Exogenous proline and hormone in combination with compost improves growth and tolerance of maize under heavy metal stress

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Abstract: Abiotic stress such as heavy metal contamination of agricultural soil limits crop production by disrupting plant's physiological activities and seed germination. Exogenous proline, hormone and compost have been used to induce growth and stress tolerance in crop but little is known about their interactive and anti-oxidative roles in crop exposed to heavy metal stress. This study assessed the effects of pre-sowing seed soaking with exogenous proline (P) and hormones (Gibberellic acid; GA and Indole-acetic acid; IAA) on the Pb accumulation, osmolytes (P, phenolics; PH and Glycine betaine; GB), Photosynthetic pigments (chlorophyll and carotenoids) and stress indexes (H_2O_2 and malonialdehyde; MDA) of maize grown on compost amended Pb (23,959 mg kg^{-1} Pb) contaminated soil. Compost was applied at 20 t ha^{-1} and 40 t ha^{-1}, P, GA and IAA were applied at 10 mM and 5 mM solely and in combinations to give a total of 34 treatments including control (Distilled water only) and replicated 3 times. Except in IAA treatment, seed pre-treatments with exogenous P and GA and soil amendment with compost generally enhanced chlorophyll production in maize and reduced GB, carotenoids, H_2O_2 and MDA in treated seeds compared to control. With regards to stress indexes G2+C1 gave the lowest values. Sole application of P1, P2, C2, G1 and I2 enhanced the production of proline in treated plants compared to combined application. Combination with compost was better than hormonal combination. A combination of P2 with C (40 t ha^{-1}) (P2C2) gave the maximum chlorophyll content and reduced the endogenous GB and P. Treatment with P2C2 also reduced Pb accumulation in plant. In conclusion, exogenous application of hormones and proline in combination with organic amendment induces heavy metal tolerance in maize and increased protection against oxidative stress compared to contaminated control. The technique that can enhance crop growth on contaminated soil, reduce metal uptake and provide osmo-protection could be a promising approach.

Keywords: Contamination, Hormones, Osmolytes, Organic amendment, Seed soaking, Heavy metals.

INTRODUCTION

Soil contamination with heavy metals is one of the abiotic stresses that cause a reduction in crop production (Sandalio *et al*., 2001). They affect plant physiological processes and induce production of Reactive Oxygen Species (ROS) that cause oxidative damage and lipid peroxidation (Eun *et al*., 2000; Siedlecka & Krupa, 2002; Verma & Dubey, 2003; Souguir *et al*., 2011). The contamination of agricultural lands from different anthropogenic sources is most worrisome. Apart from reducing the amount of cultivable lands, it increases the risk of animal and human contamination with toxic heavy metals through food chain and poses a threat to food security. Heavy metal also causes reduction in photosynthesis and retard crop growth by competing with essential mineral nutrients and inducing phytotoxicity (Siedlecka, 1995; Sharma & Dubey, 2005, 2007). Different efforts have been made to remediate heavy metal contaminated soils (Adejumo *et al*., 2010) but none of the strategies proposed can give complete remediation. The development of metal-tolerant plants has also not been possible due to the complexity of the procedures involved. There is need therefore, for the development of simple and cost-effective methods for containing the contaminants, reduce/prevent plant uptake and enhance crop tolerance.

Though the use of immobilizing agents to reduce metal solubility has been used effectively for reducing metal uptake by cultivated crops (Adejumo *et al.* 2010, Salati *et al.* 2010, Adejumo *et al.* 2018a) but, the first challenge in crop production on contaminated soil is seed germination and seedling development. Seed germination is the most crucial step in plant's life cycle that ensures the survival of the next generation. If these hurdles can be overcome, plants have natural inbuilt ability to induce anti-oxidative mechanisms that can be used for survival in the face of different environmental challenges (Hossain *et al.*, 2012). In this regard, the technique that can stimulate seed germination, induce seedling development, reduce metal uptake, ensure nutrient availability for crop development and provide osmo-protection could be an acceptable and comprehensive method for enhancing crop tolerance to heavy metal toxicity.

Seed priming technique with osmo-protectants and growth elicitors like hormones have been implicated in the activation of some pre-germination metabolic processes and stress tolerance inducement (Tsegay & Andargie, 2018).

Proline, an amino acid that participate in the osmotic adjustment of plant cells under stress has been reported to improve plant resistance to oxidative stress induced by heavy metal toxicity by scavenging ROS (Martins *et al.*, 2011; Hossain *et al.*, 2012; Adejumo *et al.*, 2015). Similarly, plant hormones which are chemical substances produced by the plant to stimulate growth and development (Zhang *et al.*, 2007) are also involved in signaling activity for inducing plant stress responses (Iqbal & Ashraf, 2013). Plants' responses to environmental stresses have also been reported to include up or down-regulation of phytohormones (Ishii *et al.*, 2010; Strader *et al.*, 2010). Similarly, positive responses to exogenous application of hormones have been reported (Yuan & Xu, 2001; Ashraf *et al.*, 2002; Iqbal & Ashraf, 2013). Gibberellic acids (GAs) and ABA are a group of plant growth hormones generally reported to be involved in the abiotic stress tolerance and developmental processes (Yuan & Xu, 2001; Magome *et al.*, 2004; Iqbal & Ashraf, 2013). Gibberellin is known for seed germination while auxin hormone has been reported to induce rooting especially in stem cuttings when exogenously applied and induce synthesis of endogenous cytokinnin (Zhang *et al.*, 1995; Camolesi *et al.*, 2007; Singh & Khan, 2009; Wang *et al.*, 2009). Their effectiveness however, varies depending on the concentration and interactions with other treatments.

Uptake, transport and accumulation of metal by plant are strongly influenced by soil conditions and activities taking place at the root zone (Bolan *et al.*, 2008; Adejumo *et al.*, 2018b). The uptake of Pb for instance, is especially influenced or determined by the level of organic matter in the soil (Adejumo *et al.*, 2010, 2011, 2012, 2018c). Pre-sowing seed treatments with an osmoprotectant like proline and growth regulators to induce tolerance and germination could be a promising approach to enhance crop growth on contaminated soil and ameliorate abiotic stress in crop plants. This can then be coupled with soil amendment with organic materials like compost to immobilize heavy metals in the soil (Adejumo *et al.*, 2011). In our previous study, seed germination, maize growth and yield, as well as duration of survival, responded positively to proline, hormone and compost treatments (Awoyemi & Adejumo, 2018). Under this study, osmolytes production, photosynthetic pigments and oxidative stress tolerance of maize crop in response to pre-sowing seed soaking with hormones and proline were investigated. Understanding the anti-oxidative roles and the interplay between exogenous proline, hormones and compost on crop will guide in the development of appropriate strategies for enhancing crop tolerance to heavy metal stress and ensuring food security.

