pb-treated soil at 0.64 g and 0.60 g, respectively.



(a)



(b)

Figure 4. The concentration of Pb (a) in the soil before and after planting *A. auriculiformis* plant, and (b) in the different part of the plant.

3.5. Impact of Pb on the distribution of macronutrients

The effect of Pb on the distribution of selected macronutrients in *A. auriculiformis* plant is shown in Figure 5. It is clearly presented that Pb induced changes in the uptake of macronutrients by *A. auriculiformis* plant. Where in general, an increase in Pb concentration on soil had produced declined pattern in the distribution of micronutrient in *A. auriculiformis*. The results showed no significant difference in the macronutrients concentrations in plants grown in 1g/kg Pb-treated soil and the control treatment. The concentration of 2 g/kg Pb-treated soil reduced the concentrations of N, P and K by 77%, 60% and 48%, respectively compared to the control, while Ca and Mg were relatively stable. The high concentration of Pb (3g/kg) affects all macronutrients content in *A. auriculiformis* plant as the concentration of all macronutrient had been decreased compared to the control (88% N, 86% P, 48% K, 19% Ca and 80% Mg).

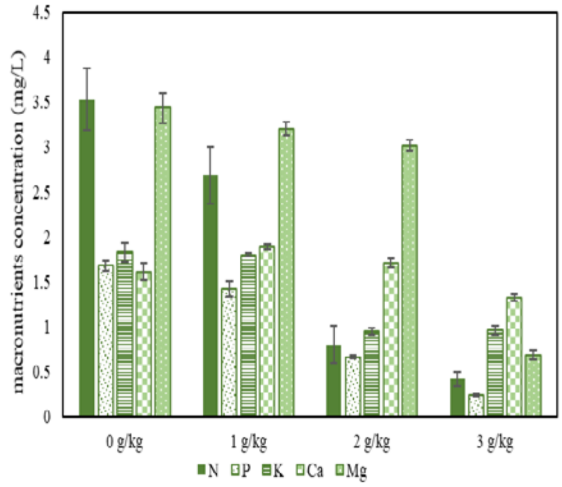


Figure 5. The effect of Pb toxicity on the absorption of macronutrients in *Acacia auriculiformis* plants.

3.6. Evaluation on potential to absorb and accumulate Pb

The bioconcentration factor decreased gradually from 3.55 to 1.20 with increasing Pb concentration from 1g/kg to 2 g/kg respectively (Figure 6). The bioconcentration factor was greater than one in all treatments (BCF > 1) indicating the plant's capability to translocate Pb from the soil to the roots which suggests that *A. auriculiformis* is a potential candidate for Pb phytoremediation. The translocation factor (TF) in A. auriculiformis plant is shown in Figure 6. Plants grown in 1 g/kg Pb-treated soil showed highest TF value (1.28) followed by plants grown in 2 g/kg Pb-treated soil (0.33), whereas the lowest TF value was observed in plant grown in 3 g/kg Pb-treated soil (0.08).

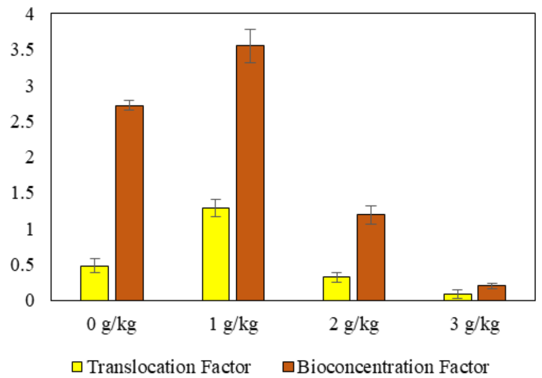


Figure 6. Values of the bioconcentration factor (BCF), and translocation factor (TF) in *A. auriculiformis* under different Pb concentration.

