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Vegetative Growth Response of Rice in Heavy Metal Contaminated Soil Due to NPK Fertilizer Based Ameliorant Application

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ABSTRACT: Heavy metal pollution in paddy fields has been recognized as a global environmental problem that must be addressed. Exposure to heavy metals causes stunted growth of rice plants. A safe and environmentally friendly approach is done through the use of a combination of ameliorants and inorganic fertilizers. NPK Co-Ameliorant fertilizer is an innovation in bio-agent multifunctional fertilizer production technology that is expected to be an alternative solution to the widespread pollution of rice fields by industrial waste. This study aims to examine the effect of NPK Co-Ameliorant fertilizer on vegetative growth of rice plants on industrial waste polluted soil. The research was conducted in a greenhouse with soil media from Jelegong village, Rancaekek which was polluted with heavy metals. The research period was from September 2023 to February 2024. The experimental design used a Randomized Group Design with eight treatments, four replications and two units consisting of: 0 (control); NPK Phonska 225 kg ha^{-1}; and various doses of NPK Bio-Organomineral fertilizer 150; 250; 350; 450; 550; 650 kg ha^{-1}. The results showed that the application of NPK Co-Ameliorant fertilizer effectively increased rice plant height, number of tillers, chlorophyll content and plant biomass weight. The dose of NPK Co-Ameliorant fertilizer 350 kg ha^{-1} was the best dose in increasing the height of rice plants reaching 95,63 at 70 DAP, increasing the chlorophyll content of plants by 24.20 CCI and increasing the dry weight of plants reaching 33,98 g.

KEYWORDS: Zeolite, Activated charcoal, Heavy metals, Biofertilizer

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INTRODUCTION

The decline in rice productivity in Indonesia is due to the fact that many rice fields have decreased fertility due to heavy metal pollution. Rancaekek is one of the areas in Bandung Regency where rice fields experience heavy metal pollution due to textile industry waste discharges (Nugraha *et al.,* 2017). Pollutants generated from the textile industry are heavy metals derived from the bleaching process (Handayani *et al.,* 2022). Research results reveal that textile industry waste contains various heavy metals such as Pb, Cr, Cd, As, Cu, and Zn that can accumulate in the soil (Komarawidjadja *et al.,* 2017). The paddy fields of Jelegong Village, Rancaekek District have been found to have heavy metal concentrations of Cr up to 174,7 mg kg^{-1}, Pb by 30,03 mg kg^{-1} and Cu by 90,91 mg kg^{-1} (Komarawidjadja, 2017; Agustine *et al.,* 2022).

Heavy metals contained in paddy fields will adversely affect the growth of rice plants, rice productivity and public health. The response of rice plants exposed to heavy metals is to have a stunted plant height, easily lodged, yellowing leaves and stems and a small number of tillers, thus reducing the productivity of rice plants (Laoli *et al.,* 2021). The decrease in rice productivity experienced by farmers in Rancaekek from 6-7 t ha^{-1} to 2-4 t ha^{-1} or even cause crop failure (Sudirja *et al.,* 2017). This happens because heavy metal accumulation has disrupted the process of plant respiration, photosynthesis rate, and transpiration, resulting in disruption of plant growth and decreased productivity (Shengwei *et al.,* 2023). In addition, human exposure to heavy metals can cause cardiovascular disease, kidney damage, cognitive impairment, immunological deficiencies, reproductive disorders, and cancer (Agency For Toxic Substances and Disease Registry, 2020).

Efforts to deal with the impact of heavy metal pollution on rice plant growth need to be carried out with effective mitigation strategies to maintain plant health and food safety. The use of ameliorants has been proven to reduce the solubility of heavy metals either alone or in combination with inorganic fertilizers. NPK Co-Ameliorant fertilizer is a multifunctional fertilizer product

innovation that contains N, P and K elements coated with ameliorants so that it is environmentally friendly. The formulation of NPK Co-Ameliorant fertilizer is NPK (60%); Zeolite (20%); Activated charcoal (10%) and Compost + Grilled Shark + Bacillus subtilis (10%). The formulation refers to the best formulation results by Sudirja *et al,* (2016) on ameliorant-based NPK fertilizer treatment in absorbing heavy metals and improving soil fertility.

