



**DYNAMIC PARTITIONING MODEL OF PLANT GROWTH
UNDER VARYING EXPOSURES OF LEAD (Pb) AND
COPPER (Cu) -
A STEP TO ENVIRONMENTAL SUSTAINABILITY**

Thesis

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UNIVERSITY OF JEMBER**

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COPPER (Cu) -
A STEP TO ENVIRONMENTAL SUSTAINABILITY**

A Thesis Submitted

By

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in partial fulfilment of the requirements for the degree of Master of Science

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2017

DEDICATION

This work is dedicated to
God Almighty, for making everything possible for me to accomplish my work

My Father and Mother for their unconditional love
My husband and children for their patience and always believing in me,

to the university and

to the
Locals farmer who are willing to sustain their precious land and their dedication in
working,

And lastly to the country and the readers that need it.

DECLARATION

The manuscripts entitled Dynamic Partitioning Model of Plant Growth under Varying Exposures of Lead (Pb) and Copper (Cu)-A Step to Environmental Sustainability, is an interdisciplinary research on Mathematical modeling or applied mathematics and physics /biophysics research. The manuscript tried to show that modeling growth is not just a work for one discipline but also a tool for physics or biophysics that could assist in aiding real environmental problems on the local area or global environmental such as lead pollution in soil. This experimental based model could be a tool that can be used in maintaining environmental sustainability.

This work is my own work and has never been submitted for a degree at this or any other university. I declare that this thesis on the part does not contain any material that has been accepted on any degree or diploma at any university. To the best of my knowledge and belief, this work does not contain a copy of paraphrase on this part, except when due to reference in the text of the thesis.

Some parts of the thesis have been submitted to the journal and presented on the conference.

Arry Y Nurhayati

APPROVAL

**DYNAMIC PARTITIONING MODEL OF PLANT GROWTH
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SUMMARY

Dynamic Partitioning Model of Plant Growth under Varying Exposures of Lead (Pb) and Copper (Cu) -A Step to Environmental Sustainability; Arry Y Nurhayati, 151820101022, 2017:138 pages; Mathematics and Natural Sciences University of Jember

Environmental stress has caused reduction on plant growth and is threatening food production sustainability. Heavy metal contamination, such as lead (Pb), is the main environmental stressor that is increasing in developing countries. Pb contamination has led to the increase of food poisoning, the reduction of productivity and triggering farmers to abandon their land. The pressure of maintaining sustainability has also been driven by the potential risk of food insecurity due to the increase of global population. With lower average annual productivity of staple food, the dependency on import markets to fulfill insufficient supplies to boost food production is imperative. For this reason, the research is dedicated for a step forward to environmental sustainability by a proposed framework to achieve two general objectives of maintaining the quality of productivity and safety of product.

Throughout observation on the locals and learning from various problems globally, the research was aimed to obtain three overall objectives of the research; (i) to seek simple mathematical models for the root-shoot partitioning of Pb and Cu effect for plant growth models based on the experimental data; (ii) to predict the relationship of organ partitioning and limitation of growth on stress conditions and its relation to environmental sustainability; (iii) and to attain a profile of growth response of biomass under different exposures of Pb and Cu, and the parameter growth of *Limnocharis flava* as a candidate to be used for phytoremediation.

The current problems of rice production could be done by a strategic frame which involves interdisciplinary research i.e. mathematical and physical-biophysical, to strengthen the environmental sustainability of agricultural land. Under proposed framework, three models based on experiments was developed. First, the shoot root ratio models on the projection doses of Pb and Cu. The

second model was the shoot to root ratio of rice plant under Pb and Cu stress on time projection. And third, the dynamic partitioning model of root, shoot, and leaves under exposure Pb and Cu. Using multi criteria selection including PRESS statistic and index of agreement, the cubic model was selected to be the appropriate model. Further research has also found allometry relations between partitioning organ.

Leave One Out (LOO) was used for a cross validation of the model in achieving consistency within its capability of the model. Using independent data, the Let One In (LOI) approach was used for analyzing the behavior change of the model. The back-casting technique was done for predicting of the data in agreement to the current dynamic partitioning model. The three approaches were found promising to be used in the model for assessing environmental sustainability.

The warning of lead and copper indications are observed by the employment of biophysical measurement. It is done using electrophysiological methods such as by measuring electrical surface potential of leaves of *Limnocharis flava*, measuring electrical conductivity and pH of soil, and measuring and observing the growth parameter and change in leaves. The results found that the availability of Pb and Cu on the soil could change the soil properties and distribution of Fe on the partitioning of plant and this in return could affect the yield. In the success of finding the capability of *Limnocharis flava* in absorbing and distributing Pb through the plant's organ partitions, and through these techniques, we have a bridge for the phytoremediation process.

The research has made a complete effort on ensuring the dynamic partitioning model to be applicable for agricultural benefits or as a frame for environmental perspectives based on experiments and the power of modeling. It is encouraged to make use of the mathematical models in the various applications while the experiments with the collective data should be provided. In accordance to the proposed framework a continuous work is needed on both modeling and environmental sustainability.

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CHAPTER 1 INTRODUCTION

1.1 Background of the Research

1.1.1 Motivation and the Importance of Environmental Sustainability in Increasing Food Productivity

Environmental stress has caused reduced plant growth and is threatening food production sustainability. Heavy metal contamination, such as lead (Pb), is the most common and primary environmental stressor that continues to increase in developing countries. Pb contamination has led to the increase of food poisoning incidences, the reduction of productivity and triggering farmers to abandon their land. This research is dedicated in strengthening environmental sustainability, by taking a step in helping to reduce environmental stress such as global warming, both fulfilling its current urgency in Indonesia and Globally.

The pressure of maintaining sustainability has also been driven by the potential risk of food insecurity due to the global population increase, from around 7.8 billion in 2015 to 9.8 billion in 2050 (Population Reference Bureau, 2015). According to this prediction, Indonesia has an increase in population to around 110 million people, from 256 million to 366 million people. With several issues on food production e.g. lower average annual productivity of staple food i.e. under 5.1 and 4.7 tons /hectare for rice and maize between 2009 and 2015 (Direktorat Pangan dan Pertanian-Bappenas, 2013, BPS Jawa Timur, 2015) and the dependency on import markets to fulfill insufficient supplies (i.e. Indonesia imported around 3 million tons of maize during 2015 as reported by Kompas, 2015), therefore boosting food production is imperative.

Several strategies should be done to fulfill the daily needs of carbohydrates of the population; around 1821 calories or equal to 0.88 kg of rice (Deptan). The dependency of food available in the global market may incur huge costs and should it fail to meet demands, developing countries such as Indonesia will have a third of its population jeopardized due to food shortages. Thus it is imperative to keep the fertile soils in the paddy fields in Java safe and sustainable. Even though

farmer owned rice fields only encompasses 0.5 ha of total rice fields (Swastika, 2008), and keeping environmental sustainability will also mean keeping jobs for the people in rural areas. For the case of local farmers in Jember, East Java with 72% of farmers each only having 0.342 ha of land (Nurhayati *et al.*, 2016; Hariadi *et al.*, 2016) keeping agricultural soil will also mean keeping jobs for the locals and controlling the cost for land and in turn would benefit the agricultural system and promote biodiversity. If the local farmers can sustain productivity, then 15% of national rice production, nearly 30% of production rice of Java would be fulfilled just by farmers in East Java alone, with Java currently producing 48% of rice nationally (BPS Jawa Timur, 2015). Assuming that the size of land would not increase, then sustained productivity could be done through sustained soil growth, maintaining food quality, and strengthening the product chain and or implementing zero waste management by turning the agricultural residue into valuable products. Therefore, the first priority of this research is to shape a standard for quality of soil, yield and product safety. To do so, it needs a model or a frame model for environmental sustainability to be assessed.

Keeping environmental sustainability is not an easy task for farmers. The increase of lead contamination is a major risk to environmental health (Zhai *et al.*, 2015). The persistent use of untreated waste water (Nurhayati *et al.*, 2015); improper balance of fertilizer use (Tiamiyu, *et al.*, 2012); volcanic eruptions (Nurhayati *et al.*, 2017 in progress); and mining could become sources of lead. Persistent lead interaction to the agricultural soil could threaten environmental sustainability, (Nazir *et al.*, 2015, Bhatti *et al.*, 2013; Flora *et al.*, 2012; Nurhayati *et al.*, 2015, Hariadi *et al.*, 2016) as the effect of lead on plants could be lethal.

The potential risk of lead to health (Ahamed *et al.*, 2011; Sharma *et al.*, 2012; Flora *et al.*, 2012), has made lead as the number one heavy metal that should be removed from the surrounding environment (Volesky, 2001; Zhai *et al.*, 2015). Lead can cause many deleterious effects such damage to organs and the nervous system.

Remediation of soil for heavy metal contamination is expensive, but without it, the soil may never return to normal (Nurhayati *et al.*, 2015). Several

methods has been proposed, one of them is by using plants for phytoremediation such as Dukweed (Chaudary *et al.*, 2014; Ugya, 2015; Patel & Kanungo, 2010; Kellaf & Zerdoui, 2010; 2009; Verma & Suthar, 2015). Some edible plants such as *Marsilea crenata* might also be explored as another phytoremediation alternative due to its capability in absorbing lead and distributing it through the plant's aerial parts (Nurhayati, *et al.*, 2015). It can also warn the people of lead contamination.

Limnocharis flava, since it has a similar habitat with rice and *Marsilea crenata*, it might be able to be used for phytoremediation on paddy field soil. The weed has been known for generations and is also used as a source of food by locals in East Java, such as Jember, where it is abundant during rice cultivation season. It is capable of tolerating Pb with a tolerance greater than 1, and translocation factor less than 1 (Rahmadiarti *et al.*, 2012). Learning how the plant responds to varied environmental growth conditions could help its qualities in phytoremediation or as a mean in conserving diversity (Morelli, 2011).