METHODOLOGY

Sources of soil used for the experiment

The study involved the use of contaminated soil and this was obtained from battery wastes dump site, Lalupon, Ibadan, Nigeria while the normal (uncontaminated) soil used as control was obtained from the crop garden of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria. The contaminated site was the dumpsite of the defunct Lead-acid Battery manufacturing company (Adejumo *et al.*, 2011). Top soil was collected from five equidistant points on the contaminated site and thoroughly mixed together before packing them in the sacs. The soil was first air-dried and sieved using 2 mm mesh. The composite sample was then taken for heavy metal analysis following standard procedure and the Pb concentration was 23,959.00 mg kg^{-1} in contaminated soil.

Experimental treatments and design

Compost was prepared from Mexican sunflower and poultry manure following the procedure of Akanbi (2002) and Adejumo *et al.*, (2010) while, proline, giberrellic acid and indole-acetic acid were obtained from the chemical store. As described in Awoyemi & Adejumo (2018), for this experiment, the treatments were denoted as Compost (C), Proline (P), Gibberellic acid (G) and Indole-acetic acid (I) and were applied using two rates. Compost was applied at 20 t ha^{-1} and 40 t ha^{-1} while proline and hormones were applied at 10 mM and 5 mM as sole (C1, C2, I1, I2, P1, P2, G1, G2,) and combined (I1+G1, I1+G2, I1+P1, I1+P2, I2+G1, I2+G2, I2+P1,I2+P2, G1+P1, G1+P2, G2+P1, G2+P2, I1+C1, I1+C2, I2+C1, I2+C2, G1+C1, G1+C2, G2+C1, G2+C2, P1+C1, P1+C2, P2+C1, P2+C2) applications to give a total of 34 treatments including control (Distilled water only) and replicated 3 times using Completely Randomized Design. Hormones and proline were prepared based on their molecular weights as explained by Awoyemi & Adejumo (2018). Seed soaking procedure was carried out according to the procedure of Hanan *et al.* (2013) and adopted by Awoyemi & Adejumo (2018). Plastic pots were filled with 1 kg each of both contaminated and uncontaminated soils and compost was applied to the pots that were receiving compost treatments two weeks before planting to ensure proper mineralization. Each pot was sown with four seeds of maize (DTMR). The emerging seedlings were thinned down to 2 after emergence and the soil was watered moderately till the end of the experiment which lasted for 8 weeks.

Determination of chlorophyll and carotenoid

The extraction was done using the method described by Sarropoulou *et al.* (2012). The absorbance of Chlorophylls a and b were measured at 665 and 649 nm respectively while Carotenoids was measured at 440nm using UV/VIS

Spectrophotometer (Spectrumlab 752s Ningbo, China). Total chlorophyll and Carotenoid were determined using the equation below:

$$Chlorophyll\ (a+b) = (6.10 \times A665 + 20.04 \times A649) \times 10/1000/F.W\ (mg/g\ F.W)$$

$$Carotenoid = (4.69 \times A440 - 1.96 \times A665 - 4.74 \times A649) \times volume\ of\ supernatant\ (10\ ml) \times dilution\ factor/\ 0.1$$

Determination of Proline, glycine betaine and total phenolics

Proline was estimated in the plant leaves according to Bates *et al.* (1973) using acid-ninhydrin method and the red colour intensity was measured at 520 nm. Leaf Glycine betaine was determined following the procedure described by Grieve & Grattann (1983) and the organic layer measured at 365 nm. Total phenolics was determined following Julkunen-Titto (1985) procedure using Folin Ciocalteaus and was read at 750 nm and results expressed as mg g^{-1} of fresh leaf weight using UV/VIS Spectrophotometer (Spectrumlab 752s Ningbo, China).

Hydrogen peroxide (H$_2$O$_2$) and Malononyldialdehyde (MDA) contents Determination

The hydrogen peroxide in the plants was determined following Chanda (2012) procedure while the protocol suggested by Velikova *et al.* (2000) was used for MDA determination, with some modifications. Tissue material (500 mg) was grinded with sand in a mortal containing 5 ml of 0.1 (w/v) trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 10,000 x g for 20 minutes and 1ml of the supernatant was divided into two eppendorph tubes as 0.5 ml per tube. Then, 1 ml of 0.5 % (w/v) Thiobabituric (TBA) in 20% TCA solution was added into these aliquots and they were incubated at 96ºC for 30 minutes. The reaction was stopped by placing the reaction tubes in an ice bath for 5 minutes and then centrifuged at 10,000 x g for 5 minutes. The absorbance of the supernatants was monitored at 532 nm. The amount of MDA - TCA complex was calculated from the extinction coefficient of 155 mM^{-1}cm^{-1}.

Analysis for Pb concentration in soil and plant

Soil Pb concentration before and after planting was analyzed according to the method described by Ogundiran (2007). The filtrates were made up to 100 ml in a standard flask and were read for Pb concentration using Atomic Absorption Spectrophotometer (VGP210 BUCK Scientific Model). Plant analysis for Pb concentration was carried out at harvesting using dry ashing method and Pb concentration was determined in the final filtrate using Atomic Absorption Spectrophotometer (VGP210 BUCK Scientific Model).

Data were analyzed using ANOVA of DSASSTAT version 2012. Means were separated using Duncan Multiple Range Test (DMRT) at p<0.05.

RESULTS

Effects of sole application on photosynthetic pigments, proline, glycine betaine, phenolics and stress indexes (H$_2$O$_2$ and MDA) of maize grown on Pb contaminated soil

Table 1. Effect of sole application of different concentrations of proline, hormones and compost on photosynthetic pigments, stress indexes (H$_2$O$_2$ and MDA), phenolics, proline and glycine betaine contents of maize grown on Pb contaminated soil.

Treatments	CHL	CRD	GB	PHNL	PROLINE	MDA	H$_2$O$_2$
C1	2.741e	797.813b	0.768d	0.713c	0.25bcd	0.046c	0.403c
C2	2.699e	800.07a	0.456f	0.926b	0.54d	0.016c	0.401c
P1	5.459a	618.054i	1.099c	0.314e	0.363bcd	0.03c	0.596b
P2	3.243d	641.306h	0.715de	0.91b	0.386ab	0.011c	0.517bc
G1	1.692f	744.686f	0.587ef	0.478d	0.472a	0.597a	0.34c
G2	3.98b	790.813c	0.543f	0.615cd	0.196cd	0.26b	0.777a
I1	3.125d	778.86d	1.272b	1.11a	0.174d	0.035c	0.376c
I2	2.825e	711.481g	1.174bc	0.557cd	0.385ab	0.04c	0.709a
CNS	5.566a	598.858j	0.883d	0.516d	0.178d	0.115bc	0.335c
CLCS	3.597c	751.638e	1.488a	0.57cd	0.241bcd	0.052c	0.461bc

Note: C= Compost, P= Proline; G= Gibberellic acid; I= Indole acetic acid; CNS= Control uncontaminated soil; CLCS= Control contaminated soil, 1= Concentration at 5 mM and rate at 20 t ha^{-1}, 2= Concentration at 10 mM and rate at 40 t ha^{-1}.

