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Amendment with Spent Mushroom Substrate Improved the Tolerance of Soybean (*Glycine max* L. Merr.) to Excess Soil Zinc

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ABSTRACT

This study was conducted to evaluate the effect of spent mushroom substrate soil amendments on soybean growth and zinc (Zn) phytoaccumulation in Zn-polluted soil. In a completely randomized factorial layout, soil Zn (0 and 400 mg Zn/kg soil) and spent mushroom substrate (SMS) amendment (0, 5, and 10 % w/w) were applied as treatments and soybean plants were maintained in the greenhouse for five weeks. Stunted growth and mild chlorosis were observed in soybean plants in the Zn-polluted soil with 0 % SMS amendment, but not in those with 5 and 10 %. Plants in the 0% SMS groups in polluted soil had higher root and shoot Zn concentrations and significantly reduced plant growth indices. While SMS amendment at 5 and 10 % reduced the Zn concentrations in plants in the polluted soil, it had no effect on the root-to-shoot Zn translocation, indicating that the SMS amendment affected Zn accumulation more than in planta distribution. While Zn treatment significantly reduced the pH of the soil, SMS amendment generally increased the soil pH. It is concluded that the amendment of Zn-polluted soil with spent mushroom substrate countered the Zn-induced increase in soil acidity, reduced Zn concentrations in plant tissue, and increased soybean tolerance to excess Zn. It may therefore be considered for use in reducing heavy metal uptake and stress in Zn polluted soils.

Keywords: Zn nutrition, soil pH, growth, remediation, stress tolerance.

1.0. Introduction

Trace element contamination can be a serious problem affecting plant production and overall ecosystem health. In many countries, trace element pollution of soil and water around communities where mining occurs can become a problem (Kabata-Pendias, 2011). In general, elevated trace element levels in soils could be due to natural or anthropogenic sources, including runoff from waste sites. In parts of Nigeria, soils around current and former mines have been reported to contain elevated levels of one or more trace elements, including lead (Pb) and zinc (Zn) (Oyebamiji *et al*., 2018). According to a study of several waste sites in Nigeria, Zn, chromium (Cr) and Pb were the top three metal contaminants, exceeding permissible limits for soils and vegetation (Nwaogu *et al*., 2017). In both the northern and southern parts of the country, heavy metal contamination of soils has been reported as an ongoing problem. For example, in parts of Zamfara State where mineral exploration occurs, the washout of metals from heaps of tailings has been revealed as a source of increased levels of trace elements including Zn, in the soils in five surrounding villages (Yahaya *et al*., 2021). And in Ebonyi state, some farmlands around the Enyigba lead-zinc mining site show elevated levels of Pb, cadmium (Cd), copper (Cu) and Zn that indicate moderate to high levels of contamination (Obasi *et al*., 2020). In addition to drainage from mines and chemical industry inputs, Zn contamination in farm soils may become extensive even when not highly excessive, due to long-term animal manure and sludge application (Alloway 2008; Ogiyama *et al*., 2010; Tóth *et al*., 2016). Although an essential trace element, Zn can

be toxic to plants when in excess. Excess Zn may affect plant growth by inducing reactive oxygen species (ROS) imbalance and inhibiting chlorophyll biosynthesis and iron (Fe) utilization in leaves (Chaney 1993; Petrov *et al*., 2015). It is capable of displacing divalent cations like Fe^{2+} and manganese (Mn^{2+}) at binding sites, when in excess (Van Assche and Clijsters, 1990). Some of the phytotoxicity symptoms of Zn include stunted growth, leaf chlorosis, and reduced biomass, amongst others (Rout and Das, 2003).