4. Discussion

Acacia auriculiformis seedlings grown in 1 g/kg Pb-treated soil showed high tolerance toward Pb toxicity, and this tolerance is ensured by various defence systems responsible for capturing and neutralizing the metal, and for eliminating and replacing damaged molecules (Pan *et al.*, 2011). These defence systems are usually present in the cytosol, and in different organelles, such as chloroplasts, mitochondria and peroxisomes (Del Rio *et al.*, 2006). Plants grown in 2 g/kg and 3 g/kg Pb-treated soil showed symptoms of Pb toxicity, which was observed in a reduction of plant height, leaves number, basal diameter, and root length. These results are concurring with Kanwal *et al.*, (2014) who found that high Pb doses reduced plant height and biomass, and long-term Pb exposure, even at non-lethal doses, led to necrosis at the root apex and leaves, as well as leaf chloroses. In the present study, all observed disturbances could be the result of Pb interaction with different cellular components and macromolecules, disrupting many physiological processes such as water status regulation, mineral nutrition, respiration, or photosynthesis (Ali *et al.*, 2012). According to Muhammad *et al.*, (2008) the reason for seedlings' high decrement could be the reduction in meristematic cells present in the shoot and some enzymes contained within the cotyledon and endosperms. When a metal is present in assimilable form and in very large quantities, the plant will be enriched with this metal, and above a certain rate, the plant metabolism will be reduced, the yield of the crop decreases and in extreme cases the death of the plant can occur (Ochonogor and Atagana, 2014).

The reduction in the selected physiological parameters had been observed in this study. The Pb toxicity had caused significant disruption on the biochemical pathway of the photosynthesis by distorting the chloroplast ultrastructure by either, (i) directly disrupting chlorophyll synthesis, plastoquinone and carotenoids via the inhibition of δ-aminolevulinic acid dehydratase and protochlorophyllide reductase (Pereira *et al.*, 2006; Tang *et al.*, 2008), or (ii) acting on the transport of electrons and enzymes in Calvin cycle (Rubisco in particular) causing a reduction in the chlorophyll content (Chatterjee and Chatterjee, 2003). Subsequently, Pb can also be the main cause of deterioration of thylakoid and chloroplast structure and composition, leading to photosystems damage (Ali *et al.*, 2012; Huang *et al.*, 2013).

By closing the stomata, Pb considerably affects the electron transport chain, restricting gases exchange between the leaves and the atmosphere and leads to significant reduction in CO_2 flow and fixation (Pourrut *et al.*, 2011). Decreasing the fixation of CO_2 and the transpiration rate of the stomata by Pb led to a decrease in the water use efficiency (WUE), which refers to the ratio of water used in plant metabolism to water lost by the plant through transpiration. The present study showed that Pb stress applied at 1 g/kg Pb-treated soil had caused drop in WUE and RWC. Sharma and Dubey (2005) reported the same response on WUE and RWC in *Lathyrus sativus L.* due to Pb exposure. RWC decrement indicates that the excess concentrations of Pb affect root growth by decreasing the formation of root hairs and causing structural changes thus affecting water flow into and within roots, which reduces water uptake and its transport to the shoot (Kastori et al. 1992). The limited effect of Pb on WUE and RWC (up to 1 g/kg of soil) indicated that *A. auriculiformis* tolerates the presence of Pb by controlling water loss (15% compared to the control). This effect may result from effective stomatal regulation in cells turgidity (Chandra *et al.,* 2016). To maintain this turgidity, plants trigger other tolerance mechanisms that contribute to the adaptation of osmotic and ionic stress caused by metals, and allow the internal osmotic pressure to be adjusted due to electrolytes and organic solutes mainly from soluble sugars and amino acids, such as proline (Taji *et al.*, 2004; Denden *et al.,* 2005).