MATERIALS AND METHODS

Scale, Place and Time of Research

The research was conducted in a greenhouse scale located at Ciparanje Experimental Farm, Jatinangor owned by the Faculty of Agriculture, Padjadjaran University, at an altitude of ± 725 meters above sea level (masl). Soil sampling in paddy fields in Jelegong Village, Rancaekek District, Bandung Regency, West Java, precisely at coordinates 06º58'10.6" LS and 107º46'58.7" East and at an altitude of ± 676 meters above sea level. The soil contains heavy metals Lead (Pb) 25.41 mg kg^{-1}, Chromium (Cr) 75.44 mg kg^{-1} and Copper (Cu) 72.81 mg kg^{-1}. The research was conducted from September 2023 to February 2024.

Experiment Design

The research method used in this study is an experimental quantitative method using a Randomized Group Design. This study contained eight treatments, consisting of one negative control treatment (without fertilizer application), one positive control treatment (NPK phonska 100%) at a dose of 225 kg ha-1 and six treatments of NPK Co-Ameliorant fertilizer doses. This study used four replications and two treatment units, resulting in 64 experimental buckets. The two treatment units consisted of one experimental unit for sampling plant uptake at the maximum vegetative phase, while the second experimental unit was for observation until the generative phase. The list of treatments and doses used in the study are presented in Table 1.

Table 1. Application Dosage of NPK Co-Ameliorant Fertilizer

Symbol	Treatment	Fertilizer Dosage (kg ha^{-1})
A	Control	0
B	NPK Phonska (15:10:12) (Recommendation fertilizer)	225
C	NPK Co-Ameliorant Fertilizer	150
D	NPK Co-Ameliorant Fertilizer	250
E	NPK Co-Ameliorant Fertilizer	350
F	NPK Co-Ameliorant Fertilizer	450
G	NPK Co-Ameliorant Fertilizer	550
H	NPK Co-Ameliorant Fertilizer	650

Analysis Design

Measurement data were tested for normality using the Saphiro-Wilk test to determine whether the raw data had a normal distribution or not. Analysis of the significance of differences in the effect of treatment means used ANOVA analysis at the 95% confidence level. If the difference in treatment means is significant, the test is continued with the Duncan Multiple Range Test at the 5% real level. Statistical analysis for significance testing and further testing was carried out using SPSS software.

Research Implementation

Preparation of planting media using polluted soil put into pots measuring 20 cm in diameter 30 cm high as much as 10 kg per pot. The seeds used in the study were Inpari 32 Agritan rice seeds with 90% germination rate. The planting medium used for nursery was soil and compost in a ratio of 1:1 and sown until the age of 15 days. Transplanting was done at the age of 15 days after sowing. Planting was done by transferring 2 seedlings into the treatment bucket, at a depth of 2-3 cm from the soil surface. The application of NPK Co-Ameliorant fertilizer was done at the time of transplanting by inserting fertilizer in the planting hole at a distance of 5 cm from the plant. Maintenance activities of rice plants in the greenhouse include replanting, watering, weeding and eradicating plant disease organisms. Replanting or replacing dead plants is done three days after transplanting. The soil should always be flooded with the height of the water surface from the soil surface between 2-3 cm. Watering is done in the morning to maintain soil moisture. The first weeding is done when the plants are 12-14 days after planting. Weeding is done manually by hand, the weed plants that are pulled out are then buried back into the soil in the same pot.

