Effects of Arbuscular Mycorrhiza-Enriched Bio-compost and Organic Fertilizer on Reducing Heavy Metal Absorption in Shallots

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Muhammad Akhsan Akib[1*], Sarjiya Antonius[2], Tutik Kuswinanti[3], Syatrawati[4], Tirta Kumala Dewi[2], Entis Sutisna[2]

[1]*Department of Agrotechnology, Faculty of Agriculture, Animal Husbandry and Fishery, Muhammadiyah University of Parepare, Jl. Jend Ahmad Yani KM.6, Parepare, South Sulawesi, 91131, Indonesia.*
[2] *Center for Applied Microbiology Research. BRIN. Jl. Raya Jakarta-Bogor km 46, Cibinong, West Java, 16911, Indonesia.*
[3]*Department of Plant Pest and Disease, Faculty of Agriculture, Hasanuddin University, Jl. Perintis Kemerdekaan KM.10, Makassar, South Sulawesi, 90245, Indonesia.*
[4]*Study Program of Food Crop Production Technology, Pangkajene Islands State Polytechnic of Agriculture, Jl. Makassar - Parepare axis KM. 83, Mandalle, Pangkajene and Kepulauan, South Sulawesi, 90761, Indonesia*

[*]*Corresponding email:* akhsanbagus@umpar.ac.id/akhsanakib@gmail.com

ABSTRACT

Shallots have physiological and pharmacological effects on the human body and have been cultivated intensively using bio-compost, which might be contaminated with heavy metals. The main objective of this study is to enrich arbuscular mycorrhizal fungi (AMF) in bio-compost and add biological organic fertilizer (BOF) to reduce the absorption of heavy metals in shallot bulbs. The study was conducted in Enrekang District, South Sulawesi, where the components of heavy metal content in plant organs and the level of infection of AMF in plant roots were observed. The study was a filed research arranged in a Randomized Block Design with five treatments, including shallots cultivation method by indigenous farmers/control(B0) and using various doses of bio-compost enriched with AM fungi of 100kg plot^{-1}(B1); 200kg plot^{-1}(B2); 100kg plot^{-1}+BOF(B3); and 200kg plot^{-1}+BOF). The results showed that bio-compost enriched with AMF at a dose of 200 kg plot^{-1} and 100kg plot^{-1}+BOF could increase Cr, Cu, and Pb accumulation in roots and leaf, as well as reduce metal accumulation in shallot bulbs, with the level of AMF infection classified as very high. The novelty of this study is that organic fertilizer enriched with AMF can reduce the absorption of heavy metals accumulated in shallot bulbs.

Keywords: Colonization; Endomycorrhizae; Organic fertilizer; Spice plant

INTRODUCTION

Shallots (*Allium cepa* L) are functional plants (Bamba et al., 2020; Hawayanti et al., 2021) that contain carbohydrates (16.80 g), protein (2.5 g), fiber (3.2 g), fat (0.1 g), vitamin A (9 IU), vitamin C (31.2 mg), thiamin (0.20 mg), riboflavin (0.11 mg), niacin (0.7 mg), pyridoxine (1.2 mg), folic acid (3 ug) and several minerals, such as phosphorus, calcium, sodium, iron and potassium (Amare, 2020; Aryanta, 2019). Shallots also contain active compounds that have pharmacological effects on the body, including flavonoids, saponins, quercetin, essential oils, and allicin (Marefati et al., 2021; Setiawandari et al., 2021), which have been used as traditional medicine for the treatment of ulcers, stomach, cholesterol, diabetes mellitus, and respiratory disorders (Mustofa et al., 2020; Zafran et al.,2021).

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Shallots as a vegetable commodity have been cultivated intensively by farmers. Organic fertilizer used as a source of plant nutrition has long been campaigned to create healthy and environmentally friendly agriculture. Shallots are a vegetable commodity cultivated intensively by horticulture farmers. Organic fertilizers that provide plant nutrients have long been promoted to support sustainable, healthy, and environmentally friendly agricultural practices. However, several research results show that organic fertilizer use has a negative impact because it contains heavy metals (Li et al., 2023; Subiksa et al., 2020). Heavy metals contained in organic fertilizers include boron (B), cadmium (Cd), Cobalt (Co), Chromium (Cr), copper (Cu), mercury (Hg), Manganese (Mn), molybdenum (Mo), Nickel (Ni), lead (Pb), and zinc (Zn), which are also contained in manure (El- Shabasy et al., 2023; Gong & Tian, 2019; Priyadi et al., 2021) and compost (Pinto et al., 2020; Ramísio et al., 2023).