When the oxidative stress is too high and outstrips the antioxidant capacity, excess radicals cause damage to plant molecules leading to a disruption of many physiological processes such as photosynthesis and respiration (Cecchi, 2008; Agati *et al.,* 2012). At this point, the appearance of visible symptoms take place, such as browning of the roots, as well as chlorosis and necrosis on the leaves, leading to a disruption of growth that can even lead to the death of the organism. Proline is the most important amino acid that accumulates under heavy metals stress. Hence, proline accumulations in plants grown in 1 g/kg and 2g/kg Pb-treated soil proves that *A. auriculiformis* has a high tolerance potential against Pb toxicity because proline plays three major roles under metal stress; (i) acting as an excellent osmolyte, (ii) as a metal chelator (Ashraf and Foolad, (2007), and (iii) inhibitor of lipid peroxidation, thus protecting plants from oxidative stress and plays a key role in heavy metal tolerance (Ullah *et al.* 2019). Even though proline is not the only defences involve since total antioxidant activities were not correlated to their phenolic and flavonoid contents (Hamli *et al.* 2017), plants with high proline accumulation were able to tolerate or accumulate a higher concentration of metals (Ahmad *et al*., 2015). In the present study, the phytoremedial potential of *A. auriculiformis* plants was assessed by the CAT enzyme content, which is considered as a defence mechanism that allows the plant to combat this damage. The increment in the CAT enzyme in the plants are grown in 1 and 2 g/kg Pb-treated soil provides a piece of evidence that *A. auriculiformis* has a good antioxidant defence system to tolerate Pb stress, which is a powerful tool for the survival of metal accumulating plants (Habiba *et al.,* 2015). Catalase enzyme acts as an antioxidant against the reactive oxygen species (Nayana and Malode, 2012). Hence, when cells are in stress condition, they will generate H_2O_2 through emergency catabolic processes, but CAT will degrade H_2O_2 and result in a net gain of reducing equivalents (Afshan *et al.,* 2015).

However, when the capacity of antioxidant processes and detoxification mechanisms are lower than the reactive oxygen species (ROS) production, which was noted in *A. auriculiformis* seedlings grown in 3 g/kg Pb-treated soil, plant damage occurs. High levels of ROS cause inactivation of certain enzymes, decrease enzyme synthesis or change the assembly of enzyme subunits resulting in a reduction in CAT activity, thereby longer H_2O_2 action, which leads to cell disturbances and DNA damage (Lo *et al.,* 2011). *Acacia auriculiformis* showed high accumulation in proline and CAT enzyme content in plants grown in 1 g/kg and 2 g/kg Pb-treated soil to face the overproduction of ROS and the oxidative stress induced by the metal. This finding gave strong evidence of *A. auriculiformis* plant tolerance characteristic, which makes it a potential phytoremediation agent to resist and absorb Pb from the soil. Still, *A. auriculiformis* plant exposed to 3 g/kg Pb resulted in a lower accumulation in proline and CAT enzyme which adversely affect the plant resistance to oxidative stress by the inhibition of cytoplasmic enzymes and damage the structures of a cell (Asati *et al.,* 2016).

The Pb concentration in the soil had appreciable effects on Pb accumulation in *A. auriculiformis* plant tissues, and as the concentration of Pb increased, the transport of Pb from the root to different plant parts decreased in the following order: root > shoot > leaves. The findings pertaining to the Pb uptake in this study agree with John *et al.* (2009) study on *Brassica juncea* roots, as roots are directly subjected to Pb contamination and act as barriers to apoplastic and symplastic Pb transport. Hence, Pb translocation to the aerial part of the plant is disturbed (Page and Fuller, 2015). This may be due to a decrement in lignified cells and xylem vessels, which can be explained by occlusion of the xylem vessels (Dugé de Bernoville, 2009). The phenomena of vascular occlusion are the deposition of a fibrous polysaccharide material, which is the origin of the formation of plugs in the xylem, preventing water supply and consequently, reduction in the vessel's lumen diameter. This obstruction is a defensive reaction of the plant to prevent the flux of Pb which follows the water movement in the plant (Dugé de Bernoville, 2009) and that explains the reduction in the water content observed in Table 4.1. Vessel occlusion has been reported as plant response under metal stress in *Vicia sativa* (Pérez-de-Luque *et al.*, 2006).

According to Wierzbicka, (1987) only a small fraction of Pb presented in the root will be transferred to the aerial parts because more than 90% is found in insoluble form and strongly bound to the outer cellular envelopes. In the present study, 20% and 14% of Pb absorbed by *A. auriculiformis* plants were translocated to the areal parts in 1g/kg and 2 g/kg Pb-treated soil, respectively. This limited transport from roots to leaves was caused by the barrier formed by the root endoderm where casparian strip bands is the major factor restricting the movement of Pb from the endoderm to the central cylinder (Sharma and Dubey 2005),. This restriction of transport to aerial parts represents a tolerance factor for some plants to the presence of contaminants in their growing medium, and it is important for their survival where only a small portion of Pb absorbed and transferred to the leaves, as Pb is a toxic element for photosynthetic activity, chlorophyll and antioxidant enzymes synthesis (Kim *et al.,* 2003).