Observations included measurement of plant height, number of tillers, chlorophyll content and measurement of plant biomass base and dry weight. Measurement of rice plant height was carried out using a meter starting from the part of the plant that was above the planting medium (base of the stem) to the highest part of the leaf when straightened vertically. Counting the number of tillers was done by counting the number of tillers of rice plants in one clump. Observations of plant height and number of tillers were made at the age of 14, 28, 42, 56, and 70 DAP. Chlorophyll measurement using a chlorofilmeter tool during the maximum

vegetative phase (70 DAP). Measurement of plant wet and dry weights using scales during the maximum vegetative phase (70 DAP).

RESULTS

Plant Height

Plant growth is characterized by an increase in the number and size of plants resulting in changes in appearance and plant height. The results of the measurement of rice plant height in all treatments are presented in Table 2.

Table 2. The results of measuring the height of rice plants during the vegetative period.

	Treatment (kg ha^{-1})	Average Height of Plants (cm)				
		14 DAP	28 DAP	42 DAP	56 DAP	70 DAP
A	Control	24,90 a	35,38 a	54,40 a	69,25 a	80,38 a
B	NPK Phonska (15:10:12) 225	26,33 b	36,45 ab	59,20 b	79,13 b	89,35 b
C	NPK Co-Ameliorant Fertilizer 150	30,38 d	39,53 cd	63,08 c	83,75 c	90,23 b
D	NPK Co-Ameliorant Fertilizer 250	30,45 d	41,28 d	65,08 c	88,80 d	94,00 cd
E	NPK Co-Ameliorant Fertilizer 350	29,93 d	41,08 d	63,75 c	91,45 e	95,63 d
F	NPK Co-Ameliorant Fertilizer 450	28,20 c	38,40 bc	58,93 b	89,68 de	94,10 cd
G	NPK Co-Ameliorant Fertilizer 550	25,75 ab	37,58 abc	56,98 b	85,18 c	92,50 c
H	NPK Co-Ameliorant Fertilizer 650	26,05 b	36,98 ab	57,23 b	85,15 c	93,10 c

Notes: Values followed by the same letter are not significantly different based on Duncan's multiple range test at 5% real level.

Based on Table 2, the effect of NPK Co-Ameliorant fertilizer application began to increase the average plant height significantly against the control (A) and the recommended fertilization of NPK Phonska 225 kg ha^{-1} (B) at 14 DAP, namely in the treatment of NPK Co-Ameliorant doses of 150 kg ha^{-1} (C), 250 kg ha^{-1} (D), 350 kg ha^{-1} (E) and 450 kg ha^{-1} (F). Plant height continues to increase until the observation of maximum vegetative time (70 DAP), where the application of NPK Co-Ameliorant dose of 350 kg ha^{-1} (E), has a plant height tends to be higher at 95.63 cm, but not significantly different from the treatment dose of 250 kg ha^{-1} (D) and 450 kg ha^{-1} (F). The lowest plant height was in the control treatment (A), which was 80,38 cm. In addition, the application of NPK Co-Ameliorant doses from 250 kg ha^{-1} to 650 kg ha^{-1} was able to provide results that were significantly different from the recommended NPK fertilizer treatment (B). This means that at these doses the NPK Co-Ameliorant treatment can effectively replace the recommended fertilizer to increase the height of rice plants. Economically, the application of NPK Co-Ameliorant at a dose of 350 kg ha^{-1} is the best treatment in increasing plant height.

Number of Tiller

The application of various doses of NPK Co-Ameliorant formulations showed a diverse response to the number of vegetative tillers of rice plants. The observation results of the number of tillers in each treatment are presented in Table 3.

Table 3. The results of the calculation of the number of vegetative tillers and the number of productive tillers of rice plants.