Mushrooms are a nutritious delicacy in many parts of the world, in addition to their medicinal value. They are often cultivated commercially on a base of straw, hay, or wood sawdust, supplemented with additional substances (Rinker, 2017). It has been estimated that approximately 5 kg of by-product remains for every kilogram of mushroom produced (Lin *et al*., 2014). Spent mushroom substrate (SMS) refers to the biomass remaining after the harvest of a crop of mushrooms. It is typically considered a waste product of the mushroom industry and might present a disposal problem (Muchena *et al*., 2021). Exploiting it to potentially enhance soil fertility, remediation, and tolerance to pollutants in contaminated soils could improve its utility in soil management. Some reports indicate the roles for mushroom substrate in agriculture to include as soil conditioner, source of organic matter, nutrients, and beneficial microbes, for adsorption of organic and inorganic pollutants, as material for raising seedlings in a nursery, and for plant protection against disease (Zhang *et al*., 2012; Othman *et al*., 2020; Ahlawat *et al*., 2022). Mushroom substrates have been studied for environmentally beneficial uses, such as amelioration of heavy metal toxicity in soil (Shuman and Li, 1997), degradation of hydrocarbons (Li *et al*., 2010), and remediation of mining polluted soils (Courtney and Harrington, 2012). While these studies provide impetus for evaluating its use in mitigation of metal toxicity in Zn polluted soils, such studies are scarce with respect to soybean production in Nigeria. Soybean is an important legume crop cultivated in parts of Nigeria for use as food, feed, and green manure, but its growth is reduced in heavy metal polluted soils. Strategies for enhancing its performance in such suboptimal soils will be beneficial, especially where food safety concerns can be mitigated by reduced element accumulation in crop tissue. This study was conducted to evaluate the effect of SMS soil amendments on the phytoaccumulation and tolerance to excess Zn, of soybean cultivated in Zn-polluted soil by measurements of plant growth, element nutrition and soil pH.

2.0 Methodology

2.1 Zn addition to soils

The soil utilized for this study was sandy-loam topsoil collected from the experimental farm site of Department of Genetics and Biotechnology, University of Calabar, Nigeria, with pH (6.3), EC (1.3 mS m^{-1}) organic carbon (0.85 %), total N (0.21 %) and available Zn (0.67 µg g^{-1}). The bulk soil was first sieved using a lab mesh (2 mm) to remove stones and other debris, then kept undisturbed for one week prior to mixing. $ZnSO_4 \cdot 7H_2O$ was artificially applied to the soil at the rate of 0 mg Zn kg^{-1} of soil (Zn-) and 400 mg Zn kg^{-1} of soil (Zn+) to induce excess conditions. Based on reports that the maximum allowable concentrations (MAC) of Zn in agricultural soils range from 120 to 300 mg Zn kg^{-1} (Alloway, 2008; Kabata-Pendias, 2011), 400 mg Zn kg^{-1} of soil was selected as excess treatment in this study (Ibiang *et al*., 2017). The Zn salt was first dissolved in distilled water then applied with mixing to the soil. All soils after mixing were bulked and stored in non-transparent bags for one week prior to amendment with SMS and seed sowing. NPK fertilizer was not added to the soil.

2.2 Mushroom substrate application and seeding

The SMS consisted mostly of wood sawdust and was obtained from the Department of Plant and Ecological Studies, University of Calabar, Nigeria. The edible mushroom farmed on it was *Pleurotus ostreatus*. Preliminary microbial analysis of the SMS indicated the tentative presence of the following bacterial species: *Pseudomonas sp., Micrococus leuteus, Shigella* sp., *Bacilllus substilis, Klebsiella* sp., *Salmonella* sp. and *Clostridium* sp., while fungal species included *Aspergillus* sp., *Fusarium* sp.,

Penicillium sp., *Alterneria* sp., *Mucor* sp. and *Rhizopus* sp. The SMS was collected for this study after two months of mushroom harvest, it was sundried in a glass house for one week and sieved to remove all pellets, prior to use for soil amendment. SMS addition to soils was performed one week after Zn treatment, at the rates of 0 % w/w (SMS0), 5% w/w (SMS5) and 10 % w/w (SMS10). The soils were then filled into respective plastic bags for seeding. Soybean seeds were sterilized in 70% ethanol and 10% H_2O_2 solution and rinsed in distilled water. The seeds were germinated on wet filter paper in petri dishes for 72 h, and those with protruding radicles were randomly picked for seeding in the bags (two seeds per pot later thinned to one after one week).