Heavy metals, such as Cu, Zn, Mn, Fe, Mg, and Mo, can play an important role in plant metabolic processes (Angulo-Bejarano et al., 2021; Bhat et al., 2020) within a certain concentration range and become harmful at concentrations exceeding the maximum limit, especially for heavy metals such as Hg, Pb, Cd, As, Cr, and Ni (Ali et al., 2019; Balali-mood et al., 2021). Heavy metals such as Hg, Pb, Cd, As, and Cr are potentially toxic to plants, animals, and humans when contaminated soil is used for crop production. Lindawati et al. (2023) stated that Cd concentration in local Palu shallots in the planting area of Oloboju Village reached the concentration of 1.68 – 101.34 mg/kg in the roots and 0.01 – 0.04 mg/kg in the tubers, while in the planting area of Solove Village, the Cd concentration was 3.78 – 107.18 mg/kg in the roots and 0.01 – 0.03 mg/kg in the tubers; therefore, an environmentally friendly technology is needed as an effort to reduce the absorption of heavy metals by plants. One of the biological technologies that can reduce the absorption of heavy metals is arbuscular mycorrhizal fungi (AMF).

Plant roots with AMF association are known to reduce heavy metal contamination accumulated in plant organs (Dhalaria et al., 2020; Riaz et al., 2021). The results of preliminary research on post-nickel mining land have found three types of AMF spores that are resistant to heavy metals, namely *Acaulospora* sp, *Gigaspora* sp, and *Glomus* sp with different spore abundances, and they have been bred to increase the number of spores.

Several researchers have revealed that the AMF application can reduce Cd levels in *Brassica juncea* plant tissue in soil contaminated with Cadmium (Cd) (Nurlaili et al., 2021). Colonization of indigenous AMF *Glomus* sp. and *Acaulospora tuberculata* can limit Fe, Mn, and Ni levels in *Nauclea orientalis* L (Husna et al., 2021). The combination of compost and AMF applied to annual plants, such as *Nauclea orientalis,* can reduce levels of heavy metals Cr, Ni, Mn, Fe, and Zn in the soil and tissues or organs of plants that humans do not consume (Boorboori & Zhang, 2022; Putra et al., 2022). These three findings reveal the ability of tree plants to absorb metals to prepare phytoremediation plants in post-mining land but do not reveal the concentration of heavy metals accumulated in the organs of consumptive plants, such as shallots plants supported by AMF.

The mechanism of protection from heavy metal provided by AMF decomposition is carried out through the mechanism of hyphal secretion by external hyphal secretions (Chulikavit et al., 2023; Dhalaria et al., 2020); therefore, it is necessary to conduct research aiming to determine the proper dose of bio-compost enriched with AMF and biological organic fertilizer (BOF) to reduce the absorption of heavy metals accumulated in shallots bulbs; which is also the novelty of this research, supporting organic farming and a healthy environment, especially in areas central where shallots are planted which is essential information for farmers.

MATERIALS AND METHODS

Research Site

Horticultural research activities were carried out at the shallot development center in Pekalobean-Sipate Village, Enrekang District, South Sulawesi, at an altitude of 1022 m asl in the 2021-2022 dry season with an average air temperature of 30.0^{O}C and RH of 51.0%. The land area used is 1,125 m^2, with facilities including sprinkler irrigation and pest control equipment. The land area is divided into 15 sections, each with an area of 75 m^2.

Experimental design

The research was arranged in a Randomized Block Design (RBD) with three replications and five treatments: shallots cultivation method used by local farmers/control (B0); Bio-compost doses enriched with AMF of 100kg.plot^{-1}(B1); 200kg.plot^{-1}(B2); 100kg.plot^{-1}+ BOF (B3); and 200kg. plot^{-1}+ BOF (B4).

Preparation and application of bio-compost enriched with AMF

The bio-compost was goat dung produced by local breeders in the Enrekang District. Meanwhile, the AMF used resulted from early-stage research activities isolated from post-nickel mining land and bred in the Agrotechnology Greenhouse, Muhammadiyah University of Parepare. AMF are propagules containing Glomus spores, *Acaulospora* sp, *Gigaspora* sp, host plant root pieces, and carrier media (sand, zeolite, and biochar). Every 10 g of propagules contains 80-100 spores.

