



The Effect of Spent Bleaching Earth Filler-Based NPK Fertilization on Proline, Growth and Yield of Maize

Ranggi Sumanjaya Purba, Siti Nurul Rofiqo Irwan and Eka Tarwaca Susila Putra*

Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia

*Corresponding author: eka.tarwaca.s@ugm.ac.id

Abstract

Spent bleaching earth (SBE) is by-product of cooking oil processing industry of crude palm oil (CPO). Palm oil industry is growing every year, followed by population growth and consumption of cooking oil so that the greater volume of waste generates SBE. An innovation is needed to anticipate the problem of SBE waste in agricultural sector, dealing with a filler component in the production of NPK fertilizer additives. This study aims to determine proline response, growth and yield of the maize to fertilization NPK with SBE-based filler. The experiments used Randomized Complete Block Design (RCBD) with the treatments of NPK filler (15:15:15) consisting of BC (brown clay), SBE and DBE (deoiled bleaching earth) at a dose of 6 g polybag⁻¹. The results showed that the use of SBE gave the same effect on plant height, leaf number, stem diameter and 100-seed weight, but the use of SBE could increase 61.15% of proline activity. SBE can substitute filler on the additional materials of NPK fertilizer.

Keywords: deoiled bleaching earth; maize; plant growth; spent bleaching earth

Cite this as: Purba, R. S., Irwan, S. N. R., & Putra, E. T. S. (2020). The Effect of Spent Bleaching Earth Filler-Based NPK Fertilization on Proline, Growth and Yield of Maize. *Caraka Tani: Journal of Sustainable Agriculture*, 35(1), 44-53. doi: <http://dx.doi.org/10.20961/carakatani.v35i1.34166>

INTRODUCTION

The refining process of crude palm oil (CPO) into cooking oil produces solid waste involving a substance known as spent bleaching earth (SBE) (Merikhy et al., 2018). SBE is a mixture of clay and oils, which need to be handled carefully because of its flammability. SBE is also a solid waste from oil refining industry with unpleasant odor that usually consists of clay minerals and residual oil (Mu and Wang, 2019; Onoja et al., 2019). Based on the Government Regulation No. 101 Year 2014, SBE is categorized as hazardous and toxic waste with the waste codes of B413, in which the source of the waste from the process of the oleo chemical industry or processing of animal fats or vegetable with 2nd hazard categories is delayed and indirectly impacts on the environment and toxicity. Currently, the utilization of palm oil waste for energy contributes substantially

to the projected renewable energy supplies in the future (Abnisa et al., 2013). The by-products of palm oil SBE can be used to add valuable bio-products such as fertilizer which would increase the financial viability of the oil palm, reduce waste and improve healthy environment for supporting sustainable agriculture (Ofori-Boateng and Lee, 2013). The research results by Widyawati and Ufidian (2017) also revealed that the SBE contained some heavy metals such as 10.74% Al, 6.89% Fe, 20.18 ppm Pb, 8.74 ppm Ni and 23.52 ppm Cu.

In general, the oil industry will throw in an area of B3 waste in landfill (Loh et al., 2013; Prokopov and Mechenov, 2013; Beshara and Cheeseman, 2014; Boukerroui et al., 2018; Gül et al., 2018). SBE is usually burned or generally disposed to landfills after processed with water to reduce the nature of fire (Cheong et al., 2013). SBE is

* Received for publication August 26, 2019

Accepted after corrections November 4, 2019

classified as B3 waste, which is thought to contain several types of heavy metals. The potential interference of heavy metals in SBE is not only related to the issue of land pollution and food safety. If the waste is continuously without proper management, it will have a negative impact on the environment and human health. Soil contamination by heavy metals is the most important apprehension throughout the industrialized world. Heavy metal pollution does not only result in adverse effects on various parameters relating to plant quality and yield but also cause changes in the size, composition and activity of microbial community, and the contamination of a river with heavy metals may cause devastating effects on the ecological balance of the aquatic environment and the diversity of aquatic organism becomes limited with the extent of contamination (Ayandrian et al., 2009).