Copper (Cu) is a micro element needed by plants, however high concentrations could be harmful (Appenroth, 2010). Therefore, maintaining the soil under suitable levels recommended for healthy food and soil (FAO/WHO, 2011) is a step for keeping environmental sustainability. This environmental sustainability should become a frame for agricultural practice. Letting environmental sustainability as a framework on agricultural practice has a consequence including the continuity of acts (Hasna, 2007) to support a healthy physical environment (Sutton, 2004) without compromising the needs of future generations (WCED,1987). The framework itself will include adaptation, resilience and conserving diversity (Morelli, 2011).

1.1.2 The Need of Mathematical Model in in Predicting Biomass

Prediction of biomass and yield below and above ground of rice is important in agricultural management. In favourable conditions, healthy plants grow well above and below ground. They are astonishingly adapted to extract energy and nutrients from both environments. However, how environmental

factors effect biomass allocation and what is the relationship between above ground biomass and below ground biomass on the crop plant differs in each shrub (Nie *et al.*, 2015) or woody plant. Understanding the distribution, partitioning and allocation of growth will benefit on managing the strategic effort on increasing yield of productivity. The partitioning of above and below ground biomass has been accepted as the parameter on the amount of differential allocation of plant photosynthates. Predicting yields and monitoring the available soil is a step for environmental sustainability. With the common parameter shoot/root ratio or root shoot ratio as the descriptor of the response to environmental changes, the value might deviate in favorable conditions (Harris, 1992).

Distribution of organ growth or allometry and plasticity in plant (Weiner, 2004) could be used at least for prediction of biomass, global warming potential and greenhouse and bioenergy system (Cunniff *et al.*, 2015, Pawlowski, *et al.* 2017, Schmer 2014; Ben-Iwo, *et al.*, 2016). However, estimates of below ground biomass production and the allocation of total plant biomass above and below the ground in some plant were limited, notoriously difficult and time consuming to sample (Cunniff *et al.*, 2015). Roots are difficult to study because they are hidden within the soil profile and extraction methods destroy samples (Madhu and Hatfield, 2013). The study of plant root is promising, however is less explored compared to growth above ground (Fageria & Moreira, 2011).

Several models of partitioning has been offered from previous research such as from empirical biomass and N model; for forestry, and for mature hybrid poplar plantations (Yin & Schapendonk, 2004, Chave *et al.*, 2014, Poorter, *et al.*, 2015, Fortier, *et al.*, 2015; Pawlowski, *et al.*, 2017, Ben-Iwo *et al.*, 2016). Other models such as allometry are typically measured as root: to shoot ratio of dry mass (Comas, *et al.*, 2013).

The research is aimed to explore the model of dynamic growth above and below ground by measuring the growth effects due to different concentrations of lead in a subsequent time for rice (*Oryza sativa*); and investigating the behaviour of *Limnocharis flava* under the stress. In the research we sought a simple model

for predicting the above and below ground growth of rice (*Oryza sativa*) under different lead concentrations.

Plants respond differently under varying environmental stress (Walters *et al.*, 2013). Plant adaptation is a key factor that will determine the future sustainability of food production due to the severity of climate change effects (Araus, *et al.*, 2008). What the effect of environmental stress on the plant and the production of plant under stress could help people to adapt and form a resilience to such stress. Resilience could be seen as a parameter of the environmental sustainability. Understanding on how the plant survives or adapts under environmental stress could help the people in managing such stress. Monitoring the water and soil should be conducted to prevent unwanted effects in the food chain. While remediation for saline and heavy metals such as lead are expensive but using plants for phytoremediation could save more money. However it will need time and a better understanding of behaviour of plants under stress.

This research could be a step to fill in the gap in the effort on environmental sustainability. Allometry or plant partitioning ratio of above and underground can be used to better understand plant growth. The rate and pattern of plant growth can be controlled by partitioning of assimilated carbon among sink organs.

Growth is a dynamic process. The rate of the growth process is affected by several variations of physical factors such as light intensity and contact duration; temperature and humidity; water and chemistry e.g. nutrient status. Those factors generally affect plants during its photosynthesis activity. Therefore, by developing the dynamic partitioning model based on real data, may benefit the program management and serve a wide range of purposes, saving time and money for the phytoremediation process. Using real experimental data for modelling could have several benefits as the data has been quantified.

1.2 Statement of the Problems

Based on the background, the following statements are intended to describe the problems that are intended to be solved.

- a. How can the dynamic partitioning model of rice be developed from the obtained experiment data explain plant growth parameter under different lead and copper exposure?
- b. How does the dynamic partitioning model be related to plant behaviour under Pb and Cu stresses?
- c. How *Limnocharis flava* responses under different exposure of lead and copper. Could it be used to strengthen environmental sustainability?

1.3 The Objective of the Research

This research is dedicated to strengthen environmental sustainability by taking action on a partial or sub system in environmental sustainability. Therefore, every stage of the research is projected onto the scheme of environmental sustainability to fulfil the following objectives.

- a. To seek simple models for the root-shoot partitioning of new biomass of lead and copper effect for plant growth models.
- b. To predict the relationship of organ partitioning and limitation of growth on stress conditions and its relation to environmental sustainability.
- c. To attain profile of growth response of biomass under different exposures of Pb and Cu, and the parameter growth candidacy plant such as *Limnocharis flava* for phytoremediation.

1.4 Advantage of the Research

Different varieties of crops exhibit noticeable differences in the appearance on induced resistance (Walters *et al.*, 2013). Therefore sufficient data on how plants resist different environmental conditions will have a great advantage in assessing the model, since the performance of the model depends on the sound parameter of the input. This is one of the reasons of conducting the research. A deeper understanding of plant behaviour under environmental stress

and how to anticipate climate change may help in better policy making. Modelling could help on more concise patterns for species that can be used for functioning on the environment or ecosystem (Kramer, *et al.*, 2012). By having the model based on the achieved results, it may help in future multidisciplinary collaboration works resulting in multiple approaches to environmental sustainability and thus boost results for better increase in global food sustainability.

1.5 The Strategy of the Research and Its Limitations

On the first chapter, how environmental stress affects plants are discussed, summarized from the previous research and tied together on the frame of environmental sustainability. This summary will become the framework of this research, and part of the research will support the partial or global objective of the research. While the effect of lead on the *Limnocharis flava* is not modelled, it is measured accordingly as part of the environmental monitoring. The research will have a great advantage both on the preservation of plants and the prevention of loss due to lead and copper effect as a part of keeping environmental sustainability for future farming. The second chapter is a review of the literature for theoretical basis. While the general procedure of the research is described on Chapter 3, the details of the procedure are included on the chapters or sub chapters. The results and discussion will be described on the chapters four, five and six. Two of the three objectives of the research were discussed on results and discussion in Chapter 4, while the behaviour of *Limnocharis flava* will be focused in Chapter 6. Chapter 5 will contain the results on the basic problem solving and a bridge for a mathematical and environmental sustainability model. The general conclusions were summarized for all chapters to answer the general problem and objective of the research. The implication and practical work were given after the conclusion as the concluding remarks and become a recommendation for future works.

CHAPTER 2 LITERATURES REVIEW

2.1 Partitioning Model and the Review on the Previous Result

2.1.1 Concept of Partitioning

Dry matter partitioning is an important concept among ecologies and forestry and it is related to mechanistic model growth on trees and forest (Bartelink, 1998). Several terms are used in allocation of biomass partitioning such as Kage *et al.* (2004) uses the term dry matter partitioning on root growth under stress. Morgado *et al.* (2017) uses the term dynamic and allometric to refer to allocation distribution of plant part, leaves, shoot and root and total dry matter. Poorter *et al.*, (2012) uses the term allocation biomass to describe the relative biomass in each organ. While McConnaughy & Coleman (1999) and Mc Alpine & Jesson (2007) uses the term biomass allocation in plant to describe optimal partitioning theory.

Allometry is the study of the relative growth (Ledig *et al.*, 1970). Several terms are used on the allometry to predict the relation between dry weight above ground and below ground, for instance allometric scaling (Cheng *et al.*, 2014); allometry plastic (Fortier *et al.*, 2015), or both terms, allometric and partitioning (McCarthy & Enquist, 2007). The allometric growth formula to the shoot /root balance has been applied by a formula (Ledig *et al.*, 1970), therefore, if two treatments differ in their effect on shoot-root balance, the slopes of their allometric regression will differ. The slope of the equation accounts for the dynamic aspect of shoot ratio balance, and for this reason alone may be preferred to a static shoot/root ratio which applies to only one transient period in development. The formula claimed to have more biological meaning than any ratio and its use is not restricted to the shoot relationship.

Knowledge of the plant growth and development pattern of wild plants could give information of growth stages on the pattern to analyze behavior of rooting system on the plant (Morgado *et al.* 2017).

2.1.2 Review on the Previous Results

Several models have been established using root and shoot biomass (above ground) in relation to nitrogen (Ågren & Franklin, 2003, Gleeson, 1993) or Nitrogen and its allocation response of plant (Ågren & Kattge, 2017). The root–shoot partitioning can also be investigated for translocation of heavy metals such as Cu and Zn on the polluted soil (Ogunkunle *et al.* 2013). Prasad *et al.* (1996) compared crop growth and relative growth rate of dry matter production. They found that polynomial prediction in sunflower were not realistic in the early stages. The results were also differing on different stages.