Enriching AMF in bio-compost for treatment B1 was carried out by mixing 100 kg of bio-compost with 7.5 kg of AMF propagules until homogeneous, then weighing 100 kg of the homogenized material for application in the field. The same thing was also done for treatment B3. Meanwhile, for treatment B2, 200 kg of bio-compost and 7.5 kg of AMF propagules were mixed until homogeneous, then 200 kg of the homogenized material was weighed for application in the field, and the same thing was also done for treatment B4.

Preparation and application of biological organic fertilizer

The biological organic fertilizer (BOF) used in this study was produced by the Indonesian Institute of Sciences (LIPI), which is now the National Innovation Research Agency (BRIN), with Patent No: 00201601284. BOF containing a population of root microbes (*Bacillus* sp., *Pseudomonas* sp., *Burkholderia* sp., *Brevundimonas* sp., and *Brucellaceae* sp) at a concentration of 10^6-10^7 CFU ml^{-1} was used to support the growth of shallot plants in treatments B3 and B4 at a dose of 10-15 L ha^{-1} applied 3 times a week.

Heavy metal analysis

The concentration of heavy metals (Cr, Cu, and Pb) in the soil and shallot tissue was analyzed at the chemistry and soil fertility laboratory at Hasanuddin University, Makassar, using an Atomic Absorption Spectrophotometer (AAS) with the HClO4:HNO3 method.

Mycorrhiza arbuscular fungi (AMF) infection analysis

The roots infected with AMF were observed and analyzed in the Biotechnology laboratory of Makassar Environmental and Forestry Research and Development Center (BP2LHK Makassar). Mycorrhizal infections were observed using the root staining technique, which involves selecting fine, fresh roots from the roots of the sample plants. The roots were placed in a tube containing FAA solution for 24 hours. The FAA solution was discarded, and the roots were washed until clean. Next, the roots were soaked in 10% KOH solution for 24 hours. The KOH solution was discarded, and the roots were cleaned and washed. Next, the roots were soaked in a hot H_2O_2 solution for 24 hours and washed thoroughly. The roots that had been washed thoroughly were soaked in a 2% HCl solution for 24 hours. The HCl solution was discarded, and the fine roots were washed with running water. Next, the roots were soaked in 0.05% trypan blue solution for 24 hours. Then, one root sample with a length of 1 cm was taken from the colored roots and arranged on a glass slide. Root pieces on slides were observed at each angle. The percentage of root infection was calculated using the following formula (Brundrett et al., 1996):

$$\textbf{Percentage of AMF infection} = \frac{\sum \textbf{number of fields of view infectied}}{\sum \textbf{total observed field of view}} \times \textbf{100\%} \qquad (1)$$

The percentage of MA fungi infection was classified based on the following criteria (Rajapakse & Miller, 1992):

<5% = extremely low category
6 – 25% = low category
26 – 50% = medium category
51 – 75% = high category
>75% = extremely high category

Statistical analysis

Observation data were statistically analyzed using Microsoft Excel software. Effects of AMF dose on the variables observed were determined using analysis of variance (ANOVA). Differences between treatments are categorized as significant if the P-value is less than 0.05 or 0.01 in the Duncan test.

RESULTS AND DISCUSSION

Heavy metal analysis before planting showed that the concentrations of Cr, Cu, and Pb in the shallot planting area were within normal limits for farming activities (Table 1). However, suppose the land is to be planted. In that case, caution is needed because heavy metals can be absorbed and accumulated in plant organs, reducing shallots' quality and causing health problems for other organisms (Briffa et al., 2020a; Briffa et al., 2020b).

Table 1. Concentration of heavy metals in soil and plants before and after planting shallots

Component	Heavy metal concentration (ppm)		
	Cr (Chromium)	Cu (Copper)	Pb (Lead)
Critical limits in soil	2.5[a]-100[b]	60-125[ab]	100[ab]-400[b]
Critical limits in plants	5-30[c]	20-100[c]	50[c]
The concentration of heavy metals in the soil before planting	185.721	58.956	112.147
The concentration of heavy metals in the soil after planting			
Plot B0	45.26	56.32	185.32
Plot B1	63.32	85.63	211.25
Plot B2	55.85	74.32	218.63
Plot B3	69.32	65.32	197.64
Plot B4	71.25	48.32	255.25

Notes: a (Ministry of State for Population and Environment Republic of Indonesia and Dalhousie University Canada, 1992); b (Kabata & Pendias, 2011); c (Alloway, 2013).