The uptake of heavy metals by plants and subsequent accumulation along the food chain are potential threats to animal and human health. The absorption of plant roots is one of the main routes of entrance of heavy metals in the food chain. Absorption and accumulation of heavy metals in plant tissue depend on many factors, which include temperature, moisture, organic matter, pH and nutrient availability. The heavy metals contained in the waste can poison human body not only through contaminated food and drinks but also through inhaled dust or smoke (Jiwan and Ajay, 2014). Therefore, the management of B3 waste should be carried out by industries that produce the waste. This is also a suggestion contained in government regulations that this waste can be used but the use should be carried out in accordance with the 3R system (Recycle, Reuse and Recovery) as regulated by the government.

There are 65 credible oil industries in Indonesia with a total annual production capacity of 9.9 million tons year⁻¹. Based on that, 40% of the total production capacity of cooking oil uses earth bleaching as the bleaching adsorbent. The bleaching earth dosage used is about 1% of CPO weight, which will produce as much as 39,600 tons of SBE every day. Embrandiri et al. (2012) added that about 5.8 tons of fresh fruit bunch produced 1 tons of CPO. The accumulation of cooking oil waste in Indonesia is approximately 700,000-1,000,000 tons year⁻¹ (Kamilah et al., 2018). Even though several studies have been carried out to alternate sustainable management,

there is still much work to be done because the produced quantities on a daily basis exceed the use (Embrandiri et al., 2013). The excessive waste of cooking oil produced by downstream industry will impact negatively to the living things and the environment. To overcome this adverse impacts at the present and in the future, it is necessary to reuse the SBE as an adsorbent in the bleaching CPO process (Respati et al., 2017).

Maize commodity has an important role in the food security system and serves as a driver of the national economy, in which the demand for maize increases every year due to population growth, the progress of the food processing industry, and the development of the livestock sector (Kementrian Pertanian, 2018). One of the uses of fertilizer through NPK fertilization is the most important factor in maize cultivation where a source of additional NPK fertilizer is needed to meet the nutritional needs of the maize plant. According to Kheang et al. (2010), the residue of CPO oil in the SBE was 20-30% and has a similar clay shape.

The results of research by Loh et al. (2013) showed that SBE contains some macro essential nutrients such as N, P, K, which can potentially be reused as a fertilizer. An innovation is needed to anticipate the problem of SBE waste. The SBE can be utilized for agricultural activity as a filler in the addition of NPK fertilizer. Re-purification of SBE waste by reducing oil content by 15-20% is known as deoiled bleaching earth (DBE), which has the potential as an alternative source of filler for the addition of NPK fertilizer production. However, it should be investigated considering its potential in the pollution of land and agricultural products. The objective of this research was to determine the response of proline activity, growth and yield of the maize to SBE-filler in NPK fertilization.

MATERIALS AND METHOD

The research was conducted in November 2018 – March 2019 at experimental farm, Agrotechnology Innovation Center (AIC) of Universitas Gadjah Mada, Kalitirto, Prambanan, Sleman, Yogyakarta. The instruments used in this study were agricultural tools, stationery, digital scales, analytical scales, rulers and oven. The materials used were hybrid maize seed Pioneer-36, manure, NPK fertilizer (15:15:15) with a filler of brown clay (BC) 10%, NPK fertilizer with a filler of SBE 5% + BC 5%, NPK fertilizer with a

filler of DBE 5% + BC 5% at a dose of 6 g polybag⁻¹ each, urea at a dose of 3 g polybag⁻¹ (Kasno and Rostama, 2013). Polybag size was 50 cm x 50 cm. The research was arranged in non-factorial Randomized Complete Block Design (RCBD), namely NPK fertilizer filler based on BC, SBE, DBE with three replications as blocks. The treatments in each block used 12 plants as samples, with a total of 108 plants. NPK fertilizer was applied 14 days after planting.