2.3 Experimental setup and cultivation practices

The experiment was set up as a 2×3 factorial in a completely randomized design. Factor 1 was the soil Zn condition (Zn-, Zn+) while factor 2 was the SMS treatment (SMS0, SMS5, SMS10). Each treatment was replicated three times (n=3) and the planted pots were maintained for five weeks in the greenhouse of the Department of Genetics and Biotechnology. All plants were routinely supplied with borehole water and Hoagland solution (50 mL pot^{-1} $week^{-1}$) and weekly shoot lengths were measured for five weeks after which the plants were recovered.

2.4 Plant harvest and measurement of Zn concentration

Plants were wholly harvested by carefully emptying the soil from the pots, breaking apart loosely attached soil, and washing roots with water to rid it of all soil particles, and drying with a paper towel. The fresh weights were measured, and plants cut into roots and shoots for measurement of their lengths, then dried in the oven at 80 °C until constant weights were attained. Trace element concentrations were determined in dry tissues. The ground plant samples were placed in ceramic vessels in an electric furnace at 550 °C for 6 h until white-ash, then digested in 0.6 mol L^{-1} HCl acid (Ibiang *et al*., 2017), after which Zn concentrations in solutions were measured using atomic absorption spectrophotometry (AAS). The root-to-shoot Zn translocation factor (TF) was calculated as Zn concentration in shoot divided by that in root and expressed as percentage (Stoltz and Greger, 2002).

2.5 Soil pH

Soils from each pot were recovered in a bag at harvest and bulked. 10 g of air-dried soil was mixed with 50 mL of distilled water, then vortexed twice at maximum speed for 30 secs, allowed to stand for 1 h, and vortexed again before measuring the pH.

2.6 Statistical analysis

Data collected were processed statistically by two-way analysis of variance (ANOVA) using STATCEL (ver4), with significance level set at $P<0.05$. The differences between the means of the treatment groups were based on Tukey-Kramer Tests.

3.0 Results and Discussion

3.1 *Results*

3.1.1 Plant growth indices

The visible effects of excess soil zinc seen in the Zn+ treatments were chlorosis in leaves and stunted growth as evidenced in the shoot lengths of the plants in Table 1. At two weeks after seeding (WAS), the shoot lengths showed significant differences with greater values in the Zn- treatments than in the Zn+, and the shoot length was highest in SMS5 in Zn- soil, and lowest in SMS0 in Zn+. At 3, 4, and 5 WAS, shoot lengths were lowest in SMS0 in Zn+ soil, and highest in SMS0 in Zn-, as plants in SMS0 ended up taller than those in SMS5 and SMS10 groups in Zn- soils. The root lengths (Table 1) showed no significant differences, but the shortest values were observed in SMS0 in Zn+ soil. The total fresh

weights (Table 1) showed a significant effect of soil Zn treatment and Zn×SMS interaction, with generally lower values in Zn+ compared to Zn- soils. In the Zn+ soils, the smallest weights were in SMS0, while in the Zn- soils, the smallest weights were in SMS10. The number of leaves per plant (Table 1) was significantly decreased due to excess zinc, with the lowest values in SMS0 in Zn+ treatment. In Zn- soils, SMS0 had lower values than SMS5 and SMS10, while in Zn+ soils, SMS5 and SMS10 had higher values than SMS0.

Table 1: Growth indices of soybean under varying soil Zn conditions and spent mushroom substrate amendments