The partitioning allometry predicts the relation between dry biomass of organs such as its relation between dry biomass leaves to dry biomass root, shoot and root, or above ground to root (McCarthy & Enquist, 2007; Cheng & Niklas, 2007; Niklas & Enquist, 2002; Cheng *et al.*, 2015). In the ideal condition, the allocation of above dry biomass and root dry biomass will follow according to Cheng *et al.*, (2015) in the equation (2.1).

$$Dw_A = \beta Dw_R^\alpha \quad (2.1)$$

where β is normalization constant and α is exponent scaling, while Dw_A and Dw_R refers to “above ground” and root dry biomass. Here we use the term dry weight to be consistent with the model used in the following section. The transformation of the equation followed the equation of 2.2.

$$\log Dw_A = \log \beta + \alpha \log Dw_R \quad (2.2)$$

Allometric partitioning model predicts that dry weight shoot should be scaled isometrically to root, or allometry scale to the power of $3/4$ between leaves to root or leaves to shoot (McCarthy & Enquist, 2007; Cheng & Niklas, 2007) as the following equation;

$$Dw_L = \beta_1 Dw_R^{3/4} \quad (2.3)$$

$$Dw_L = \beta_2 Dw_S^{3/4} \quad (2.4)$$

where DW_L , DW_R and DW_S are referred to dry weight leaves, root and shoot respectively. β is the allometry constant, which varies between species and varies in environment (Niklas & Enquist, 2002). The value of $\alpha \approx 1.0$ for a small plant (non woody) and for a bigger plant $\alpha \approx 3/4$ (Niklas, 2005).

During seedling it is expected that shoot and root dry matter should scale isometrically (Cheng *et al.*, 2014), therefore;

$$DW_L = \beta_1 DW_R = \beta_2 DW_S \quad (2.5)$$

During the seedling, since the above ground biomass consists of shoot and leaves, the equation of (2.1)

$$DW_A = DW_S + DW_L = \beta_1 DW_R + \frac{\beta_1 DW_R}{\beta_2}$$

$$\left(\beta_1 + \frac{\beta_1}{\beta_2}\right) DW_R = \beta_3 DW_R \quad (2.6)$$

The equation (2.6) implies that the isometric relationship could be derived among the relationship between leaf, shoot and root.

Following the analogy of work of Cheng *et al.* (2014), during seedling of rice refer to the equation;

$$\beta_3 \approx \frac{\text{above dry mass}}{\text{below dry mass}} \approx \frac{\text{shoot}}{\text{root}}$$

The shoot to root ratio (SRR) would be an allometric constant if the above ground dry weight scale is isometrically to the below ground dry weight.

The shoot root ratio usually changes as the plant grows (Wilson, 1988). During vegetative growth, size and time differences are usually equivalent. The difference might be a reflection of size and not a differentiation. Allometric relationship between shoot and root biomass plant ontogeny was further measured by equation (Lohir *et al.*, 2014)

$$DW_s = \beta \times DW_r^\alpha \quad (2.7)$$

The equation (2.7) can be written as:

$$\ln(Dw_s) = \ln(\beta) + \alpha \times \ln(DW_r) \quad (2.8)$$

The allometric coefficient α is the slope of a regression of the natural logarithm of shoot dry weight on the natural logarithm of root dry mass (Lohir *et al.*, 2014). Changing the y and x to the dry weight of partitioning organ the equation also can be written as

$$\log y = \log \beta + \alpha \log x, \text{ which allows a straight-line plot.} \quad (2.9)$$

where

y and x represent the biomass organs Y and X respectively (Poorter *et al.*, 2012)

This model is empirical and does not attempt to explain growth process, but enables plants to express relations in a simple manner between weight of shoot and weight of root, and it makes it easy to see any departure from particular relations, e.g. when the plant starts to flower (Wilson, 1988). By denoting Dw_y and Dw_x as the dry weight of organs Y and X correspondingly. The parameter α represent the relative growth rate (RGR) ratio between organ X and Y, in which it is equal to 1.0 when plant grows in the steady state (Poorter *et al.*, 2012); and can deviate from the unity due to environment and species.

We summarized the relation between partitioning model and plant growth presented on the Figure 2.1. The allometry equation can be changed with respect to the equation of (2.1) to equation (2.9). All the equations from (2.1) to equation (2.9) are actually described as a ratio. The question is the difference in biomass allocation independent of plant ages. Up until now, partitioning model has been included on the crop's model. The concept serves in partitioning of assimilating base between plant organs (Connor & Fereres, 1999).

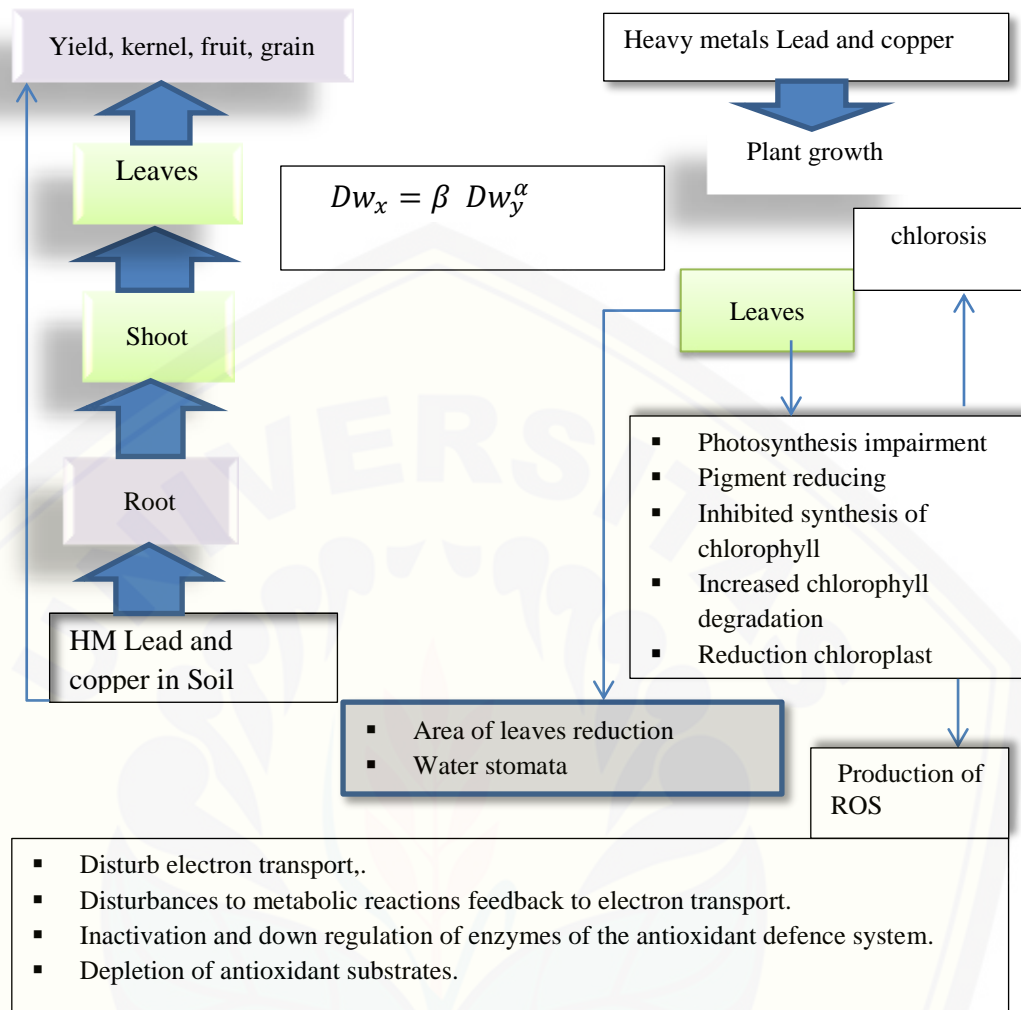


Figure 2.1. Summary of the relation between partitioning model

2.2 Modeling Process and Selection Criteria

2.2.1 Fitting the Model

Even though the effect of lead on the biological system is a more stochastic effect, there are several deterministic models that could be used for fitting the model of experimental data. These are linear model, quadratic model, cubic, exponential, compound and logistic (for modeling and forecasting rice production and price) (Hassan *et al.* 2011; Rahman *et al.*, 2013). These models could also be adapted for the partitioning models. The equation of the models is summarized on the following equation of (2.10) to (2.20) on Table 2.1.

Table 2.1 Potential Model for Partitioning Model

Model	Equation	Equation	Parameter on the predictor and
Linear	$Dw = a + bX + \epsilon$	(2.10)	Dw is dry weight (g/plant):
Quadratic	$Dw = a + bX + cX^2 + \epsilon$	(2.11)	Dw_r , Dw_s , Dw_l
Cubic	$Dw = a + bX + cX^2 + dX^3 + \epsilon$	(2.12)	for root, shoot and leaves
Logarithmic	$Dw = a + b \log_e X + \epsilon$	(2.13)	X is time
Power	$Dw = at^b e^\epsilon$	(2.14)	a, b, c and d are the coefficients of the models
Exponential	$Dw = ae^{bt\epsilon}$	(2.15)	
Compound	$Dw = ab^t e^\epsilon$	(2.16)	ϵ is regression residual
S-shape	$Dw = e^{a + \frac{b}{t} + \epsilon}$	(2.17)	
Inverse	$Dw = a + b/X + \epsilon$	(2.18)	
Growth	$Dw = e^{(\alpha + \beta X)}$	(2.19)	
Logistic	$Y_i = \frac{\alpha_0}{(1 + \alpha_1 \exp(\alpha_2 X_i))} + \epsilon_1$	(2.20)	

The model fitting equation (2.10) to (2.20) are used for predicting the behaviour of growth under partitioning model of dry weight biomass (Dw) of root, shoot and leaves. The equation is also used for shoot root ratio on the concentration base (dose). The different nature of the equation is the interpretation of growth rate that is constant in the linear equation (eq. 2.10), exponential (2.15) and compound (2.16) models. While on the quadratic, cubic, logarithmic and inverses the growth rate changes over time.