The planting media used was regosol soil, obtained from the AIC UGM farmland, which was put into polybags with a weight of 15 kg polybag⁻¹ each, then added 15 g polybag⁻¹ of goat manure and mixed evenly. Maize planting was done by direct seed planting method and then thinning activities were carried out when the plants aged 2 weeks, by pulling out the maize only seed in each polybag. Plant care included watering every morning or evening by observing the condition of plants in a polybag, weeding by manually removing weeds growing in the polybag, and cleaning weeds in the surrounding area.

The observation included analysis of heavy metals in NPK fertilizer using the Atomic Absorption Spect (AAS) method (Balai Penelitian Tanah, 2009). It was performed by weighing each 0.5 g fertilizer that had been mashed and put into a 100 ml volume measuring flask, 10 ml of 25% HCl was added with a 10 ml volume pipette, and then it was heated to a hot plate until completely dissolved and boiled for 15 minutes. It was then diluted with ion-free water and after it cooled, it was set as much as 100 ml, covered and shaken back and forth with hands until it was homogeneous. Afterwards, it was left overnight or filtered to get clear extracts. Micro elements such as Cu, Zn, Ag, Cr and Cu heavy metals were measured directly from the extracts using AAS with standard series, each as a comparison with the air-acetylene flame. Proline content was determined following Bates et al. (1973), plant height, leaf number, stem diameter and 100-seed weight. The data were analyzed using variance analysis with $\alpha = 5\%$, followed by DMRT analysis, if there were significant differences between treatments.

RESULTS AND DISCUSSION

Plant growth can be defined as an increase in volume and mass of plants with the establishment of new structures such as organs, tissues and cells

(Brukhin and Morozova, 2011). In general, plants need nutrients for growth and development of life that can be supplied from fertilizers. Fertilization is done by giving a balanced fertilizer, which means the provision of fertilizer is adjusted with the needs of the plant by considering the soil ability to provide nutrients naturally. Fertilizer application is important for maintaining soil health, as extraction rates for nutrients exceed the natural delivery from the soils. Balanced fertilization is a site specific nutrient management that depends on the environment. Maize plants require balanced fertilization to maximize their productivity, quality and efficiency of energy use (Kumar et al., 2018). Yield quality depends on contaminant content, including heavy metals. Heavy metal is a chemical element that has a metal atom density of greater than 4 or 5 g cm⁻³ (Ackova, 2018) and heavy metal is one of the major abiotic stresses that adversely affect the quantity and nutritive value of maize (Rizvi and Kha, 2018).

As illustrated in Table 1, several heavy metals were contained in NPK fertilizers with different fillers namely Ag, Cr, Cu, Zn and Pb. The Ag content in NPK fertilizer with DBE filler 5% + BC filler 5% was higher than NPK fertilizer with BC filler 10% and NPK fertilizer with SBE filler 5% + BC filler 5%. Silver metal (Ag) can be obtained through AgNO₃ compounds and is now developed in the forms of silver nanoparticles (AgNPs). AgNPs are used in a variety of industrial and agricultural activities as growth hormones, fungicides and fruit maturation boosters (Yan and Chen, 2019).

Chromium (Cr) is a heavy metal used in electroplating, textile coloring and the metal processing industry. Table 1 presents that the Cr content in NPK fertilizer with SBE filler 5% + BC filler 5% and NPK fertilizer with DBE filler 5% + BC filler 5% was higher than the content of NPK fertilizer with BC filler 10%. This metal is found in the form of Cr (VI), which is very toxic and Cr (III), which has a lower toxicity level (Rosariastuti et al., 2012). Cr toxicity causes inhibition of cell division and elongation so that the roots grow short. This can interfere with water and mineral absorption. Cr can damage plants through manipulation of several mechanisms that occur in cells (Emamverdian et al., 2015).