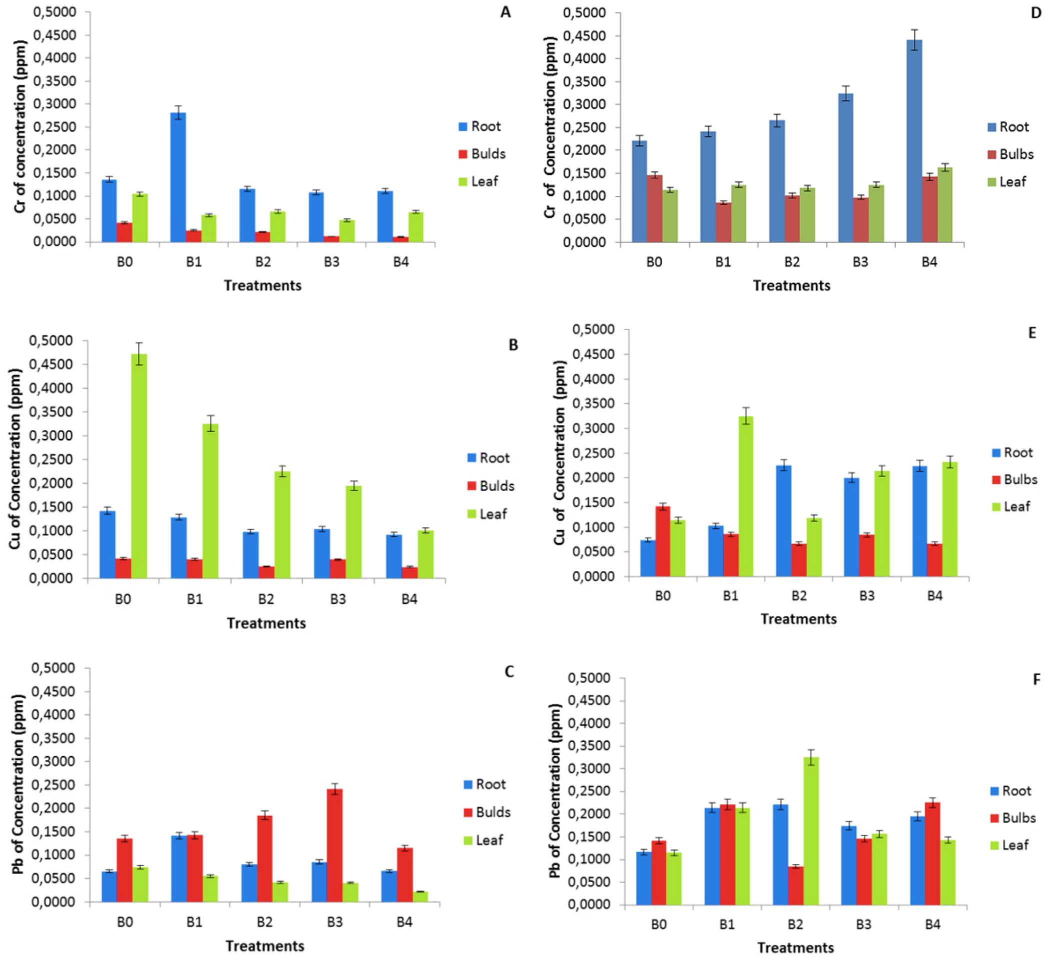

Figure 1. Average heavy metal concentrations of Cd, Cu, and Pb in shallot treated with treatment bio-compost enriched with AMF and BOF application at 30 (A, B, C) and 75 DAP (D, E, F)

The concentration of Cu and Pb in the planting area has increased after planting shallots. This increase can be caused by the use of chemical compounds (pesticides and fertilizers) by local farmers around the research area through the flow of rainwater and wind so that they can accumulate on the surrounding land. Rasman & Hasmayani (2018) stated that the types of pesticides used by