	Zn-			**Zn+**		
	SMS0	SMS5	SMS10	SMS0	SMS5	SMS10
Shoot length 2WAS (cm)	21.33^{ab} ±2.33	24.83^{a} ±2.45	22.50^{ab} ±1.61	14.00^{b} ±2.08	22.67^{ab} ±1.45	21.00^{ab} ±2.30
Shoot length 3WAS (cm)	30.67^{a} ±0.67	29.33^{ab} ±3.48	26.83^{ab} ±2.61	14.00^{c} ±2.08	27.00^{ab} ±1.52	26.83^{ab} ±2.24
Shoot length 4WAS (cm)	39.00^{a} ±1.15	33.33^{ab} ±4.67	33.67^{ab} ±3.18	15.86^{c} ±1.93	29.27^{ab} ±1.26	29.33^{ab} ±1.76
Shoot length 5WAS (cm)	55.00^{a} ±5.00	39.00^{ab} ±5.19	39.33^{ab} ±3.18	15.87^{c} ±1.93	34.00^{b} ±1.52	39.00^{ab} ±4.04
Root length (cm)	19.17^{a} ±1.87	18.50^{a} ±2.92	17.33^{a} ±3.71	6.50^{a} ±1.25	17.00^{a} ±1.15	19.00^{a} ±5.29
Total Fresh weight (g)	8.15^{a} ±0.85	6.02^{ab} ±1.12	5.19^{ab} ±1.12	0.25^{c} ±0.01	2.16^{bc} ±0.20	3.68^{bc} ±0.86
Number of leaves/plant	5.33^{ab} ±1.20	6.00^{a} ±0.57	6.33^{a} ±0.33	2.33^{b} ±0.33	4.33^{ab} ±0.33	5.33^{ab} ±1.20

Values are Mean ±SE. ^{abc}Letters denote differences on the basis of Tukey-Kramer tests.

3.1.2 Zn nutrition in plants and soil pH

There were significant effects of soil Zn, SMS amendment and interaction on shoot Zn concentrations (Figure 1a) with higher values in Zn+ compared to Zn- soils. In Zn+ soils, SMS0 plants had significantly higher values than SMS5 and SMS10. In Zn- soils, increases in shoot Zn concentrations in SMS5 and SMS10 treatments compared to SMS0 were not significant. A similar trend was observed in root Zn concentrations (Figure 1b), with significant effects of soil Zn, SMS amendment and interaction. Values were higher in Zn+ plants compared to Zn-, while SMS treatments reduced root Zn concentrations in Zn+ soils. Root-to-shoot Zn translocation (Figure 1c) was not significantly different, but SMS treatments increased Zn translocation in Zn- soils but insignificantly decreased it in Zn+ soils. A significant effect of soil Zn and SMS amendment on soil pH was observed. The soil pH (Figure 1d) was generally lower in Zn+ soils compared to Zn-, while SMS treatment tended to increase the soil pH in both Zn- and Zn+ soils.

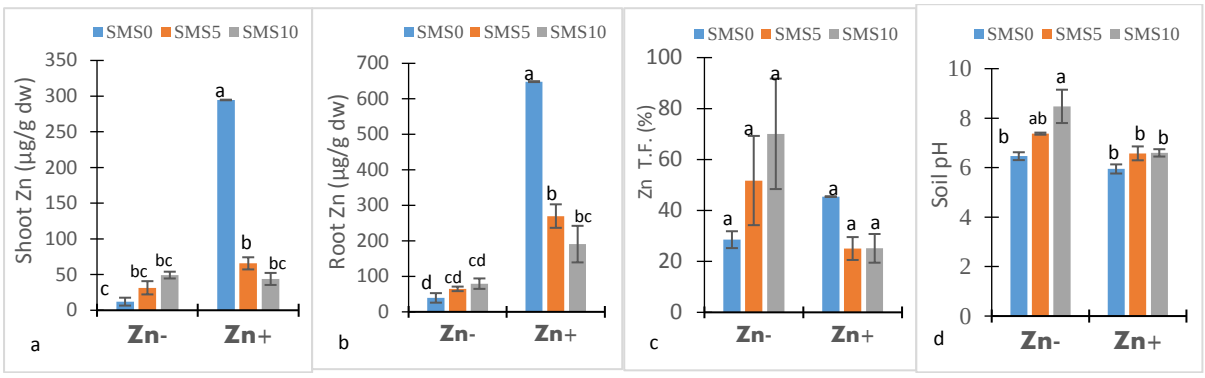

Figure 1. (a)Shoot Zn concentration, (b)Root Zn concentration (c) Root-to-shoot Zn translocation (d) Soil pH of soybean under varying soil Zn and spent mushroom substrate treatments. Values are Mean ±SEM. [abc]Values with similar superscript are not significantly different.