Reviewed by Fageria and Moreira (2011), root dry weight of rice will increase in quadratic fashion over time of planting, while shoot dry weight tended to follow linear fashion. Different treatment of rice could differ on the trend model. Table 2.1 gives a possibility of fitting model.

2.2.2 Good Practice Modeling

The first step of the modeling process is to define the objective of the model under the framework proposed. Since the objective of the model is related to the objectives of the whole research, it is consequential that the process model might not have only one model to be developed. However, it is a necessity to conduct a good practice on the modeling process, which involves three non-linear of process modeling analysis, identification and model evaluation. Referring to Cournède *et al.* (2013) the analysis model includes activities such as recognizing behavior of the model theoretically or numerically and obtaining necessary experimental data for parameterization. While on the model identification the process includes confronting the model to experimental data. The model evaluation of the activities could be (i) by checking model qualitatively e.g behavior of the model and its ability to simulate expecting phenomena;(ii) by checking quantitatively e.g. by comparing the model outputs to real data; or (iii) comparing criteria (Baey, *et al.*, 2013).

2.2.3 Objective of the Model

The objective of the model is seeking a suitable dynamic partitioning model for rice plant growth under lead and copper based on the experimental data. The hypothesis of the model growth under different lead is that it will have different behaviors on the model. The different partitioning of rice plant is behaves differently on the model. For the developing model, the collection data has already been obtained from the experiments, therefore the main process model could be model identifying, model analyzing, and model evaluating. The main problem is what models are needed to confront the experimental data.

2.2.4 Selection Criteria

Model selection is an important part of the modelling process in the selecting best fit of the model. Different selection criteria are used by researchers. Several arguments also rise on using the criterion such as for R square or adjusted R square (\bar{R}^2) or (R_{adjust}^2). Since the coefficient determination R^2 , is used in

many experiments of science such as physics, chemistry and others. Here the research is not in the position of arguing the use of R^2 , or R^2_{adjust} . But it takes advantage of using these coefficients for first selection. Then we continue for the next criteria RMSE (root mean squared error), MAE (Mean Absolute Error), MAPE (Mean Absolute Percent Error), and Efficiency model (EF) or the index of agreement (d). Several symbols might be used differently by several researchers depending on the models, and different set criteria used (Cai *et al.*, 2017; Pedersen *et al.* 2004; Mitchel & Makowski, 2013; Zhuang *et al.* 2001 and Baey *et al.* 2012). Here we presented the selection criteria for evaluation or best fitting.

RMSE was used to evaluate the models (Equation 2.21). O_i is the observed value, while P_i is the prediction value. Other equations use sim_i as the simulated value instead of P_i , and experiment value for observed value. Others such as Mitchel & Makowski (2013) use equation term Y_t as the yield at time t, and \hat{Y}_t the fitted yield value obtained at the same time with the model adjusted for all available data, and N is available yield data.

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (O_i - P_i)^2} \quad (2.21)$$

(Cai *et al.*, 2017; Pedersen *et al.*, 2004; Mitchel & Makowski, 2013)

$$RRMSE = \frac{\sqrt{\frac{1}{n} \sum_{i=1}^n (O_i - P_i)^2}}{n} \quad (2.22)$$

$$ME = \frac{[\sum_{i=1}^N (sim_i - obs_i)]}{N} \quad (2.23a)$$

or

$$ME = \frac{1}{N} \sum_{i=1}^N (\hat{e}_i - e_i) \quad (2.23b)$$

(Viana *et al.*, 2012, Cai *et al.*, 2017)

$$MAE = \frac{1}{N} \sum_{i=1}^N |\hat{e}_i - e_i| \quad (2.24)$$

Where N is the number of values in the data set, \hat{e}_i is the prediction value (Viana *et al.*, 2012).

Index of agreement (d) from Pedersen *et al.*, (2004).

$$d = 1 - \frac{[\sum_{i=1}^N (sim_i - obs_i)^2]}{[\sum_{i=1}^N (|sim_i - \bar{obs}| + |obs_i - \bar{obs}|)^2]} \quad (2.25)$$

(Cai *et al.*, 2017, Pardesen *et al.*, 2004)

$$SD = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (e_i - \bar{e})^2} \quad (2.26)$$

Where

\bar{e} is the mean of biomass values of the sample (Viana *et al.* 2012; Cai *et al.*, 2017).

2.2.5 Evaluation the Model

For evaluation of the model or comparison criteria, two criteria were used for the model comparison; the root mean squared error of prediction (RMSEP) and the modelling efficiency (EF) (Baey, *et al.*, 2012). The mean squared error of prediction (MSEP) measures the distance between observed and predicted values, and its squared root is used to obtain the same unit as the observed and predicted values.

$$RMSEP = \sqrt{\frac{\sum_{i=1}^N (obs_i - Predicted_i)^2}{N}} \quad (2.27)$$

The modelling efficiency (EF) is a dimensionless quantity which measures the overall goodness of fits between prediction and observation. It is similar to the coefficient of determination in linear regression (Mayer & Butler, 1993).

$$EF = 1 - \frac{\sum_{i=1}^n (obs_i - Predicted_i)^2}{\sum_{i=1}^n (obs_i - \overline{obs_i})^2} \quad (2.28)$$

$\overline{obs_i}$ is the mean of observed values. The modelling efficiency range between -1 to 1 . The modelling efficiency equals to 1 , in the case of perfect fit i.e. when predicted and observed value are equal. A value of zero corresponds to the case where the model's predictions are not better than the mean of the observed values, and a negative value is obtained when the predictions perform worse than the mean.

Referring to Cournède *et al.* (2013) the analysis model includes activities such as recognizing behavior of the model theoretically or numerically; and obtaining necessary experimental data for parameterization, while on the model identification the process includes confronting the model to experimental data.

On the model evaluation, the process involves checking qualitatively (checking behavior of the model and its ability in simulate expecting phenomena) or quantitatively (by comparing the model outputs to real data) (Cournède *et al.* (2013), or comparing criteria (Baey, *et al.*, 2012).

Several models are used to predict response to predict future observation. A special case for cross validation is used as a part of the data to access how well the model performs. On the leave one out approach, if the primary goal model is predicted it will use the smallest PRESS (Prediction Error Sum of Squares) statistic.

$$PRESS = \sum_{i=1}^n (y_i - \hat{y}_{(i)})^2 \quad (2.29)$$

where

y_i indicates that the $y_i - \hat{y}_{(i)}$ is the PRESS residual. $\hat{y}_{(i)}$ is the predicted value. y_i is the observed value response (Write State University, 2012).

PRESS has been used in several cross validation data, e.g. in principle component analysis (PCA) (Engelen & Hubert, 2004). PRESS is the weighted sum of squares of the predicted residual error (Xu, 2017) of mixed models in genomic prediction or for weighted predicted sum of squares of the predicted residual error.

2.3 Review on the Mathematical Model of Plant Growth

2.3.1 Mechanistic model on plant growth and Evaluating Mechanistic Models on the Effect of Environmental Stresses

Mechanistic models are based on physical selection and physical-chemical relations (Murthy, 2002; Rivera, 2007) that can be used to predict behaviour of a system (Rivera, 2007); or predict interaction of environment (e.g. forest) and interchange of mass and energy (Amthor *et al.*, 1994). On the plant growth model, the mechanistic framework allows the biological law to describe a regulatory model in a mathematical equation (De Vos, *et al.*, 2012).

Some applications of mechanistic model such as the series RLC circuit theory based model provides a relatively complete, straightforward mechanistic framework for evaluating water flow through the soil plant continuum. By analysing how plants configure and correspond to hydraulic architecture could help the plant adapt to water stress by moving sink and source (Zhuang *et al.*, 2014). Application of the RLC model could be on the steady state condition or unsteady state (Steudel, 2000; Zhuang *et al.*, 2014), by application of mass transfer or mass flow, i.e. Poiseuille's law for average rate of fluid on a cylindrical tube. The mechanistic models show the mechanisms or how the parts of the system work together, while empirical models simply describe the data, and do

not exhibit any information which are not comprised of data (Hunt, 1979). In the mechanistic models used in plant growth analysis, model's parameters have physiological significance such as crop growth rate (CGR) and relative growth rate (RGR), therefore, derivation is not necessarily required for growth analysis (Ghaudriaan *et al.*, 2011). It is important to select the best model for analyzing growth (Torabi, *et al.*, 2014) as the parameter's number and their biological concept may be different among growth of a variety over different years or of different varieties within a year. The variation of the models includes the environmental stress such as on Wang *et al.*, (2013), by allowing the possibility of different growth patterns on the mechanistic model application. This also gives a chance to develop a best suited mechanistic model on a different stage and different species growth in a variety of environmental stress. Other examples of simulation model in relation between environmental effect and multi feedback at varying resolution (Tardieu, *et al.*, 2015) should also be explained.

Torabi *et al.*, (2014) used a different mechanistic model for growth analysis of a safflower cultivar at different planting dates and selecting the best model for describing the growth pattern. The model was described by Logistic, Richards, Gompertz, Beta, Truncated expolinear, and symmetrical expolinear. The goodness of fit statistics, standard error (SE) of the parameters estimates, a , and b coefficient, correlation coefficient R and RMSD of linear regression between observed and predicted dry matter weight were calculated, and RMSD remain as the sole criterion for indicating accuracy of model prediction.

Deen *et al.* (2001) reported that the deviation between simulated model and the measured were greater during early stage of growth and development of ragweed. The accuracy is needed for influencing the model. Calibration and statistical analysis is used for evaluation of deviation of model and of experimental data. The process would ensure the model would be appropriate for growth and development of plant. The mechanistic model is currently promoted to social science (Holme & Liljeros, 2015) for understanding the mechanistic model on social science and its influence on natural science.