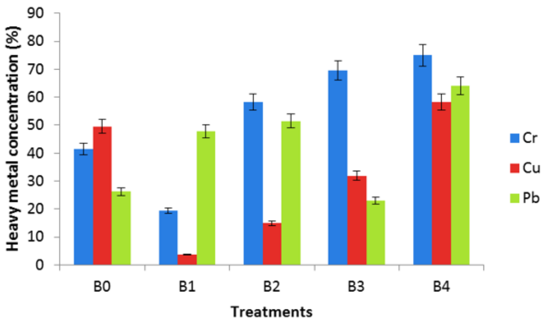

Figure 2. Percentage of the increase in heavy metal concentrations in shallot plants treated with bio-compost enriched with AMF and BOF application from 30 to 75 DAP

farmers in Enrekang that contain Pb include Antracol WP, Dithane M 45 80 WP, and Buldok EC, with Pb contents of 12.4800 ppm, 19.3710 ppm, and 2.0420 ppm, respectively, with application doses reaching 500-900 L ha^{-1} applied every 1-2 days. Meanwhile, the type of fertilizer used contains lead (Pb), namely 4.4511 ppm urea and 2.1620 ppm Nitro Ponska, which can also contribute to the heavy metals Zn and Cu. Ruhban & Kurniati (2017) stated that shallot plantations in Enrekang District, Indonesia, showed average Pb content in agricultural land aged 10 - 25 years of 15,224 - 17,523 ppm, and the cadmium content in shallots reached 0.2386 ppm. Dewi et al. (2021) added that there are 11 brands of pesticides from the avermectin, dithiocarbamate, glycine, imidazole, carbamate, organophosphate, pyrethroid, pyrrole, and triazole groups containing Pb of 2.70-22.31 mg kg^{-1} and Cd of 0.04-0.50 mg kg^{-1} and 5 brands of inorganic fertilizer containing 10.53-28.09 mg kg^{-1} of Pb and 0.07-0.52 mg kg^{-1} of Cd.

The phenomenon of Cr, Cu, and Pb metal concentrations appears to increase and decrease as the plants reach harvest time (75 DAP) (Figure 2), which are accumulated in the roots, tubers, and leaves of shallots (Figure 1). However, plants' average metal concentration is still within normal limits (Table 1).

Cr, Cu, and Pb accumulation in organ tissue occurs in two ways: absorption through plant leaves and roots. Because Cr, Cu, and Pb particles in the air fall and settle on the leaf surface, absorption through leaves happens (Hardiyanti et al., 2020). Shallot leaves have large stomatal size (long, 20-35 μm and wide, 5-15 μm) (Nur et al., 2020; Saparso et al., 2021) compared to the size of Pb particles (less than 4 μm), which allows the metal to enter leaf tissue through the stomatal pore (Hamidah et al., 2020). Once the metal enters the tissue, a buildup will occur between the cells of the leaf or root tissue (Ejaz et al., 2023; Febrita, 2020). Therefore, the content of heavy metals (Pb and Cu) in leaf tissue is higher than in other tissues, such as tubers and roots.

Metals of Cr, Cu, and Pb absorbed by hairy roots will experience binding, inactivation, and deposition as a protective strategy for plants (Giannakoula et al., 2021; Srivastava et al., 2021; Zulfiqar et al., 2019). Meanwhile, AMF protects host plants from absorbing toxic heavy metal elements through filtration, complexation, and accumulation effects (Chot & Reddy, 2022; Dhalaria et al., 2020).

Arbuscular mycorrhizal fungi can boost absorption and accumulation of heavy metal while limiting excessive absorption that enters plant cells, so they can function as a biological agent in situations when land is contaminated with heavy metals (Boorboori & Zhang, 2022; Dhalaria et al., 2020; Haider et al., 2021). Metal elements absorbed by AMF will be stored in vacuoles, cell walls, hyphae, vesicular and arbuscular (Begum et al., 2019; Dhalaria et al., 2020; Doyama et al., 2021). Thus, the concentration of heavy metals in root tissue will be higher than in the plant canopy.

The analysis of variance showed that bio-compost enriched with AMF significantly affected the percentage of shallot roots infected with AMF at the ages of 30 and 75 DAP. The Duncan test at 30 DAP demonstrated that bio-compost enriched with AMF of 200 kg plot^{-1} + BOF had a significant effect compared to the other treatments (Figure 3).