Copper (Cu) is a heavy metal that is included in micronutrients for plants. As demonstrated in Table 1, the Cu content in NPK fertilizer with

SBE filler 5% + BC filler 5% and NPK fertilizer with DBE filler 5% + BC filler 5% was higher than that of NPK fertilizer with BC filler 10%. Metal Cu is present in the form of Cu^{2+} and Cu^+ in plant physiology. Cu ions act as cofactors for many enzymes, including Cu/Zn superoxide dismutase (SOD), cytochrome c oxidase, plastocyanin and polyphenol oxidase. Cu plays a role in plant defense against disease and chlorophyll formation (Fauziah et al., 2018). Lead (Pb) is a heavy metal whose function is not yet known in plant metabolism. The Pb content in NPK fertilizer with BC filler 10%, NPK fertilizer with SBE filler 5% + BC filler 5% and NPK fertilizer with DBE filler 5% + BC filler 5% were not significantly different. Sharma and Duber (2005) states that lead toxicity can inhibit enzyme activity, interfere with nutrient absorption and water balance, change in hormonal status and damage to membrane permeability. Zinc (Zn) is an essential micro nutrient for plants. Zn solubility will increase with pH. Zn acts as a cofactor of more than 300 types of enzymes that play a role in nucleic acid metabolism, synthesis of protein compounds, hormone formation, protect cells from free radicals and plant resistance to pests (Fauziah et al., 2018). The Zn content in NPK

fertilizer with SBE filler 5% + BC filler 5% was higher than the content of NPK fertilizer with DBE filler 5% + BC filler 5% and NPK fertilizer with BC filler 10%. Plants only need a small amount of Zn that is with criteria <10 ppm declared deficient, 11-25 ppm low and >71-81ppm very high, which can cause plant toxicity. Zn toxicity causes reduced root growth and leaf widening and leaf chlorosis (Rudani et al., 2018).

Some metal elements contained in the NPK fertilizer might originate from the process of making the fertilizer and from raw materials for the SBE purification process and the refining of DBE (Kominko et al., 2019). Heavy metals can contaminate for a range of reasons and in a variety of contexts, including through modern chemical industries and fertilizer production (Fiyadh et al., 2019). The heavy metal content of NPK fertilizer with different filler can inhibit maize growth due to the increased activity of osmoprotectant compounds such as proline. From the results of this study, it is also known that the source of filler that is used in the manufacture of NPK fertilizer classified as hazardous and toxic (B3) waste, i.e. SBE, can be used or used as a source of filler in the manufacture of NPK fertilizer and does not interface with growth and maize crop yield.

Table 1. Heavy metal concentrations in NPK fertilizer with different filler

Treatments	Ag	Cr	Cu	Pb	Zn
	mg kg ⁻¹				
NPK with BC filler 10%	7.03b	17.17b	23.76b	4.59a	47.29c
NPK with SBE filler 5% + BC filler 5%	4.51c	21.76a	43.95a	4.48a	54.85a
NPK with DBE filler 5% + BC filler 5%	9.16a	20.12a	44.10a	4.26a	50.69b
CV (%)	10.99	3.80	1.26	3.79	1.58

Note: Numbers followed by the same letters in the same column do not differ significantly according to DMRT 5%

Plants can adapt to a stress of heavy metal contaminant through a mechanism by accumulating osmoprotectant compound such as proline. The proline adjusts to the osmotic plant so that water can get into the plant tissues and keep the water status in the systems for surviving life. Table 2 shows that the NPK with BC filler 10% treatment was significantly different than the treatment of NPK with DBE filler 5% + BC filler 5% and NPK with SBE filler 5% + BC 5% in proline content and NPK with SBE filler 5% + BC 5% could increase 61.15 % of proline activity. The results of the research by Rizvi and Khan (2018) revealed that proline content in the leaves of maize planted in soil processed with metal/not

processed with metal was determined. The level of proline progressively rose with the increasing dose rates of Cu and Pb. Interestingly, 64% and 52% increases in proline content in maize foliage were recorded at Cu 2007 mg kg⁻¹ and Pb 585 mg kg⁻¹, respectively, relative to uninoculated controls. This research shows that the presence of heavy metals such as Cu and Pb in maize leaves increases the activity of proline as a form of plant mechanism in neutralizing the presence of heavy metals in maize plants.