2.3.2 Biophysical Model of Root Cell Growth

The biophysical model of cell root growth has been proposed by Chavarra-Krauser *et al.* (2005). In the model, biophysical equations are used to describe growth rate. Growth rate is defined as the absolute increment in length, the increment in length per unit time, the relative change in length or the relative change in length per unit time (equation 2.30). Similar definitions were used for wall extensibility in the equation (2.31) to characterize cell wall properties of growing maize root without restricting water supply. The equation is given by Chavarra_Krauser *et,al* (2005) as from Lockart (1965).

$$g = \frac{d\ell}{dt} = \ell\phi (\psi_p - Y) \quad (2.30)$$

and

$$g = \tilde{\phi}(\psi_p - Y) \quad (2.31)$$

where

g is the growth rate (μmmin^{-1}), ℓ is the cell length (in μm), ϕ is the wall extensibility ($\text{MPa}^{-1} \text{min}^{-1}$), ψ_p is the turgor pressure (MPa) and Y is the yield threshold (MPa). $\tilde{\phi}$ is an apparent wall extensibility, given by the product of cell length and wall extensibility ϕ .

Both equations (2.30) and (2.31) with constant ϕ and $\tilde{\phi} = \text{constant}$ held true for their measurements, because these were conducted in a manner to support both equations.

2.4 Heavy Metals Stress, Lead and Copper Effect

Lead (Pb) has been known as a toxic metal, and it is difficult to degrade. Besides the long biological half-life of lead, the function of lead on the plant has not been known. While copper is needed by plants in small quantities, as it is known as a micronutrient. Pb are not essential, since they do not perform any known physiological function in plants (Rascio & Navari-Izzo, 2011). Removing Pb from environment will have a great benefit economically. Accumulation of

lead in crops grown in polluted soil may easily cause damage on human health through the food chain (Volesky, 2001, Cheng, 2003). It is one of the priority heavy metal pollutants that needs to be removed (Volesky, 2001).

Pb poisoning has been recognized as a major public health risk, particularly in developing countries (Flora, *et al.*, 2012). Lead is capable of inducing oxidative damage to brain, heart, kidney, and reproductive organs (Sharma, *et al.* 2012). It is a risk for childhood aplastic anemia (Ahamed *et al.*, 2011); induces effects on cell membranes, and degrades DNA and antioxidant defence systems of cells (Flora *et al.*, 2012, Ahamed, *et al.*, 2011). There is no safe level of lead to be found. Chronic toxicity on blood lead levels is about 40-60 µg/dL (Flora *et al.*, 2012). It affects the haematological system even at concentration below 10µg/dL (Ahamed *et al.*, 2011). Low level exposure to lead, in blood lead levels previously considered normal, may cause cognitive dysfunction; neuro behavioural disorders, neurological damage, hypertension and renal impairment (Patrick,2006; Ishiaq, *et al.*, 2011). Although lead toxicity is highly explored and a comprehensively published topic, complete control and prevention over lead exposure is still far from being achieved (Flora, *et. al.*, 2012). Use of industrial effluent and wastewater on agricultural land has become an accustomed practice in Indonesia, especially in Java, as a consequence of the persistently growing population and industry. Increase in lead concentrations in cultivated soil is detected in close proximity to industrial sites (Hussain *et al.*, 2013). Therefore controlling heavy metal discharges, and removing toxic heavy metals from aqueous solutions and soil become a challenge to the utmost importance (Ashraf & Ali, 2007).

Some methods of removal of heavy metals has been investigated such as biosorption, detoxification (Kumar, *et al.*, 2009, Volesky 2001); extraction, precipitation, ion exchange (Banach *et al.*, 2012); and phytoremediation (Dhir, 2009, Banach *et al.*, 2012), for detoxification of their substrate according to the pollutant. Some plants such as a hyperaccumulator are being proposed, including aquatic fern such as *Azolla Caroliniana wild* (Banach *et al.*, 2012).

2.5 Water Uptake

Water transport on plants involves not only purely on water, but also nutrients, metals or heavy metals, and concentration in salt. In the water scarcity due to a long dry condition or drought, water management is not merely how to use water efficiently, in this environmental condition, but also how to keep plants safe from heavy metals contamination, or saline water. In the case of agricultural activities, understanding plants adapting to a harmful effect will be important, including how to remediate saline or heavy metal contamination. Understanding the process of behaviour of plant under water stress, and the process of plant water uptake will be important in minimizing the losses.

Water uptake and nutrition availability affects root development, and plants can balance their water uptake and loss through coordinated regulation of both stomatal and root development (Hepworth, *et al.*, 2016). Water uptake and transportation play important roles on the environmental stressors such as high salinity, drought and low temperatures (Wang, *et al.*, 2015). Water resource and management of water on agricultural practice are both important in increasing food productivity and commerciality of plants. Controlling water movement could be due to dependence of shoot water potential and root water potential (Rossdeutsch *et al.*, 2016).

2.6. Stress Symptom

Chlorosis on leaf of plant could be a symptom of lead stress on plant. Many agricultural practices rely on the symptom of leaf or stem on the detection of the elemental deficiency such as necrosis or chlorosis due to too little plant nutrition research available (Wong, 2005). As Mg is the central atom of the chlorophyll molecule, the molecular basis for Mg deficiency symptom is not fully understood, and the interaction between Mg deficiency and the regulation of Mg²⁺ transport are unclear (Kobayashi & Tanoi, 2015).

Three basic tools are used for diagnosing nutrient deficiencies and toxicities for soil testing, plant analysis and visual observation on the field (Mc Cauley, 2011). Magnesium are actively involved in the photosynthesis as a

component of chlorophyll and also plays important roles in plant respiration and energy metabolism (Agronomy Fact Sheet Series, 2011). Agronomy fact sheet series 11 stated that magnesium deficiency can occur in acidic and sandy soils, or soil with high levels of Ca and K.

Plants exhibit a series of physiological response to Mg availability, including morphological and architectural responses on the root system, but little attention is given on root growth on the response of Mg deficiency compared to the excess Mg deficiency (Niu *et al.*, 2014). Reviewed by Guo *et al.*, 2016, Mg deficiency involves leaf chlorosis activities, photosynthesis, antioxidative enzyme, sucrose accumulation, transpiration, and root activities such as antioxidative enzymes, root hair and primary root, Ca^{2+} , transpiration. Mg is a key element for cell metabolism, and deficiency of it leads to inactivity in critical metabolic points such as CO_2 fixation, carbohydrate up-loading to phloem, ATP –ases activity (Esfandiari *et al.*, 2010).

Root fresh biomass of *Arabidopsis thaliana* decreases from its control on the high concentration of Mg and increases on the lower concentration Mg than its control (Niu *et al.*, 2014), this shows that it might be consistent with the decrease of root hairs. Root transport is the result of various root membranes with distinct transport properties that can be nutrient and plant species dependent.

2.7 Environmental Sustainability: The Proposed Framework Model

It has been mentioned on the chapter 1, that environmental sustainability became a frame work on the model developed. Numerous concepts of sustainability has been used up until now, many arguments has rose due to different concepts of the researchers' and institutions. Here we used the following concepts of sustainability; i.e the broad concept of sustainability of Brundtland (WCED, 1987), physical concept of Sutton (2004), Folke *et al.* (2002), Morrelli, (2011) and Hasna (2007).

The following concepts are some references of environmental sustainability concept, development sustainability or environmental sustainability.

“A development to meet the needs of the present without compromising the ability of future generations to meet their own needs” (WCED, 1987).

A similar concept of environmental sustainability from Morelly (2011) is,

“a meeting resources and services needed by the current and future generation without compromising the health of the ecosystem..”

According to Sutton (2004), sustainability is

“ the ability to maintain quality in which valued from the physical environment”.

According to Morelly (2011),

“A condition of balance, resilience and interconnectedness that allow society to satisfy its needs while neither exceeding the capacity of its supporting ecosystem to continue to regenerate the services necessary to meet those needs nor by our actions diminishing the biological diversity”

From Hasna (2007), sustainability is,

“Continually act responsible within the limits of environment”.

Referring to four above concepts, it appears that increasing food productivity, keeping the yield quality, protecting local plants (as source of food, nutrient and energy) and keeping the capability of soil in producing is the important component of environmental sustainability.

The relationship between plant and the growth process is summarized on the Figure 2.2. The figure has also become the proposed frame for guiding the research or roadmap, with focus in feasibility of time and resources. In the Figure, safety products from heavy metals contamination is a part of the keeping environmental sustainability. In the increasing population and industrial activity, untreated industrial waste water might contaminate water. Heavy metal contamination such as lead (Pb) causes plants to be poisoned and can be harmful through the food chain. Lead contamination, is not merely due to agricultural practice on urban area by using irrigated water from the industry source of Pb (Nurhayati, *et al.*, 2015), but could also be from using improper fertilizer as in the rural areas (Hariadi *et al.*, 2016). Keeping plants from interacting with a source of

heavy metal by proper water management is to the utmost important on agricultural practice in developing countries. Plants may respond differently to environmental stress. In adverse conditions or harmful environments, it is possible for a wild, native, or indigenous plant to survive or go extinct. Therefore the need for environmental sustainability by preserving plant growth is urgent while keeping the availability of food supply.