	Treatment (kg ha^{-1})	Average number of tillers				
		14 DAP	28 DAP	42 DAP	56 DAP	70 DAP
A	Control	1,00	2,25 a	12,00 a	13,25 a	15,25 a
B	NPK Phonska (15:10:12) 225	1,00	3,00 ab	15,50 b	18,00 b	20,00 b
C	NPK Co-Ameliorant Fertilizer 150	1,00	3,25 bc	14,75 b	19,00 b	20,50 b
D	NPK Co-Ameliorant Fertilizer 250	1,00	4,00 c	17,25 b	21,50 c	23,50 c
E	NPK Co-Ameliorant Fertilizer 350	1,00	4,00 c	17,50 b	23,50 c	25,25 c

F	NPK Co-Ameliorant Fertilizer 450	1,00	3,00 ab	16,75 b	26,00 d	28,25 d
G	NPK Co-Ameliorant Fertilizer 550	1,00	3,50 bc	16,75 b	22,50 c	24,50 c
H	NPK Co-Ameliorant Fertilizer 650	1,00	3,50 bc	15,75 b	22,25 c	25,25 c

Notes: Values followed by the same letter are not significantly different based on Duncan's multiple range test at 5% real level.

Based on Table 3. the average number of tillers began to show significant differences at 42 DAP, where all fertilizer treatments showed significantly higher results than the control (A). NPK Co-Ameliorant fertilizer treatment at a dose of 450 kg ha^{-1} (F), at 56 DAP and 70 DAP produced the highest average number of tillers significantly against other treatments, namely 26 tillers at 50 DAP and 28.25 tillers at 70 DAP. This shows that the NPK Co-Ameliorant treatment at a dose of 450 kg ha^{-1} (F) is the best treatment significantly in producing vegetative tillers, while the control treatment (A) is the lowest treatment significantly in producing vegetative tillers.

Kandungan Klorofil

Based on Table 3, the chlorophyll content value in each fertilization treatment has a significantly higher chlorophyll content than the control without fertilization (A), which only amounted to 11 CCI. This shows that fertilization of plants is very important to increase chlorophyll content. The NPK Co-Ameliorant fertilizer treatment also showed an increase in the amount of chlorophyll against the recommended NPK fertilizer treatment of 225 kg ha-1 (B) significantly at doses of 250 kg ha^{-1} to 650 kg ha^{-1}. The treatment of NPK Co-Ameliorant fertilizer at a dose of 350 kg ha^{-1} (E), from an economic point of view, is the best treatment, because at a smaller dose level but can significantly increase the amount of chlorophyll to reach 24,20 CCI.

Table 3. Measurement results of chlorophyll content of rice plants aged 70 DAP

Symbol	Treatment (kg ha^{-1})	Chlorophyll Content (CCI)
A	Control	11,15 a
B	NPK Phonska (15:10:12)	16,30 c
C	NPK Co-Ameliorant Fertilizer	14,83 b
D	NPK Co-Ameliorant Fertilizer	21,00 d
E	NPK Co-Ameliorant Fertilizer	24,20 e
F	NPK Co-Ameliorant Fertilizer	24,25 e
G	NPK Co-Ameliorant Fertilizer	23,40 e
H	NPK Co-Ameliorant Fertilizer	23,30 e

Notes: Values followed by the same letter are not significantly different based on Duncan's multiple range test at 5% real level.

Plant Biomass

Table 4. Measurement results of plant biomass weight at 70 DAP.

Symbol	Treatment (kg ha^{-1})	Wet Weight	Dry Weight
A	Control	100.25 a	23,64 a
B	NPK Phonska (15:10:12)	148.75 b	29,24 b
C	NPK Co-Ameliorant Fertilizer	171.25 c	31,28 bc
D	NPK Co-Ameliorant Fertilizer	191.25 d	33,24 cd
E	NPK Co-Ameliorant Fertilizer	206.50 e	33,98 d
F	NPK Co-Ameliorant Fertilizer	203.25 e	34,01 d
G	NPK Co-Ameliorant Fertilizer	180.25 cd	33,14 cd
H	NPK Co-Ameliorant Fertilizer	182.50 cd	33,53 cd

Notes: Values followed by the same letter are not significantly different based on Duncan's multiple range test at 5% real level.