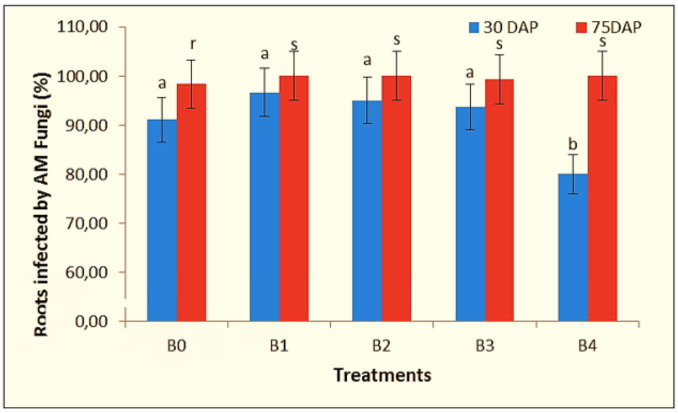

Figure 3. Levels of shallot root infection treated with bio-compost enriched with AMF and BOF application at 30 and 75 DAP

The availability of nutrients causes this phenomenon to occur through the application of bio-compost and BOF, which is at quite high doses, so it can reduce the effectiveness of AMF infections at 30 DAP, especially at a dose of 200 kg plot^{-1}+BOF. According to Muchoka et al. (2020), Robifahmi et al. (2020), and Prosanti et al. (2023), there is an inhibition of the performance of the AMF in fertile soil, especially the availability of P. In contrast, in soil with infertile conditions, the AMF is very active (Huey et al., 2020; Robifahmi et al., 2020). When the plants reached 75 DAP, all bio-compost treatments enriched with AMF responded similarly. AMF enriching bio-compost is thought to show positive performance. In addition to the availability of nutrients for plants and the ability of AM fungal spores to adapt to heavy metals, it is also possible that AM fungal spores have developed and grown in more significant numbers. However, this did not happen in the control treatment, even though many indigenous AM fungal spores were found.

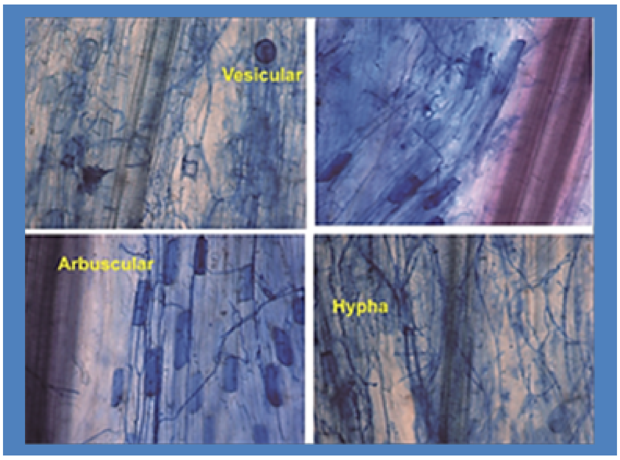

Figure 4. Cross section of shallot plant roots infected with AMF

The results of observations of roots infected with AMF at 75 DAP reached 76% - 100% and were classified in the highly high category. The performance of the AMF in carrying out infections from 30 to 75 DAP increased in each treatment, namely 7.34% (B0), 3.33% (B1), 5.00% (B2), 5.70% (B3), and 20.00% (B4). Figure 4 illustrates that the AMF organelles in vesicular, arbuscular, and hyphae found in root epidermal cells infecting shallot roots may bind heavy metals. This happens because the AMF is known to be able to bind heavy metals in carboxyl groups and pectic compounds (cellulose chemicals) in the matrix between the contact the surface of the AMF and the host plant, in the polysaccharide sheath and hypha cell walls (Begum et al., 2019; Dhalaria et al., 2020). AMF can bind metal ions to the cell walls of their hyphae and can protect plants from metal ions. Heavy metals are deposited in crystalloids in the fungal mycelium and mycorrhizal plant cortex cells (Dhalaria et al., 2020; Li et al., 2023).

CONCLUSION

Bio-compost enriched with AMF of 200 kg $plot^{-1}$ and 100 kg $plot^{-1}$+BOF increased metal accumulation in the roots and leaves and reduced Cr, Cu, and Pb accumulation in shallot bulbs.

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AUTHORS CONTRIBUTIONS

MAA, SY, and SA designed and conceived the experiments. MAA and SY conducted the experiment. MAA, TK, TKD, and ES contributed to the preparation of samples and interpretation of the results. The manuscript was primarily composed of MAA, TK, and SA. All authors provided critical feedback and contributed to developing the research, analysis, and manuscript.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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