On the effect of the sole application on total chlorophyll contents of maize, it was observed that the application of proline at a lower rate performed better than other treatments including uncontaminated treatment. This was followed by those pre-treated with G2 and those planted on uncontaminated soil (CNS) while maize treated with G1 had the least chlorophyll content (Table 1). Carotenoid, unlike chlorophyll, was low in the maize pretreated with P compared to other treatments. Maize planted on soil amended with compost had the highest value but not different from hormone treatments. This was followed by that of maize grown on contaminated control (CLS) and treatment with G2. Similar to what was observed for carotenoid, glycine betaine was reduced in response to seed pretreatment with proline and

hormone as well as soil amendment with compost. Maize pre-treated with G had the lowest while maize planted on contaminated soil only had the highest. Compared to the control plants (Distilled water), sole application of P1, P2, C2, G1 and I2 enhanced the production of proline in treated plants compared to untreated seeds in contaminated control. The lowest value of proline was recorded in maize planted on uncontaminated soil. Seed pretreatment with I1 gave the highest concentration of phenolics in maize leaf compared to other treatments. This was followed by those pre-treated solely with P2 and C2 while it was low in maize pre-treated with P1. On hydrogen peroxide content, the highest value was recorded in maize pre-treated with G2 followed by those of I2. Surprisingly, the lowest value was recorded in maize grown on control soil followed by those pre-treated with a lower rate (5 mM) of hormone and those grown on soil treated with C1 and C2. Malonylaldehyde (MDA) value was reduced in maize pre-treated with P2 but was high in maize pre-treated with I1 followed by that of G2 (Table 1).

Photosynthetic pigments, proline, glycine betaine, phenolics and stress indexes (H_2O_2 and MDA) of maize grown on Pb contaminated soil as affected by combined application of proline and hormone without compost

Table 2. Effect of combined application of different concentrations of proline and lower rate of hormones on photosynthetic pigments, stress indexes (H_2O_2 and MDA), phenolics, proline and glycine betaine contents of maize.

Treatments	CHL	CRD	GB	PHNL	PROLINE	MDA	H_2O_2
P1I1	4.494b	331.065h	0.97c	0.715b	0.316bc	0.048ab	0.586cd
P2I1	3.667d	666.463e	0.989c	0.173d	0.402b	0.062ab	0.937a
G1I1	3.942c	743.465c	0.763d	1.131a	0.296bc	0.021b	0.805ab
G2I1	1.643f	713.878d	0.76d	0.411c	1.238a	0.055ab	0.754abc
P1G1	3.041e	1010.467a	0.96c	0.223d	0.279bc	0.143ab	0.66bc
P2G1	3.502d	655.986f	1.262b	1.113a	0.209c	0.233a	0.403e
CNS	5.566a	598.858g	0.883cd	0.516c	0.178c	0.115ab	0.335e
CLCS	3.597d	751.638b	1.488a	0.57bc	0.241bc	0.052ab	0.461de

Note: C= Compost, P= Proline; G= Gibberellic acid; I= Indole acetic acid; CNS= Control uncontaminated soil; CLCS= Control contaminated soil, 1= Concentration at 5 mM and rate at 20 t ha^{-1}, 2= Concentration at 10 mM and rate at 40 t ha^{-1}.

Table 3. Effect of combined application of different concentrations of proline and higher rate of hormones on photosynthetic pigments, stress indexes (H_2O_2 and MDA), phenolics, proline and glycine betaine contents of maize.

Treatments	CHL	CRD	GB	PHNL	PROLINE	MDA	H_2O_2
P1I2	5.126c	636.116e	0.945bcd	0.326c	0.254ab	0.025b	0.697ab
P2I2	4.569d	701.892d	0.834d	0.819a	0.248ab	0.038b	0.654abc
G1I2	6.786a	137.968h	0.987bcd	1.002a	0.304ab	0.015b	0.621abcd
G2I2	3.39g	527.505g	0.941bcd	0.231c	0.199b	0.012b	0.782a
P1G2	4.305e	708.084c	1.122b	0.21c	0.157b	0.738a	0.504cde
P2G2	4.241e	769.71a	1.026bc	0.341c	0.431a	0.811a	0.528bcd
CNS	5.566b	598.858f	0.883cd	0.516b	0.178b	0.115b	0.335e
CLCS	3.597f	751.638b	1.488a	0.57b	0.241b	0.052b	0.461de

Note: C= Compost, P= Proline; G= Gibberellic acid; I= Indole acetic acid; CNS= Control uncontaminated soil; CLCS= Control contaminated soil, 1= Concentration at 5 mM and rate at 20 t ha^{-1}, 2= Concentration at 10 mM and rate at 40 t ha^{-1}.

On the interactions between hormones in combination with proline, though the highest value of chlorophyll was recorded in uncontaminated control maize, but among the pretreated seeds, P1+I1 gave the highest value followed by maize pre-treated with G1+I1. Lowest chlorophyll content was recorded in maize pre-treated with G2+I1. For carotenoid, highest value was recorded in maize pre-treated with P1+G1 while, maize pre-treated with P1+I1 had the lowest. On the GB content, maize planted on contaminated soil had the highest value followed by those pre-treated with P2+G1 and the lowest value in maize pre-treated with G1+I1. On proline content seed pre-treated with G2+I1 had the highest value and lowest value of proline was found in maize grown on uncontaminated soil. Similarly, highest value of phenolics was recorded in maize pre-treated with G1+I1 followed by that of P2+G1 and those pre-treated with P1+I1. For hydrogen peroxide content, compared to control, seed pre-treatment with P2+I1 conversely gave the highest value followed by those pre-treated with G1+I1, while lowest hydrogen peroxide value was recorded in those grown on uncontaminated soil as well as maize pre-treated with P1+G1. Combined P2+G1 gave the highest malonyaldehyde (MDA) value followed by P1+G1 treatment. The lowest MDA value was recorded in maize pre-treated with G1+I1 followed by that of uncontaminated control (Table 2). The higher concentration of indole-acetic acid in combination with G and P gave different results compared to a lower rate. The result showed that the highest chlorophyll value was recorded in maize pre-treated with G1+I2 followed by maize grown on uncontaminated soil while lowest chlorophyll value was found in maize pre-treated with G2+I2. For carotenoid content, highest value was found in maize pre-treated with P2+G2, followed by maize grown on contaminated soil and those pre-treated with P1+G2. The lowest mean value was recorded in maize pre-treated with G1+I2. Except in maize grown on contaminated soil, GB was reduced in other

treatments and the lowest value was recorded in maize pre-treated with higher concentration of proline combined with higher concentration of indole-acetic acid. On the proline content, maize pre-treated with P2+G2 had the highest value, followed by those pre-treated with G1+I2 and of contaminated control. However, the lowest proline value was recorded in maize pre-treated with P1+G2. The phenolic content of Pb stressed maize plant was found to increase when seeds were pre-treated with G1+I2 compared to that of untreated maize grown on contaminated soil. This was followed by the treatment with P2+I2. The lowest value was recorded in maize pre-treated with P1+ G2 and maize pre-treated with G2+I2. On the stress indexes, more of hydrogen peroxide was produced in maize pre-treated with I2 in combination with G and P (i.e G1+I2, G2+I2, P1+I2 and P2+I2) compared to control. Lowest value was recorded in maize grown on uncontaminated soil. Similarly, MDA content was more in maize pre-treated with lower concentration of proline and gibberellin when combined with higher concentration of indole-acetic acid while reduced values were recorded in other treatments including control (Table 3).