The competition on the sorption place between Pb ions and the macronutrients in the roots surface especially those with the same valency such as N, P, and K which affects their absorption (Küpper and Kochian, 2010). Therefore, Pb will interfere with nutrient uptake by affecting membrane transport processes and altering the permeability of the plasma membrane (Dong *et al.,* 2006). When Pb attaches to membrane wall components in large quantities, it changes the physical and chemical properties of the wall, and its plasticity. This plasticity reduction affects many cellular mechanisms such as cell division and elongation, which affects the proper functioning of plant cells (Pourrut, 2008). As shown in Figure 2, the symptoms of high Pb toxicity in *A. auriculiformis* plants were expressed by growth inhibition, particularly root growth, by reducing the absorption of water and similarly the absorption of essential nutrients, such as N, P, and K which play a significant role in the metabolism of plants including chlorophyll synthesis, protein analysis, stem and root growth, and enzyme cofactors associated with metabolites transport (Tripathi *et al.,* 2014).

The crucial factors determining *A. auriculiformis* phytoremediation either as phytostabilisation or phytoextraction may lie in the translocation process of Pb from the roots to the areal parts. According to Bongoua-Devisme *et al.* (2019), TF and BFC value will determine the type of phytoremediation agent either as phytoextraction (TF and BCF > 1) or phytostabilisation (TF and BCF <1) or phytostabiliser but act as phytoextract at lower concentrations (BCF > 1 and TF < 1). Oseni *et al.* (2018) reported herbaceous plant, *Sida acuta* and *Chromolaena odorata* only managed to exhibit TF value at 0.9 and 0,7 respectively with most of extracted Pb stored in the roots system. Thus, both plants can only be considered as phytostabilisations agent since the Tf value was less than 1. In the present study, the BCF and TF in 1 g/kg Pb treatments were higher than 1. The high tolerance of *A. auriculiformis* shown previously in growth result, with the BCF and TF greater than 1 makes it an excellent candidate to phytoextract Pb from the soil. However, BCF and TF decreased with the increment of Pb concentration as increasing the concentration of Pb to 2 g/kg and 3g/kg Pb-treated soil hampered normal physiological and metabolic activities. Subsequently *A. auriculiformis* avoids translocation of this metal by the implementation of several mechanisms to reduce the transfer of Pb to the aerial part of the plant. This is an important protective mechanism against the spread of this toxic metal to green tissue (Sinha *et al.,* 2013). Majid *et al.,* (2012) observed a BCF of 1.02 and a TF of 1.89 in *A. mangium* grown in a sewage sludge containing only 19.2 ppm of Pb. However, the concentrations of metals experimented in the media are very low compared to the present study, where *A. auriculiformis* plants grown in 1 g/kg Pb-treated soil showed high tolerance, absorbed 0.64 g in their roots and translocated 0.38 g to the shoots. With the BCF and TF more than 1, *A. auriculiformis* could be a perfect phytoremediation candidate as a Pb phytoextractor in a contaminated soil with no more than 1 g/kg Pb. Although increasing Pb concentration to 2 g/kg of soil reduced and hampered different plant morphological and physiological functions, *A. auriculiformis* plants were able to grow and absorb 0.97 g of Pb in its roots; thus it can be used as a phytostabilizer in a 2 g/kg Pb contaminated soil.

5. Conclusion

Lead accumulation induced both physiological and biochemical changes in *A. auriculiformis* with Pb tolerance proportional to the increased of Pb concentrations and significantly increased level of antioxidative enzymes (catalase). The results demonstrated that *A. auriculiformis* can tolerate Pb toxicity up to 2 g/kg Pb of soil, and hyper–accumulate a significant amount of Pb content in roots and shoots through phyto stabilisation and phyto-extraction.

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