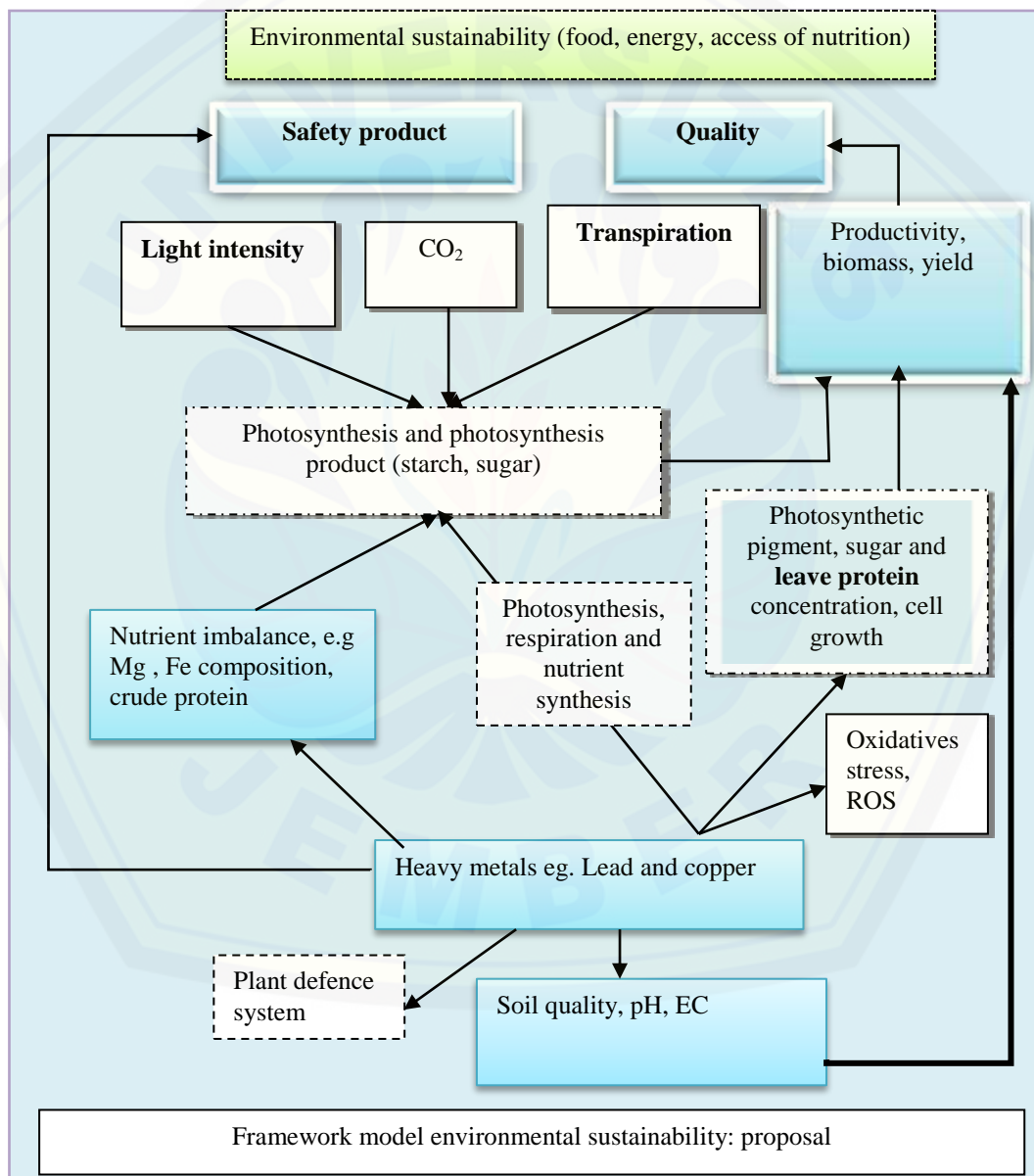
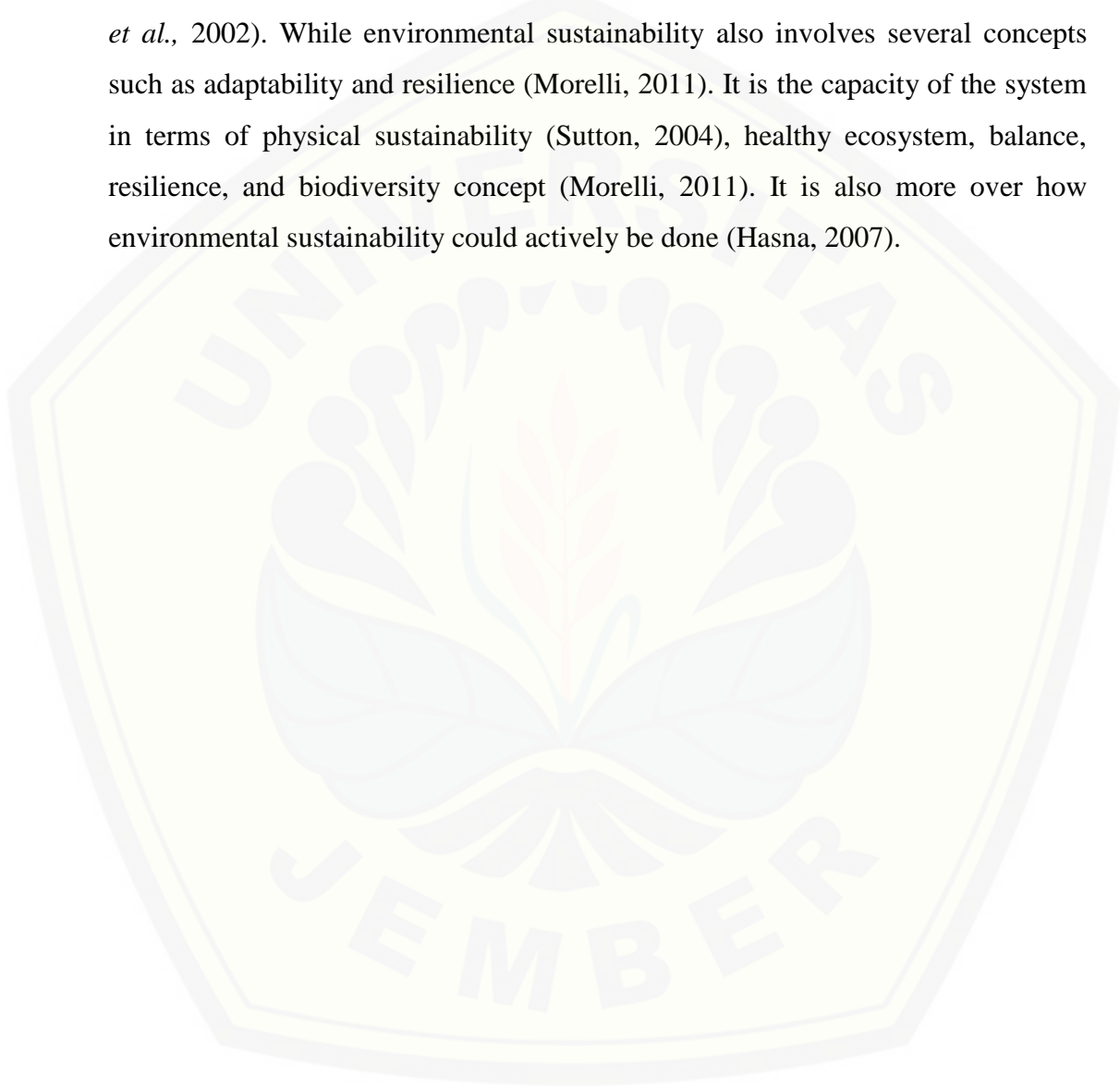


Figure 2.2 Summary of environmental stress and environmental growth of plant and framework model of environmental sustainability

Preserving native plants for future generations or keeping environmental sustainability are both part of sustainable development that may also strengthen the local farmers on keeping their land for food. Sustainability here is the ability of a system to bounce back from shock and stress and adopt stable states (Folke, *et al.*, 2002). While environmental sustainability also involves several concepts such as adaptability and resilience (Morelli, 2011). It is the capacity of the system in terms of physical sustainability (Sutton, 2004), healthy ecosystem, balance, resilience, and biodiversity concept (Morelli, 2011). It is also more over how environmental sustainability could actively be done (Hasna, 2007).



CHAPTER 3 MATERIAL AND METHOD

3.1. The Experiment Procedure

All the experiments were conducted at the University of Jember-East Java Indonesia. The experiments were conducted on two time bases, first during June to October 2016, and second were conducted on May to August 2017. Glass house experiment and Biophysics measurement were conducted in a Biophysical lab while content analyses were done on the chemistry lab. The stages of research followed Fig. 3.4 on the end of the Chapter 3, and guided by our proposed sustainability frame (as roadmap) presented on Fig. 2.2.

3.2 Plant Material and Growth Condition

3.2.1. Rice Cultivation

Three weeks old (22 day-old) uniform and healthy seedlings of rice (*Oryza sativa*) obtained from local nursery of Mayang Jember were replanted on plastic pots filled with all purposes growth fertile soil obtained from Puspa Alam Nursery Jember and watered with tap water.



Fig. 3.1 Rice (*Oryza sativa*) recultivated and adapted in approximately 8 hours of full light

After four weeks of growth the plants were watered with 200 ml modified Hoagland solution with different compositions of lead (0 ppm for controlled plant, 3, 10, 20 and 50 ppm), and different copper composition (0.04 ppm for controlled plant, 10 ppm, 50 ppm, 100 ppm and 200 ppm). All treatments were five times replicated. The composition of modified Hoagland solution is presented in Table 3.1 (Hariadi and Shabala, 2004).