Photosynthetic pigments, proline, glycine betaine, phenolics and stress indexes (H_2O_2 and MDA) of maize grown on Pb contaminated soil as affected by combined application of proline, hormone and compost

Using lower rate of compost, highest value of chlorophyll was recorded in maize pre-treated with G2+C1. This was followed by maize pre-treated with I2+C1 and maize grown on uncontaminated soil. The lowest value of chlorophyll was found in maize pre-treated with I1+C1. However, maize pre-treated with I1+C1 had the highest carotenoid value followed by those pre-treated with P1+C1 and P2+C1. The lowest value was recorded in maize pre-treated with G2+C1. Compared to control, maize pre-treatment with proline in combination with C1 had no significant effect on chlorophyll content. As observed above, GB was more in untreated maize planted on contaminated soil without amendment. This was followed by that of maize plant pre-treated with P1+C1 and those pre-treated with G2+C1. The lowest value was recorded in maize pre-treated with G1+C1 and maize pre-treated with I2+C1. Among the combinations, for the leaf proline content, the highest value was recorded in maize pre-treated with P1+C1 followed by maize pre-treated with G2+C1 while, the lowest value was recorded in maize grown on uncontaminated soil. Phenolic content was more in maize treated with G1+C1 followed by I1+C1 and I2+C1. Compared to other treatments, maize pre-treatment with the combination of proline at both rates with a lower rate of compost, however, reduced the phenolic content in maize. On stress indexes of maize, hydrogen peroxide, was more in maize pre-treated with P2+C1 followed by those pre-treated with P1+C1. Lowest hydrogen peroxide content was recorded in maize pre-treated with G2+C1. Meanwhile, malonylaldehyde (MDA) content was recorded to be the highest in maize pre-treated with I1+C1 followed by maize pre-treated with P2+C1. The lowest value was found in G1+C1 and G2+C1 and not significantly different from those grown on contaminated and uncontaminated soil (Table 4).

Table 4. Effect of combined application of different concentrations of proline, hormones and lower rate of compost on photosynthetic pigments, stress indexes (H_2O_2 and MDA), phenolics (PHNL), proline and glycine betaine (GB) contents of maize.

Treatments	CHL	CRD	GB	PHNL	PROLINE	MDA	H_2O_2
P1C1	3.626e	781.226b	1.249b	0.416bc	0.371a	0.167cd	0.588ab
P2C1	3.659e	762.436c	0.828cd	0.331c	0.209ab	0.371b	0.71a
G1C1	4.824d	652.681e	0.694d	0.903a	0.241ab	0.569a	0.485bc
G2C1	7.79a	302.498h	1.14b	0.516b	0.349ab	0.27bc	0.232e
I1C1	2.914f	816.344a	0.914c	0.825a	0.23ab	0.022d	0.291de
I2C1	5.881b	591.947g	0.711d	0.794a	0.251ab	0.027d	0.448bcd
CNS	5.566c	598.858f	0.883c	0.516b	0.178b	0.115cd	0.335cde
CLCS	3.597e	751.638d	1.488a	0.57b	0.241ab	0.052d	0.461bcd

Note: C= Compost; P= Proline; G= Gibberellic acid; I= Indole acetic acid; CNS= Control uncontaminated soil; CLCS= Control contaminated soil; 1= Concentration at 5 mM and rate at 20 t ha^{-1}; 2= Concentration at 10 mM and rate at 40 t ha^{-1}; CRD= Carotenoids; CHL= Chlorophyll.

Amendment of contaminated soil with higher compost rate (C2), gave a remarkable increase in the chlorophyll content of maize pre-treated with P2 and P1 while the lowest was recorded in maize pre-treated with I2. However, the highest value of carotenoid was recorded in maize pre-treated with G2 and G1 but the lowest value was found in maize pre-treated with P2. A combination of higher rate of compost and seed pretreatment with exogenous proline and hormone also reduced the glycine betaine production in stressed maize compared to contaminated control and were not significantly different from those grown on uncontaminated soil. The highest value was recorded in maize grown on contaminated soil. For proline content, the highest value was found in maize pre-treated with indole-acetic acid. Meanwhile, seed pretreatment with proline in combination with higher rate of compost reduced the amount of endogenous proline and lowest value was recorded in maize pre-treated with higher concentration of proline combined with higher rate of compost. For the phenolic content of maize, the highest value was recorded in maize pre-treated with G1+C2 and lowest in maize pre-treated with G2+C2 followed by that of P2+C2. Hydrogen peroxide content was

recorded to be the highest in maize pre-treated with I2 in combination with C2. Lowest value was recorded in those pre-treated with G2 and grown on soil amended with C2. Similarly, the lowest MDA value was recorded in maize pre-treated with G1 while the highest was in maize pre-treated with I1. This was followed by maize pre-treated with P2 and combined with higher rate of compost (Table 5).

Table 5. Effect of combined application of different concentrations of proline, hormones and higher rate of compost on photosynthetic pigments, stress indexes (H_2O_2 and MDA), phenolics, proline and glycine betaine contents of maize.

Treatments	CHL	CRD	GB	PHNL	PROLINE	MDA	H_2O_2
P1C2	6.412b	490.17g	0.886c	0.737a	0.211b	0.035b	0.538ab
P2C2	8.286a	(-)341.123h	0.826c	0.537b	0.156b	0.281a	0.591ab
G1G2	3.902e	811.697b	0.515d	0.914a	0.276b	0.345a	0.415bc
G2C2	2.638g	826.996a	1.093b	0.331c	0.262b	0.343a	0.343c
I1C2	5.136d	525.359f	0.853c	0.847a	0.664a	0.012b	0.57ab
I2C2	2.358h	747.494d	0.598d	0.923a	0.602a	0.025b	0.625a
CNS	5.566c	598.858e	0.883c	0.516b	0.178b	0.115b	0.335c
CLCS	3.597f	751.638c	1.488a	0.57b	0.241b	0.052b	0.461abc

Note: C= Compost; P= Proline; G= Gibberellic acid; I= Indole acetic acid; CNS= Control uncontaminated soil; CLCS= Control contaminated soil; 1= Concentration at 5 mM and rate at 20 t ha^{-1}; 2= Concentration at 10 mM and rate at 40 t ha^{-1}.