The results of the analysis showed that the weight of dry and wet biomass of plants in the control treatment (A) had the lowest weight significantly, which was 23, 64 g and 100.25 g, respectively. The wet weight of plants in all treatments ranged from 100.25 g to 206,50 g. The treatment of NPK Co-Ameliorant fertilizer application doses of 350 kg ha^{-1} and 450 kg ha^{-1} had the highest wet biomass weight of 206,50 g and 203,25 g, respectively. This value is significantly different from the recommended NPK fertilizer application which is only 148.75 g. While the dry biomass weight of the plants showed a range of dry weights in all treatments from 23,64 g to 34,01 g. The treatment of NPK Co-Ameliorant application has a dry weight tends to be higher at doses of 350 kg ha^{-1} (E)

and 450 kg ha^{-1} (F), which amounted to 33,98 g and 34,01 g, respectively. However, these figures were not significantly different from the NPK Co-Ameliorant treatments of 250 kg ha^{-1}, 550 kg ha^{-1} and 650 kg ha^{-1}. Economically, the application of NPK Co-Ameliorant at a dose of 350 kg ha^{-1} is the best treatment in increasing plant biomass weight.

DISCUSSION

The application of NPK Co-Ameliorant fertilization gives a better plant height than the treatment without fertilization and NPK recommendations (Figure 1). The nutrient that plays an important role in influencing the height of rice plants is Nitrogen (N). The element N is needed to compose amino acids, proteins and increase leaf green substances that are useful in the photosynthesis process to support vegetative growth such as branches, stems, leaves and tillers to grow more optimally (Saputra *et al.,* 2021). The increase in the number of tillers is produced due to the provision of nutrients that are balanced between the application of inorganic and organic fertilizers. The presence of N nutrients that are easily leached and volatilized in paddy field soils causes the treatment of inorganic NPK fertilizer recommendations to be unable to provide sufficient nutrients for increasing plant height and the number of rice tillers.