Proline is a multifunctional amino acid, which acts as an osmoprotectant molecule that safeguards the plants from the damaging effects of reactive oxygen species (ROS) generated under

environmental stresses like drought, salinity or heavy metals (Singh et al., 2014). The biosynthesis and degradation of proline and its accumulation in plants are regulated by different abiotic stress. Proline synthesis in plants consists of two different cycles. The first is glutamate cycle, in which, glutamate is phosphorylated to γ -glutamyl phosphate and reduced to glutamate- γ -semialdehyde (GSA), which is spontaneously cyclized to Δ^1 -pyrroline-5-carboxylate (P5C). The second is the ornithine γ -aminotransferase (OAT). Proline biosynthesis from glutamate comprises two enzyme reactions, involving Δ^1 -pyrroline-5-carboxylate synthetase (P5CS) and glutamate dehydrogenase (GDH). On the other hand, the proline accumulation depends on its degradation rate, which is catalyzed by the mitochondrial enzyme proline dehydrogenase (PDH) (Yang et al., 2009).

In plants, both PDH and Δ^1 -pyrroline-5-carboxylate dehydrogenase (P5CDH) are attached to the matrix side of the inner mitochondrial membrane. Proline synthesis initiates the generation of NADP⁺, which acts as the backbone for ribose 5-phosphate required for the purines synthesis, and proline oxidation yields the reduced electron carriers, which provide energy for the numbers of biochemical reaction such as nitrogen fixation (Kim and Nam, 2013). The other line to produce proline is through the involvement of the enzyme ornithine by ornithine aminotransferase and pyroline-R-5-carboxylate reductase (Lestari, 2015). Increase in protein content under heavy metal stress may contain a larger proportion of proline. Binding with metal ions due to the chelating ability of proline can also be a defense mechanism for survival.

Table 2. The content of proline activity in maize plants

Treatments	Proline (g mol ⁻¹)
NPK with BC filler 10%	0.54b
NPK with SBE filler 5% + BC filler 5%	1.39a
NPK with DBE filler 5% + BC filler 5%	1.08a
CV (%)	19.27

Note: Numbers followed by the same letters in the same column do not differ significantly according to DMRT 5%

Balanced fertilization ensures the appropriate application N, P, K and trace elements based on crop requirements and soil fertility performance (Li et al., 2019). The use of NPK fertilizer in this study has included several nutrients so that it is more efficient than a single fertilizer (Himmah et al., 2018). Nutrients of N, P, K are essential elements in plants. N is needed by plants for the production of proteins, nucleic acids and chlorophyll (Shehu et al., 2019). Plants also need P for the development of energy, sugar and nucleic acids, while K is used by plants in enzyme activation, photosynthesis, protein formation and sugar transportation (Mccauley, 2011).

The response of maize plants can be seen in observations of plant growth such as plant height, leaf number and stem diameter. Plant height is the most obvious plant growth variable observed because it can be measured simply and becomes a determinant of yield quality. Plant heights measured at 4, 6 and 9 weeks after planting (WAP) are shown in Table 3. The plant height in NPK fertilization with different filler did not cause any significant difference in NPK fertilizer with BC filler 10%, NPK fertilizer with SBE filler 5% + BC filler 5%, and NPK fertilizer DBE filler 5% + BC filler 5% at 4, 6 and 9 WAP.