Effect of sole and combined application of different concentrations of proline, hormones and compost on soil and plant lead (Pb) concentrations.

Contaminated soil without compost amendment had more post-cropping soil Pb concentration compared to un-amended soil and the soil where proline treated seeds were planted had the highest. Apart from the uncontaminated soil, the lowest value was recorded in soil amended with 40t/ha compost (Fig. 1A). The same trend was observed under combined treatments, the post-cropping soil lead content was the highest in the unamended contaminated soil compared to amended soil. Unlike what was observed under sole application where treated seeds were sown on unamended soil, the soil Pb content was reduced in the soil that received compost and was sown with pretreated seeds (Fig. 1B). For example, under sole proline treatment (P2) without compost, the post-cropping soil Pb was 46854 mg kg^{-1} Pb but the value was reduced with soil amendment with 20 and 40 t ha^{-1} to 25108 and 10925 mg kg^{-1}, respectively. Addition of higher rate of compost to Pb contaminated soil generally reduced post-cropping soil Pb compared to untreated contaminated soil except in P1C2 treatment (maize pre-treated with lower proline concentration) and grown on soil combined with higher compost rate (Fig. 1C). Apart from the uncontaminated soil, the lowest value for post-cropping Pb concentration was found in P2C2 treatment.

Conversely, with regards to the Pb accumulation in the shoot and root, the untreated control plant grown on untreated contaminated soil had the lowest compared to the pretreated maize plant. On the effect of sole treatment, Pb accumulation in the shoot of maize was found to be the highest in the untreated maize seeds planted on contaminated soil amended with C2. This was followed by the concentration in the maize pre-treated with a higher concentration of indole acetic acid (Fig. 2A). The lowest mean value for lead in the shoot was recorded in maize pre-treated with gibberellic acid at 10 mM and the control maize plants (Fig. 2B). Treatment with gibberrellic acid and higher compost rate also gave the lowest values though not significantly different from the contaminated control (Fig. 2C). The shoot lead content was however low in the two proline treatments compared to other treatments while the highest shoot lead content was recorded in maize pre-treated with G1C1 followed by that of I1C1 followed by maize pre-treated with higher concentration of indole-acetic acid in combination with lower concentration of compost (Fig. 2C).

Pb concentration in the root was generally more than that of the shoot in most treatments. Sole treatments of C1 and P2 treatments increased the lead concentration in the root while the lowest was recorded for G2 treatment (Fig. 3A). Seed pre-treated with a lower and higher concentration of proline and grown on soil amended with compost, however, had the highest mean value which were not significantly different from each other while the lowest mean value was found in maize pre-treated with lower gibberellic concentration combined with higher compost rate (Figs 3B, C).