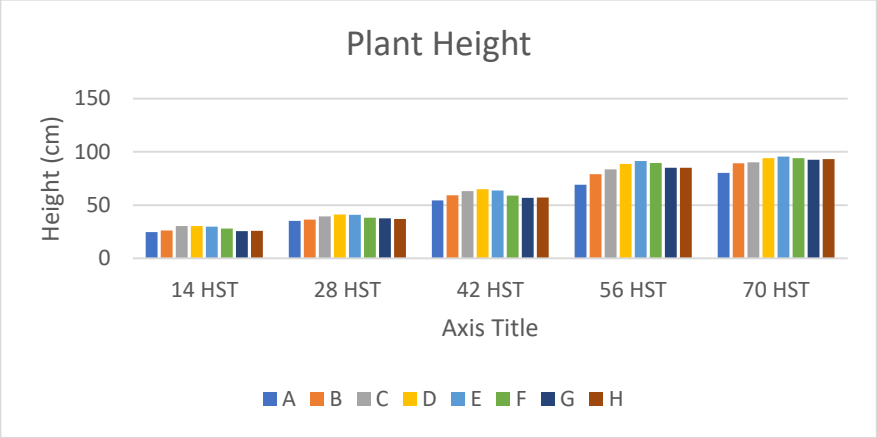

Figure 1. plant growth chart

Unlike the recommended NPK fertilizer, the NPK Co-Ameliorant formulation containing zeolite, activated charcoal and compost can retain N in the soil, so that its nutrient needs can be optimal until the final vegetative period. The addition of zeolite and activated charcoal to NPK Co-Ameliorant fertilizer makes the nutrient content in the soil available more optimally. The provision of zeolite can prevent leaching of nutrients out of the root area, so that nutrients in the root area can meet the needs of rice plants (Rajiman *et al.,* 2021). Activated charcoal is also able to keep ammonium in the fertilizer from being dissolved directly and undergoing rapid nitrification (Tarigan *et al.,* 2021). This makes the Bio- Organomineral NPK treatment proven to be better in increasing the number of tillers compared to the application of recommended NPK fertilizers that do not contain ameliorants.Kandungan klorofil dalam tanaman dapat mencerminkan tingkat pertumbuhan dan kesuburan tanaman yang nantinya dapat dikaitkan dengan prediksi produksi dari tanaman. Ada hubungan linier yang kuat antara nilai klorofil dan konsentrasi nitrogen (N) total daun karena nitrogen memiliki fungsi utama sebagai bahan sintesis klorofil (Shahzad *et al.,* 2023). Perlakuan NPK Co-Ameliorant menunjukkan kandyngan klorofil yang lebih tinggi karena dapat menyediakan N secara optimal untuk tanaman karena bersifat *slow release* dengan zeolit dan arang aktif membuat unsur N tidak mudah hilang akibat pencucuan dan volatilisasi. Tercukupinya unsur N membuat daun tanaman terlihat hijau yang menandakan kandungan klorofil yang tinggi, jika kandungan nutrisi tercukupi dengan baik maka produktivitas tanaman juga akan semakin tinggi.

Rendahnya tinggi tanaman, jumlah anakan dan kandungan klorofil pada perlakuan kontrol selain karena tidak adanya pemupukan juga disebabkan karena media tanah yang digunakan tercemar oleh logam berat. Konsentrasi logam berat yang tinggi dalam tanah maupun air irigasi juga dapat menghambat pertumbuhan tanaman padi dengan menghambat proses penyerapan nutrisi oleh akar tanaman. Media tanah yang digunakan mengandung kandungan logam berat Timbal (Pb) 25,41 mg kg-1, Kromium (Cr) 75,44 mg kg-1 and Tembaga (Cu) 72,81 mg kg-1. Logam berat Cr dan Pb yang berlebihan dapat menghambat pertumbuhan tanaman, merusak sel-sel akar, penghambatan pada klorofil dan membran sel dengan cara menghambat penyerapan unsur hara essensial maupun menggatikanya untuk diserap tanaman sehingga tanaman menunjukkan gejala toksisitas (Lidiana, 2022). Tanaman yang mengalami gejala toksisitas logam berat menunjukkan pertumbuhan tanaman yang kerdil, mudah rebah dan jumlah anakan sedikit (Laoli *et al.,* 2021)

The wet weight and dry weight of rice plants are affected by the moisture content in the plant. Wet weight is the total weight of the plant including the water in it, while dry weight is the weight of the plant after the water in it is removed. Plant dry weight is a determinant in the rate of photosynthesis and respiration. Plant dry weight reflects how much nutrients are absorbed by the plant,

which then helps the growth and development of the vegetative parts of the plant. The higher the plant dry weight, the more efficient the plant is in producing dry matter, increasing growth, and development and absorbing nutrients for vegetative growth (Karlinah *et al.,* 2023). NPK Co-Ameliorant treatment has a better plant dry weight than recommended fertilization. Increased fertilizer efficiency due to ameliorants contained in NPK Co-Ameliorant fertilizer allows plants to absorb nutrients optimally. Zeolite and activated charcoal are supporting materials for controlled nutrient release substances so that they can be optimally available (Aziz, 2023). In addition, compost in the formulation of NPK Co-Ameliorant fertilizer is able to fertilize the soil because it increases C-organic, cation exchange capacity, soil water holding capacity, solubility of N, P, K, Ca, and Mg elements, and reduces Al saturation and soil content weight (Nurmegawati *et al.,* 2020).

CONCLUSION

The application of NPK Co-Ameliorant fertilizer effectively increased rice plant height, number of tillers, chlorophyll content and plant biomass weight. The dose of NPK Co-Ameliorant fertilizer of 350 kg ha^{-1} was the best dose in increasing the height of rice plants reaching 95,63 at 70 DAP, increasing the chlorophyll content of plants by 24,20 CCI and increasing the dry weight of plants reaching 33,98 g. The application of NPK Co-Ameliorant fertilizer effectively increased the height of rice plants, number of tillers, chlorophyll content and biomass weight of plants.

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