Table 3.1 Composition of modified Hoagland's solution (adapted from Hariadi and Shabala, 2004) for strength growth

Chemical compound	Concentration
Macro-nutrients (in mol m⁻³)	
Ca(NO ₃) ₂ ·4 H ₂ O	5.0
KNO ₃	5.0
KH ₂ PO ₄	1.0
(NH ₄) ₂ SO ₄	1.0
MgCl ₂	0.8
Micronutrients (in 10⁻³ mol m⁻³)	
H ₃ BO ₃	1.0
MnCl ₂ ·4 H ₂ O	1.0
ZnSO ₄ ·7H ₂ O	1.0
CuSO ₄ ·5 H ₂ O	1.0
NaMO ₄ ·2H ₂ O	1.0
Fe EDTA	1.0

3.2.2 *Limnocharis flava* Cultivation

Samples of the plant *Limnocharis flava* with similar growth were collected from local farmers of Jember area, East Java Indonesia. The plants were then replanted in a shade house for adapting and kept for healthy growth for later use. Homogenous plants were selected, replanted on soil media and then were exposed under different concentration of lead (PbO). The levels are 0-ppm as controlled plant, 3 ppm, 10 ppm, 20 ppm and 50 ppm. Every treatment was replicated three times for statistical purposes. The second group treatment was conducted by watering the plant with different concentrations of Cu (0.04 ppm; 10 ppm, 50 ppm, and 100 ppm, and 200 ppm).

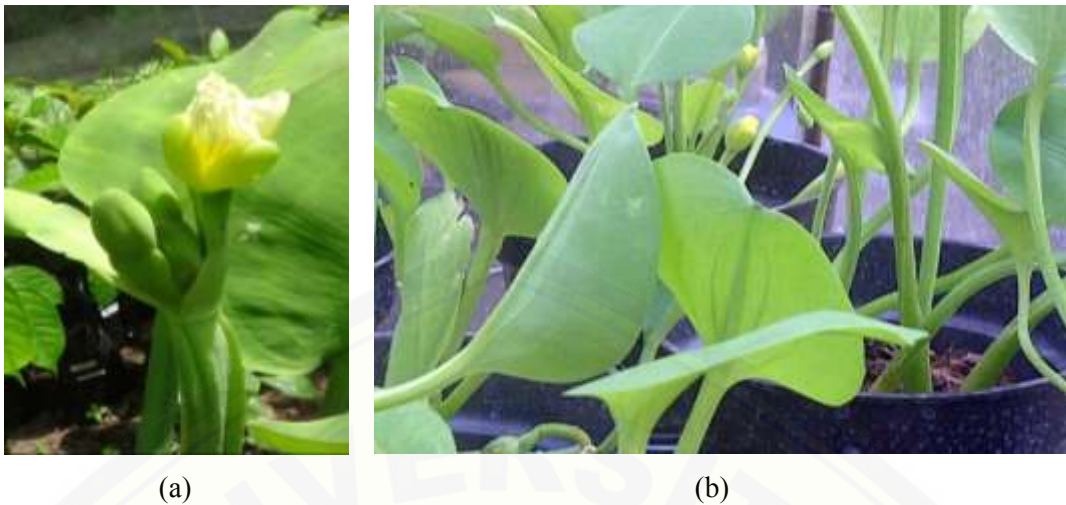


Figure 3.2 *Limnocharis flava* L Buchenau –yellow velvet (Indonesian-Genjer) , Yellow colour of flower, a monocod weed in the limnocharitaceae family (Chandran and Ramasamy, 2015). Wildly grows in a rice paddy area in Mayang (a) and rembangan (b), Jember East Java, Indonesia. The green colour of leaves is slightly different in the areas in between which is slightly yellow.

Growth observation was done by measurement of the plant on four consecutive weeks. The parameter of growth used is using the average leaf areas. The leaf area was measured by multiplying the length, width, and correction factors. The correction factor was done by comparing between the area of leaf measured by millimetres paper and the area from multiplying length and width. All the experiment for *Limnocharis flava* were triplicated.

3.3 Electrophysiology Behaviour of *Limnocharis flava* under Lead and Copper

The electrophysiology behaviours were observed weekly in four consecutive weeks by measuring the leaves' surfaces electrical potential difference (PD). The leaves chosen were from similar positions of plants to make consistent measurements. The electrode wick used cotton and a micropipette with 2 μm of tip of borosilicate glass. The tips were made by pulling the hot glass. Afterwards, the measurement was done according to the method by Hariadi & Shabala, (2004) and Nurhayati *et al.* (2015).

3.4 Rice Growth Measurement For Data Modeling

3.4.1 First Collection Data Samples

The first collection of data were obtained from the first experiment of lead in five different levels of lead (0, 2, 10, 20 and 50 ppm) and copper with five different doses of exposure (0.04, 10, 50, 100 and 200 ppm). It was gathered after first exposure of one week, 3 weeks and one month, by harvesting plant on day-7; day-24 and day -31 (destructive data). The plants were harvested after the correlated above ground measurement such as leaf area, number of leaves, and height of plant measured. Around 150 individual plants were used for destructive measurement. After harvesting, the plants were cleaned and separated from root, shoot and leaves and grain. It was then weighed and oven dried for 103° C for 6 hours.

3.4.2 Second Collection Data Samples

The second collection of data were obtained by planting plants under three different doses of lead (0, 20 and 50 ppm), and three different doses of copper (0, 100 and 200 ppm). Above ground and below ground measurement were done on the same day on a 6 days basis. The plants were harvested, separated, and weighed for fresh weight and dry weight after oven dried for 103° C for 6 hours. In this second experiment (for stress model) the response of rice was also observed after 2 hours exposure. All treatment were five times replicated (around 270 individual plant has been assessed for the model). Data collected was for 0 day (2 hours), 6, 12, 15, 18, 24, 30, 36 days and extended day of day-42.

For the allometry relation, we used separated data that has been collected in the time period (two different harvested times). Around 82 individual plants were used in the allometry. In total around 502 individual rice plant used for destructed measurements.

3.5 Monitoring of Safety Plant

The plant safety is measured by measuring the lead and copper content after the plant being exposed of lead or copper through daily watering for one week (7 days). Distribution of lead and copper on the partitioning organ is analyzed for root, shoot and leaves. The results were compared to the previous results (Nurhayati *et al.*, 2015, Nurhayati and Hariadi, 2014), and consulted to the WHO/FAO codex Alimentarius commission 2011.

The effect of the lead and copper exposure to nutrient content is measured by AAS for Iron (Fe) on the leaves and fruit for *Lymnocharis flava* as both are edible parts.

3.6 Calculation Bio Concentration Factor Estimation (Biological Absorption Coefficient), Translocation Factor and Transportation Relative Index

a. Translocation factor (TF), defined as the ratio of the total concentration of elements in the aerial parts of the plant $[C]_{shoot}$ to the concentration in the root $[C]_{root}$ is calculated as follows:

$$TF = \frac{[C]_{shoot}}{[C]_{root}} \quad (3.1)$$

b. Transportation index (T_i) for Pb and Cu was calculated using the following equation

$$T_i = \frac{Pb \text{ or } Cu \text{ content of the leaves } mg \text{ kg}^{-1}}{Pb \text{ or } Cu \text{ content of root } mg \text{ kg}^{-1}} \times 100 \quad (3.2)$$

c. Bio-concentration factor (BCF) was calculated as Rashid *et al.* (2014).

$$BCF = \frac{\text{Average Pb or Cu content on the plant tissue } (mg \text{ kg}^{-1})}{\text{Spiked Pb or Cu in the soil } (mg \text{ kg}^{-1})} \quad (3.3)$$

d. Dynamic Stress Index (DSI)

The DSI is proposed in an attempt to have a general index for stressor. The index is analogous to the previous index of salinity, that counted by the expression of Dalton *et al.* (2001).

$$DSI = \frac{\text{accumulation of shoot lead}}{\text{total shoot biomass}} \quad (3.4)$$

3.7. Visual Change Observation

Visual change observation is conducted on weekly basis. Qualitative discussion is presented for visual change. Effect on cell growth is observed under microscope for different stress.

3.8 Assessing the Model

The data is processed using SPSS program. The difference between observation and model was compared using criterion on the Table 2.1.