DISCUSSION

Plant productivity depends on different environmental and endogenous factors which in turn control plant's behaviour and responses. These factors may act independently, additively, interactively or synergistically. The survival of crop therefore, depends on the complex interactions among the various environmental factors and processes taking place within the plant. In this study, sole and combined application of different concentrations of proline, hormones and compost induced different physiological and biochemical responses in maize crop grown on Pb contaminated soil. The response was found to depend on the type of treatment and concentrations. A combination of these treatments was more
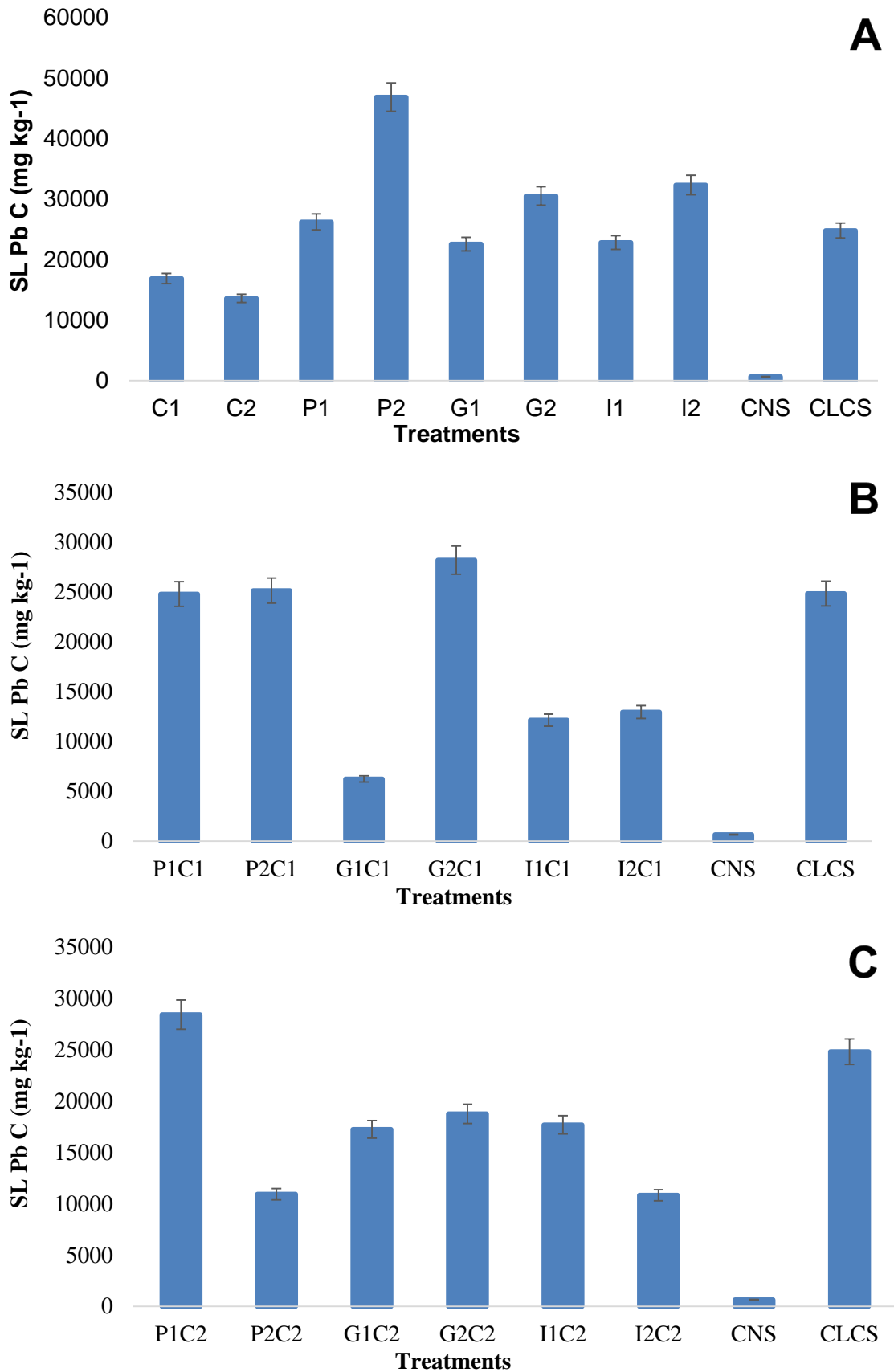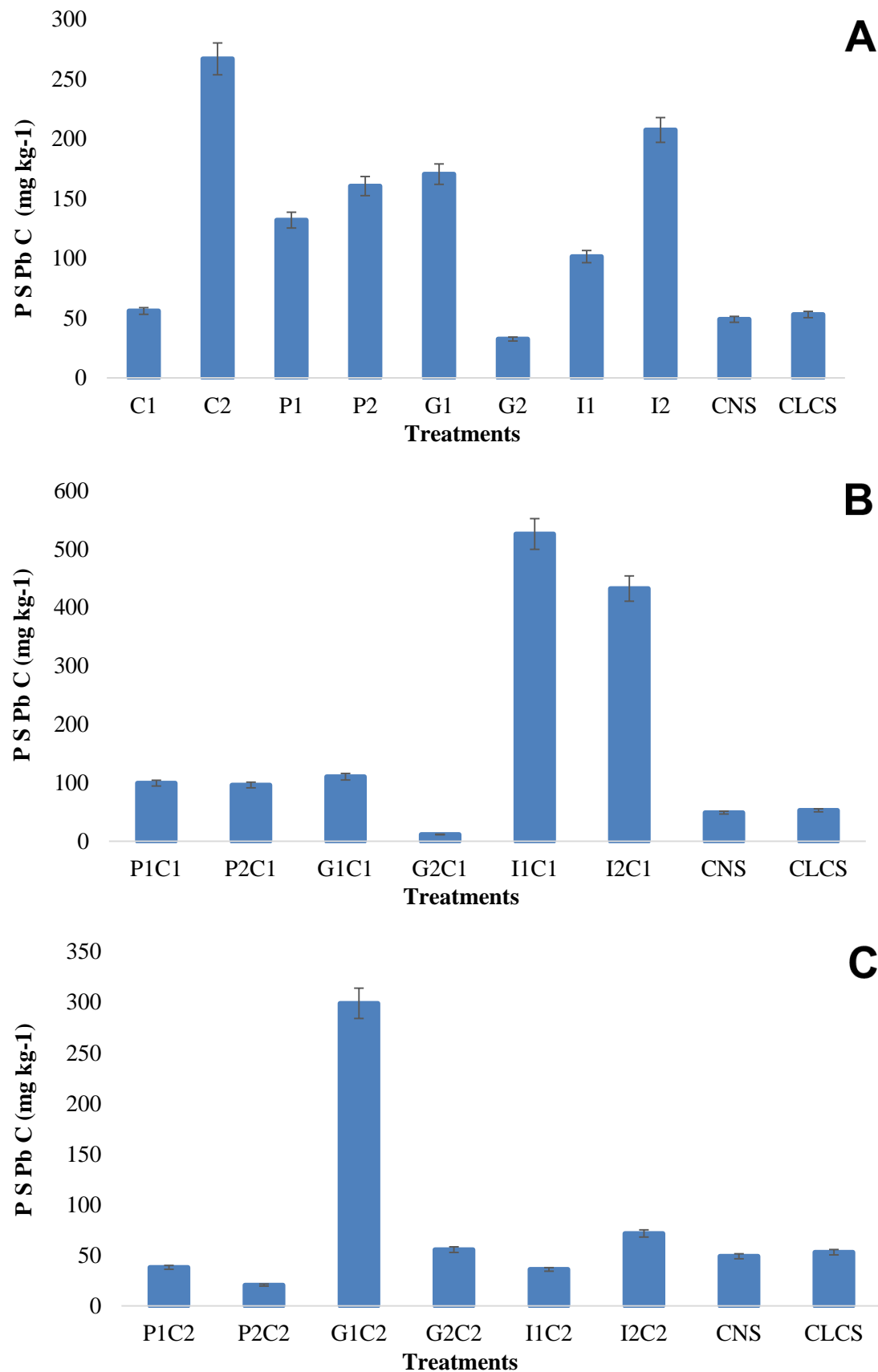
Figure 1. Effect of sole and combined application of different concentrations of proline, hormones and compost on post cropping soil lead contents (Bars on chart represent standard error).
[**Note:** Chart A = Sole effect of treatments on soil Pb concentration, Chart B = Interactive effect of Proline and hormones with lower rate of compost, Chart C = Interactive effect of Proline and hormones with higher rate of compost; C= Compost; P= Proline; G= Gibberellic acid; I= Indole acetic acid; CNS= Control uncontaminated soil; CLCS= Control contaminated soil: 1= Concentration at 5 mM and compost rate at 20 tha^{-1}, 2= Concentration at 10 mM and compost rate at 40 t ha^{-1}; S Pb C= Soil lead content]
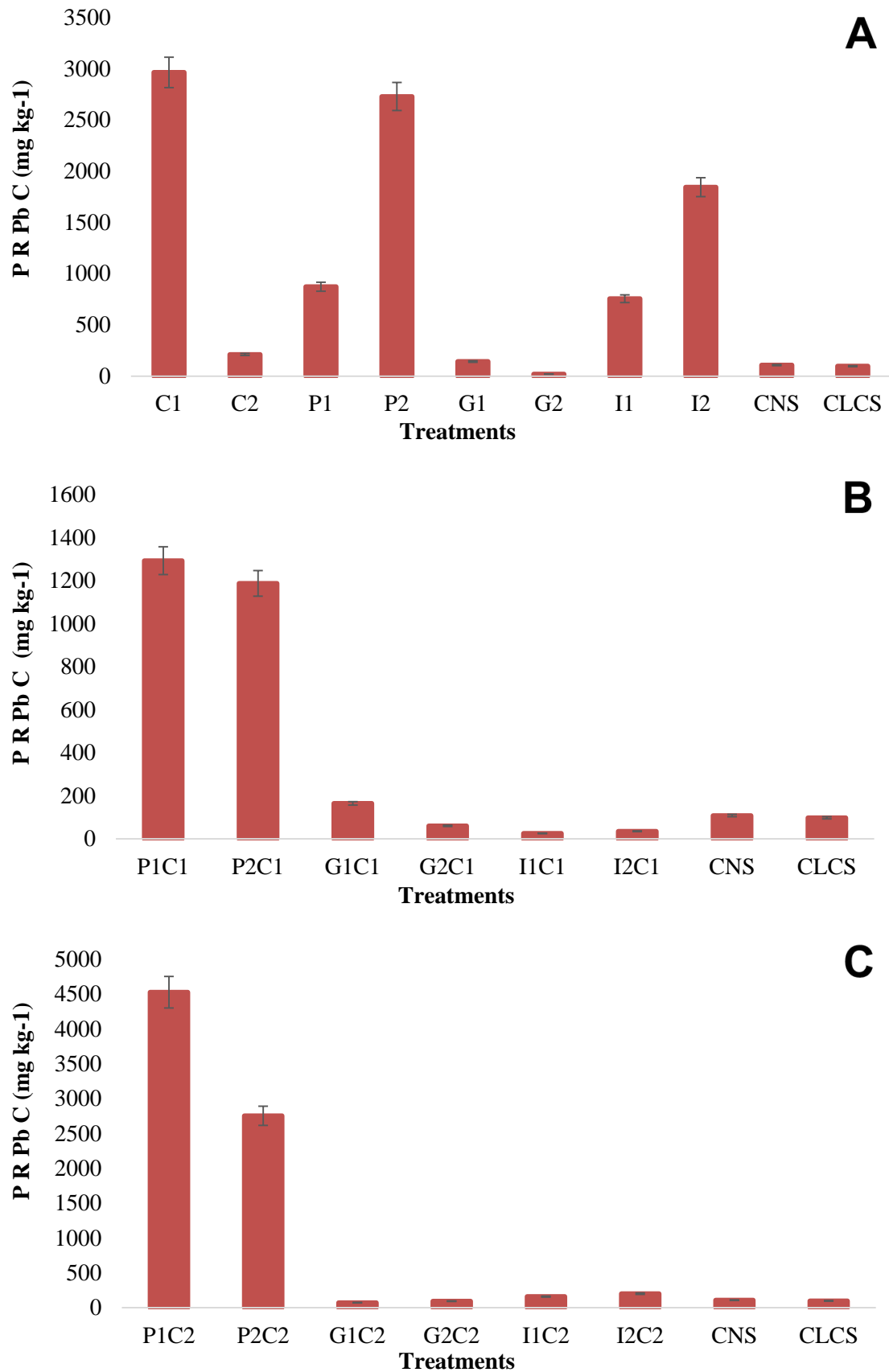
Figure 2. Effect of sole and combined application of different concentrations of proline, hormones and compost on shoot lead content of maize (Bars on chart represent standard error).