3.2 *Discussion*

This study demonstrated that the amendment of Zn-polluted soil with spent mushroom substrate reduced the toxicity occasioned by excess soil Zn in soybean. Higher plant Zn concentrations in Zn+ soils compared to Zn- are obviously due to the excess Zn content in the soil, in line with the treatment. The reduction in the shoot lengths, fresh weights, number of leaves, and mild chlorosis observed in Zn+ soils with 0 % SMS amendment are among the typical symptoms of Zn phytotoxicity (Rout and Das, 2003; Shi *et al*., 2015). Zinc toxicity symptoms in plants may become very visible at leaf concentrations ≥ 300 mg Zn kg^{-1} dry weight (Marschner, 1995), in line with our observations for SMS0 groups in Zn+ soil. Chlorosis of the leaves indicate that Zn is likely interfering with Fe and chlorophyll metabolism, and this could occur due to Zn-Fe antagonism playing out as Zn interference in Fe translocation from roots to shoots (Kabata-Pendias, 2011). Such interference is partly due to competition in the chelation processes involved in plant Fe translocation from roots to tops, and the existence of shared transporters such as the Iron Regulated Transporter 1 (IRT1) (which can transport Fe and Zn) (Korshunova *et al*., 1999; Kim and Guerinot, 2007; Krohling et al. 2016). The absence of significant effect of SMS soil amendment on root-to-shoot Zn translocation indicates that the mechanism of mitigating Zn toxicity has more to do with limiting Zn phytoaccumulation than by modulations of element homeostasis in the plants. In other words, the SMS amendment affected Zn uptake rather than *in planta* Zn distribution. Trace element accumulation is highly dependent on the bioavailability of the element, which in turn, is strongly influenced by the soil pH (Rieuwerts *et al*., 1998; Rengel, 2015). Zn addition to soils increases soil acidity since Zn is a lewis acid and is mostly mobile and available to plants in the form of soluble free and complex ions (Kabata-Pendias and Sadurski, 2004). Consequently, soil amendments that counteract excess Zn-induced soil acidity should reduce the element availability and uptake. According to Kalembasa and Becher (2012), spent mushroom substrates may strongly bind metals with organic and mineral compounds, fulvic and more stable humic acids. The calcium carbonates, ash and high organic matter in spent mushroom substrates can increase the pH of soils as well as bind to heavy metals such as Zn ions, rendering them less bioavailable. The decreased soil acidity due to application of spent mushroom substrate will make Zn less available for plant uptake and limit Zn nutrition in Zn+ soils. This is in line with the significantly lower root and shoot Zn concentrations observed in the plants cultivated in SMS5 and SMS10 groups in Zn+ soils. Aside organic matter enrichment, mushroom substrates increase the soil microflora and enhances soil enzymatic activities, which both have the potential to impact element nutrition of plants (Zhang *et al*., 2012). The preliminary microbiological screening of the substrate did indicate the presence of some bacterial and fungal species therein. In Zn- soils, however, SMS amendment did not appear to benefit soybean growth, judging by the plant growth indices. Although some reports indicate that mushroom substrate might be a source of extra nutrients

to plants and potentially increase plant growth (Muchena *et al*., 2021), this was not generally the case in this study. It must be noted that effects on plant growth vary based on the species cultivated, the compost material, the amount and age after mushroom harvest, of the substrates applied to the soil (Çatal and Aysun, 2020; Andrews *et al*., 2021).

4.0 Conclusion

In conclusion, the amendment of Zn-polluted soil with spent mushroom substrate countered the Zn-induced increase in soil acidity, reduced Zn concentrations in plant tissue, and increased soybean tolerance to excess Zn. It may therefore be considered for use in mitigating heavy metal stress in Zn polluted soils in Nigeria.

Conflict of interest
None declared.

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