Table 3. Plant height of maize plants aged 4, 6 and 9 WAP

Treatments	Plant height (cm)		
	4 WAP	4 WAP	4 WAP
NPK with BC filler 10%	56.78a	56.78a	56.78a
NPK with SBE filler 5% + BC filler 5%	62.79a	62.79a	62.79a
NPK with DBE filler 5% + BC filler 5%	61.69a	61.69a	61.69a
CV (%)	10.22	10.22	10.22

Note: Numbers followed by the same letters in the same column do not differ significantly according to DMRT 5%

The leaves are plant organs that play a role in the photosynthetic process. The number of leaves in maize can indicate the distribution of assimilation and photosynthetic product in the plant. Table 4 presents that the number of maize

leaves was not significantly different at the age of 4, 6 and 9 WAP among the treatments. Thus, the number of leaves in each maize plant gives the same results in producing leaves.

Table 4. Leaf number in maize plant aged 4, 6 and 9 WAP

Treatments	Number of leaves per plant		
	4 WAP	4 WAP	4 WAP
NPK with BC filler 10%	6.67a	6.67a	6.67a
NPK with SBE filler 5% + BC filler 5%	7.44a	7.44a	7.44a
NPK with DBE filler 5% + BC filler 5%	6.44a	6.44a	6.44a
CV (%)	12.03	12.03	12.03

Note: Numbers followed by the same letters in the same column do not differ significantly according to DMRT 5%

The growth of maize plants in the canopy, in addition to being indicated by plant height, can also be indicated in stem diameter. The distribution of photosynthetic during the growth in the canopy is also used by plants to increase the

diameter of the stem. Table 5 demonstrates that the diameter of maize stalks was not significantly different at the age of 4, 6 and 9 WAP among the treatments.

Table 5. Stem diameter in maize plants aged 4, 6 and 9 WAP

Treatments	Steam diameter (mm)		
	4 WAP	4 WAP	4 WAP
NPK with BC filler 10%	12.69a	12.69a	12.69a
NPK with SBE filler 5% + BC filler 5%	12.09a	12.09a	12.09a
NPK with DBE filler 5% + BC filler 5%	11.86a	11.86a	11.86a
CV (%)	12.78	12.78	12.78

Note: Numbers followed by the same letters in the same column do not differ significantly according to DMRT 5%

In this observation, the alleged heavy metals contained in SBE and DBE did not affect the growth either in plant height, number of leaves and stem diameter of maize plants in treatment with NPK with BC filler 10%, NPK with SBE filler 5% + BC filler 5% and NPK with DBE filler 5% + BC filler 5%. Therefore, maize plants are equally good at absorbing nutrients and water from the soil used as photosynthetic materials. The leaves are able to properly capture photons of sunlight and produce glucose and oxygen for

supporting plant growth and development (Yahia et al., 2019).

100-seed weight is the final photosynthetic yield that has economic value. Seed dry weight will reach maximum physiological maturity, and the transfer of food substances has been stopped and dry weight is only influenced by environmental conditions, especially air humidity (Cao et al., 2019). Table 6 shows the 100-seed weight of maize plants. The NPK fertilization with different filler did not cause a significant difference in the dry weight variable of 100 seeds.

Table 6. 100-seed weight in maize plants

Treatments	100-seed weight (g)
NPK with BC filler 10%	27.47a
NPK with SBE filler 5% + BC filler 5%	26.19a
NPK with DBE filler 5% + BC filler 5%	26.18a
CV (%)	7.90

Note: Numbers followed by the same letters in the same column do not differ significantly according to DMRT 5%

The use of SBE and DBE-based fillers did not have a significant effect on the growth and yield of maize. This indicates that the filler can be used in the process of making NPK fertilizer because it is not harmful to maize plants. SBE and DBE fillers have abundant availability and can be used as a substitute for BC filler which is generally used as a filler for NPK fertilizer. SBE based fillers are recommended for use because production costs are lower and the resource can be renewed.

CONCLUSIONS

The use of SBE-based NPK fertilizer gives the same effect on plant height, leaves number, stem diameter, 100-seed weight and can increase 61.15% of proline activity so that SBE waste can be used as a source of filler in NPK fertilizer because it does not have a negative impact on the growth and yield of maize, abundant availability and reduce effects if not managed properly.

ACKNOWLEDGEMENTS

This research was fully funded by Wilmar International Ltd project 2018-2019.

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