[**Note:** Chart A = Sole effect of treatments on shoot Pb concentration, Chart B = Interactive effect of Proline and hormones with lower rate of compost, Chart C = Interactive effect of Proline and hormones with higher rate of compost; C= Compost; P= Proline; G= Gibberellic acid; I= Indole acetic acid; CNS= Control uncontaminated soil; CLCS= Control contaminated soil; 1= concentration at 5 mM and compost rate at 20 t ha^{-1}; 2= Concentration at 10 mM and compost rate at 40 t ha^{-1}; P S Pb C= plant shoot lead content.

Figure 3. Effect of sole and combined application of different concentrations of proline, hormones and compost on root lead content of maize (Bars on chart represent standard error).
[**Note:** Chart A = Sole effect of treatments on Root Pb concentration, Chart B = Interactive effect of Proline and hormones with lower rate of compost, Chart C = Interactive effect of Proline and hormones with higher rate of compost; C= Compost, P= Proline, G= Gibberellic acid; I= Indole acetic acid; CNS= Control uncontaminated soil; CLCS= Control contaminated soil; 1= Concentration at 5 mM and rate at 20 t ha^{-1}; 2= Concentration at 10 mM and rate at 40 t ha^{-1}; P R Pb C= Plant root lead content]

effective and synergistically enhanced maize growth and survival on contaminated soil more than sole treatments. This was reflected in the different parameters considered. For instance, chlorophyll production and osmolytes accumulation were found to be more in the combined treatments of proline, hormones and compost compared to sole treatments. These might be attributed to the interactions between these treatments with one compensating for the lapses of the other or causing each other to become more active. Hormones have been reported to have an interplay among themselves that usually results in better performance when used at the appropriate concentration (Mayank *et al.*, 2015). Similarly, lower concentration of hormones was also found to perform better than higher concentration since hormones have been known to be required in minute concentration.

High concentration of heavy metals disrupt the metabolic activities in plant due to increase in the production of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide (H_2O_2) (Verma & Dubey, 2003; Souguir *et al.* 2011). ROS can cause oxidative damage to the biomolecules when produced in larger amounts leading to cell membrane peroxidation, loss of ions, protein hydrolysis, and even DNA strand breakage (Cakmak & Horst, 1991; Hossain *et al.*, 2012). Plants have developed enzymatic and non-enzymatic anti-oxidative system to scavenge the ROS. Carotenoid, Glycine betaine and proline are examples of compatible osmolytes known to induce abiotic stress tolerance in plants (Sakamoto & Murata, 2002; Claussen, 2005). Exogenous supply of proline in this study was found to induce stress tolerance in heavy metal stressed maize plants. This observation confirms the assertion that proline is capable of inducing stress tolerance in crops (Islam *et al.*, 2009; Adejumo *et al.*, 2015; Noreen *et al.*, 2018). The effect of treatments was also reflected in the endogenous concentration of the stress-related metabolites in the treated and untreated maize plants grown on contaminated soil.

For example, carotenoids unlike chlorophyll, has been identified as one of the non-enzymatic mechanisms of stress responsiveness in plant (Hossain *et al.*, 2012). All the seeds pretreated with sole application of proline and in combination with soil amendment with a higher rate of compost had reduced concentration of carotenoids compared to the untreated seeds. A combination of gibberellin with a lower rate of IAA and compost also reduced the carotenoid content. This high carotenoid concentration in the untreated maize (control) grown on unamended Pb contaminated soil agrees with the report of Adejumo *et al.* (2018c) that carotenoid production was enhanced in the plant exposed to drought stress than the unstressed plant. However, the reduction in carotenoid concentration in the pretreated plants might be connected with the stress amelioration as a result of maize pretreatment with proline and organic amendments. Compost amendments has also been reported to confer tolerance on crop grown on contaminated soils by making nutrients available for crop (Adejumo *et al.* 2011). Similar to what was observed for carotenoid, production of glycine betaine which is another stress related metabolite (Prasad & Saradhi, 2004; Chen & Murata, 2011), was also reduced in response to seed pretreatment with proline and hormone as well as soil amendment with compost. Glycine betaine content was high in maize planted on contaminated soil without seed pretreatment or soil amendment. This therefore implies that seed pretreatment with proline and hormones coupled with soil augmentation with compost was able to reduce the heavy metal stress in maize crop. Tolerance to extreme osmotic stress and drought has been reported to be due to the production of plant metabolites like glycine betaine, proline and trehalose as compatible solutes (osmoprotectants) (Nyyssölä & Leisola, 2001; Sakamoto & Murata, 2002; Adejumo *et al.* 2018a). Accumulation of Glycine betaine and phenolics have also been found to play a major role in the tolerance mechanism under stress in higher plants (Takahama & Oniki, 2000; Shen *et al.*, 2002; Ashraf & Foolad, 2007). Glycine betaine protects the photosynthetic apparatus under stress conditions by stabilizing the PSII complex (Banu *et al.*, 2009; Adams *et al.*, 2013). In all, combined treatments was more effective in terms of stress amelioration and GB production compared to sole treatment.