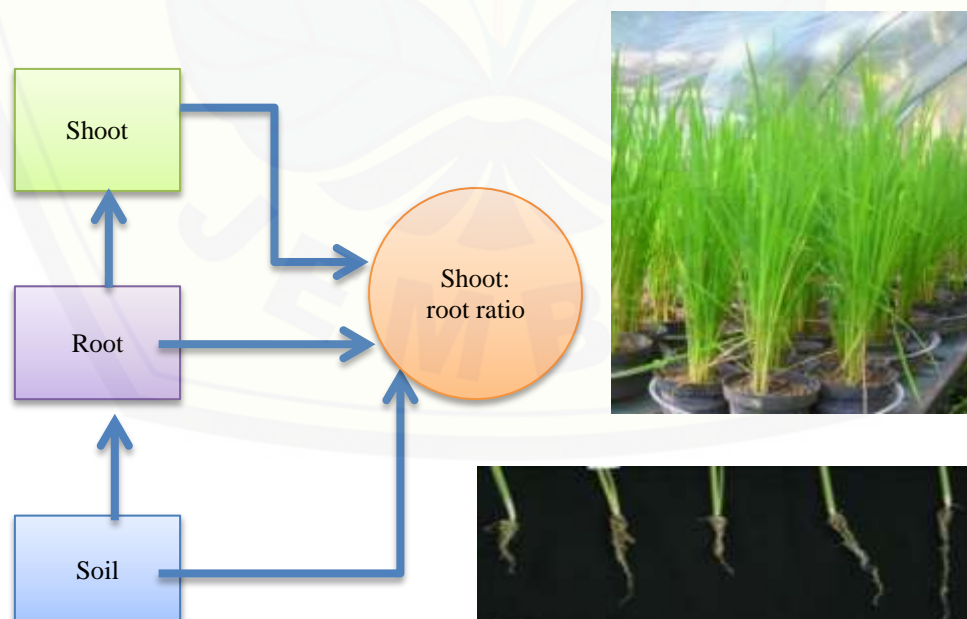


Figure 3.3 The partitioning of shoot and root

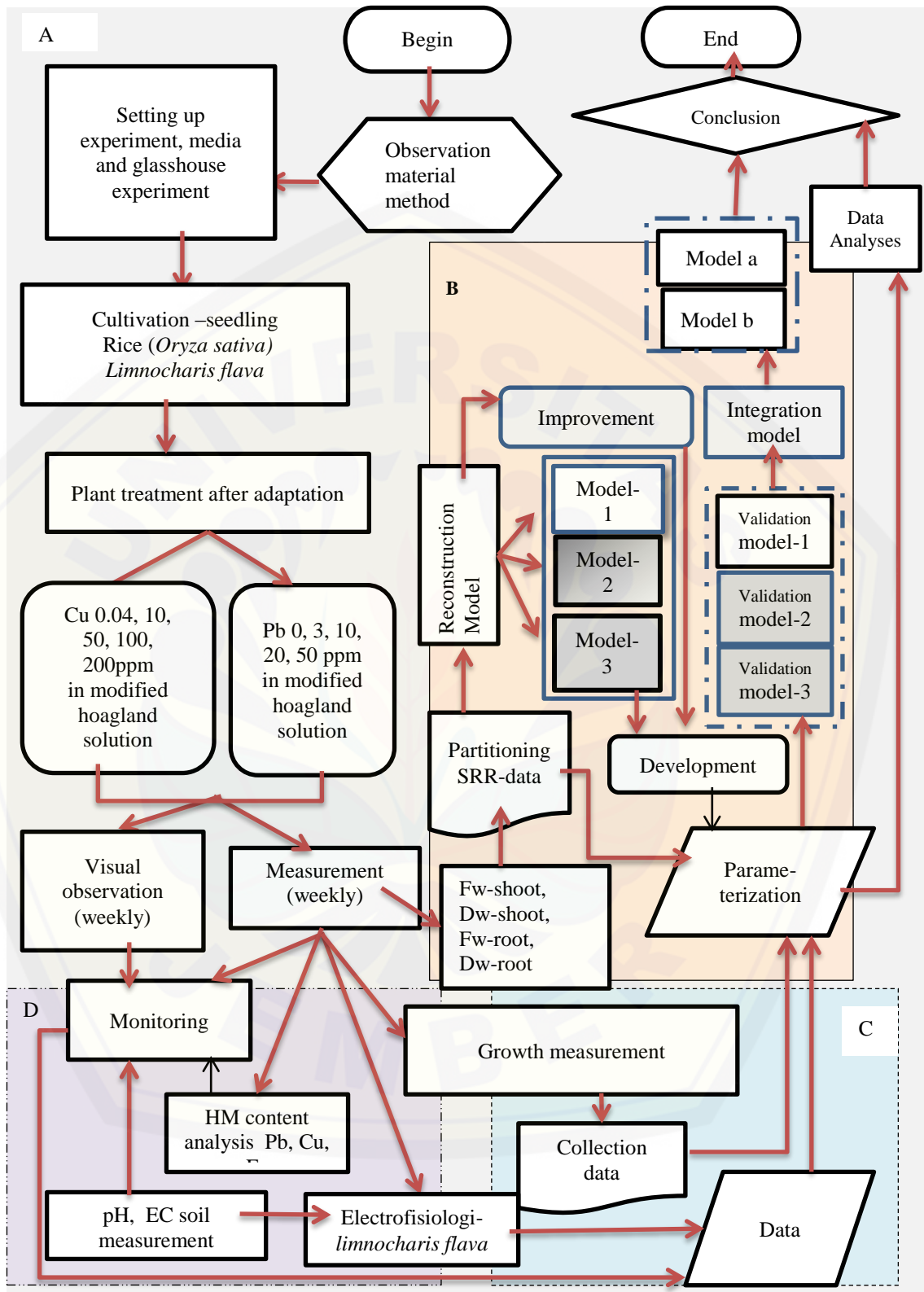


Figure 3.4 The experiment procedure. A setting experiment and cultivation , B-Process model, C. Data collection and growth measurement, D. Monitoring-biophysical measurement and chemical content analysis

**CHAPTER 4 RESULTS AND DISCUSSIONS:
SEEKING DYNAMIC PARTITIONING MODEL OF RICE GROWTH
UNDER VARYING EXPOSURE OF LEAD (Pb) AND COPPER (Cu)**

The aim of this part of the experiment is to seek a feasible dynamic partitioning model of rice plant growing under varying doses of lead (Pb) and copper (Cu) by investigating the effect separately. The model was sought by constructing the model from the profile data of shoot to root ratio from five different exposures of Pb (Pb 0, 3, 10, 20 and 50 ppm), and five different doses from exposure of Cu (Cu 0.04, 10, 50, 100 and 200 ppm). The data were obtained from the first experiment on harvesting during 16 September to 3 October 2016 (Model-1).

The successful results on Shoot to Root Ratio (SRR) model of Pb and Cu on a time exposure given were important mostly in the biological meaning. Here we assumed that the SRR could be a parameter of growth. By this assumption therefore, the value of SRR will differ in plant growth with respect to the age of plants and the phases of the state of life from growth in favoring conditions. The change of value in SRR could also be an indicator of stressor effects of Pb and Cu to the plant. It is important to develop models of simple SRR on the several doses of Pb or Cu (model-1) and the SRR model on the bases of the Pb and Cu that might affect the physiological change on plant growth (i.e. stress) and on the bases of time (model-2).

The models-1 and models-2 (SRR of stress of Pb and Cu on the variation of time) both have consequences since they are actually mere ratio of variables on dry mass or dry weight of two different partitioning organs above ground i.e. shoot) and below ground (i.e. root). The first consequence is related more on the mathematical than biological aspect that improves model-2 with the development model-3 of dry weight partitioning organ root, shoot and leaves. On the process of collection of new data for the process model, we have to carefully consider the physiological change that allows the future enrichment of the models.

On every individual plant as a sample on every treatment, we have to correlate data above and below ground on different variables of growth such as in leaves; average dry weight of leaves, number of leaves, and leaves area. Other variables are the dry weight of shoot and the height of plant. We also consider the grain yield, and physiological change or visual change. This includes below ground measurement of the roots, number of roots and length of root. Our emphasis here is the dry weight, therefore several data were not shown here otherwise stated for discussion. Readers could follow more of the discussion on the following sub chapter on Chapter 5.

The second consequence is from the SRR model-1 as the SRR could be a parameter of growth, therefore the relation of shoot and root could be explained by the allometry relation of partitioning organs, e.g. the relative growth (Ledig *et al.*, 1970; Prasad *et al.*, 1996) and changes of the SRR due to plant growth process (Wilson, 1988); the shoot-root balance; and the relation of shoot or root to another partitioning organ such as leaves with respect to the concept of allometry or isometric on the condition of equations 2.1 to equations 2.9. The discussions were presented on the last section of the Chapter 4.

On the fitting model, the deterministic models of Table 2.2 were used. Three models have been selected, cubic, quadratic and linear. From the three models we further analyze to get the best representative of the model.

4.1 The Dynamic Model of Partitioning under Projection of Pb and Cu (Model 1)

4.1.1 Profile of Fresh and Dry weight of Shoot to Root Ratio of Rice Plant Due to Different Exposures of Pb

Graphical result on the Figure 4.1 portrays plant organ growth above and below the ground. From the figures can be seen that the shoot to root ratio (SRR) of fresh weight has a differing trend to that of shoot to root ratio of dried weight on the 7 days exposure to 24 days or 31 days exposure of Pb.

4.3 Dynamic Partitioning Model of Below and Above Ground Rice under Stress Pb and Cu on the Projection of Time (Model-3)

The results showed that different models caused different time of decreasing dry matter in the partition. It also showed different times of the optimal value of plant yield of dry weight. Under the prediction, those times are important for management of time for reducing stress.

The cubic model on the dynamic partitioning model of rice growth under Pb-20 ppm were best fit with the criteria of smallest RMSE, RRMSE, MAE, MAPE and higher efficiency of model (EF). According to the criteria of goodness fit model (Table 4.10) under Pb 50 ppm, partitioning organ rice plant performed better on the cubic model compared to quadratic and linear.

Plants tended to behave upon the cubic model on plant growth under Pb 50 ppm compared to quadratic and linear models. With the efficiency of fit greater on cubic model on root, shoot and leaves and lower in MAPE on cubic. From a lower MAE, RRMSE and RMSE, it could be concluded that the model is the best fit with respect to the criteria.

Under cubic models, root, shoot and leaf model are fitted with the lowest value of RMSE, RRMSE, MAE and MAPE. While the value should be the highest EF for plant growth under exposure of 100 ppm and 200 ppm of Cu. The cubic model showed various results in accordance to the criteria on the plant growth under Cu 0.04 ppm

4.6 Conclusion-Eligibility of the Models

Three models have been developed on seeking the dynamic partitioning models of rice plant for Pb. Based on the results and the best fit of the model; we conclude the cubic model is a representative model for rice growth under influence of stress lead and copper on the explaining of the dynamic partitioning of root, shoot and leaves.

The dynamic partitioning model could be used to predict the dry weight of the partitioning organ of root, shoot, and leaves of three conditions of growth

- a. Conditions that assumed to be in normal soil or normal growth of Cu-0.04 ppm. Both equations accordingly could be used for prediction of growth on the vegetative phase and early reproductive time.
- b. Condition with stress of the Pb, which is assumed of Pb 20 and Pb 50 ppm.
- c. Condition where the soil is of copper stress which is assumed on the doses of 100 and 200 ppm of copper.

The developed dynamic partitioning models (Model-3), could be used to describe parameter growth such as the shoot to root ratio under stress of lead and copper. It also could be used on the prediction of the yield in terms of the biomass to get the expression of the plant behavior under stress lead and copper. This relation and partitioning part could be used on the strategy planning or designing on effort for keeping environmental sustainability.

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