The production of proline which is another stress tolerance amino acid was also affected by the seed pretreatments with proline and hormone and soil amendment with compost. Probably, due to stress reduction, the lowest proline value was recorded in maize pre-treated with a higher concentration of proline and combined with a higher rate of compost. Combined treatments, however, reduced the proline level more than the sole treatments compared to control plant due to enhanced reduction in stress. This could have been responsible for the survival of maize plant on contaminated soil up to 10 weeks after sowing compared to the untreated maize on contaminated soil. Meanwhile, as observed by Noreen *et al.* (2018) that exogenous application of proline enhanced proline production in wheat, seed pretreatments with sole proline induced endogenous production of proline more than other treatments but by combining the treatments, the concentration of proline was reduced, though, this was rate dependent. Proline treatment at any rate in combination with a higher rate of compost was more effective in reducing proline concentration as observed for GB. The reduction in the concentration of these metabolites in the pre-treated maize plants and compost amendment treatments could be attributed to stress amelioration in the metal stressed maize and reduced synthesis under stress conditions (Kavi Kishor *et al.*, 2005). Exogenous supply of proline to osmotically stressed callus of rice also increased the growth of callus in

vitro by increasing the stress tolerance. This is because these metabolites especially, proline seem to have diverse roles under osmotic stress conditions, such as stabilization of proteins, membranes and subcellular structures and protecting cellular functions by scavenging reactive oxygen species (Vanrensburg *et al.*, 1993; Kavi Kishor *et al.*, 2005). Stress-induced oxygenase activity of Rubisco is also said to be suppressed by proline thereby protecting photosynthetic activity under stress while its activity is enhanced as a result of glycine betaine accumulation in the chloroplast during stress (Zhang *et al.*, 1997; Prasad & Saradhi, 2004). Sole application of lower rate of proline for seed soaking and in combination with hormones also reduced the phenolic content in maize leaf compared to other treatments. This indicates that proline transporters exist in plants that probably transport the exogenous proline in and within the treated plant thereby inducing the tolerance in plant.

It is noteworthy that, indole-acetic acid behaved as stress inducer in plants when compared to proline and gibberellic acid treatments. Indole acetic acid rather than ameliorating the stress effect in maize, it reduced germination and growth of maize on contaminated soil in our previous study (Awoyemi & Adejumo, 2018). The negative effect of this hormone was also reflected on the stress indexes under this study. Hydrogen peroxide and malonylaldehyde which are stress indexes were more in plants pre-treated with higher and lower concentrations (5 mM and 10 mM) of indole-acetic acid as well as in combination with proline and gibberellin which signifies an increase in stress (Hossain *et al.* 2011). This agrees with the finding of Sarvesh *et al.* (1966) that plant growth regulators such as indole-3-butyric acid (IBA), kinetin but not gibberellic acid (GA) initiated proline accumulation in seedlings of *Guizotia abyssinica* whereas, NaCl-induced growth inhibition was alleviated by an exogenous supply of GA and ABA, but not by IBA and kinetin in seedlings of Niger as was also observed in this study. However, compared to sole application, combining other treatments with IAA enhanced tolerance in maize and reduced the stress indexes and GB concentration possibly due to interactions among the different treatments but maize pre-treated with a lower concentration of gibberellic acid and combined with indole-acetic acid and compost increased the maize phenolic and proline contents. Seed pretreatment with IAA and sown on soil amended with high rate of compost increased proline concentration in maize leaf more than other treatments. Sole treatment with indole acetic acid unlike other treatments also increased the GB concentration in maize leaf. This can be related to the induced increase in the activity of enzymes involved in the synthesis of these compounds under heavy metal stress (Lattanzio *et al.* 2006).

Conversely, it was recorded that post cropping soil Pb was more in the soil-grown with the seeds pretreated with sole application of proline at 10 mM and was more than that of control. Therefore, survival of maize plant on this soil must have been really assisted with the seed pretreatment with exogenous proline. The addition of compost to the contaminated soil however, reduced the soil Pb concentration compared to the soil without amendment but with the seed treated with proline and hormone only. This observation agrees with the findings of the previous researchers on the ability of compost to increase soil nutrients and reduce heavy metal concentrations in the soil. Composts have been able to improve soil fertility and quality because of the increase in organic matter content which has been reported to have greater affinity for heavy metals (Rennevan *et al.*, 2007; Fleming *et al.*, 2013). Apart from increasing soil organic matter content, composts improve soil physical properties such as structural stability, total porosity and hydraulic conductivity (Tejada *et al.*, 2009). Meanwhile, soil amendment with higher concentration of compost increased Pb translocation to shoot more than other treatments as observed in the previous findings where Pb was found to be more in the maize grown on soil amended with higher compost rate (Adejumo *et al.*, 2010). The uptake of any element by plant, either toxic or non-toxic is said to be biomass dependent. This could have also explained the lower Pb content recorded for contaminated control plant that had the lowest plant biomass. Besides, crop response to environmental stresses like heavy metal contamination at molecular, cellular and physiological levels is very complex. It is not surprising that the maize response to heavy metal contamination is a complex process in plants. This is because, presence of a toxic element in the growing medium will influence the selective absorption/uptake of essential elements in the soil as well as disrupt the metabolic processes that make use of these nutrients all of which will results in yield reduction as observed in the control plant grown on contaminated soil alone. This again might have led to the disruption in the normal physiological processes which in turn could have also resulted in the accumulation of the toxic metal in the plant at the expense of the essential mineral nutrients. Stress tolerance and resistance are said to be controlled at the transcriptional level by an extremely intricate gene regulatory network (Chen *et al.* 2002, 2004). The effective strategies for ensuring crop production in the face of soil contamination challenges are said to be immobilization in the soil, restriction to the root and tolerance enhancement. Plants' defenses to metal toxicity may also constitute avoidance of metal entry into the cell through the exclusion principle or binding of metal to the cell wall. The binding to cell wall has been described as a major detoxification mechanism of preventing the toxic metals from entering into the cell and damaging the sensitive organelles (Antosiewicz & Wierzbicka, 1999).

CONCLUSION AND RECOMMENDATION

This study showed that pre-treatment of seeds with a higher concentration of proline (10 mM) and application of higher rate of compost (40 t ha^{-1}) on the soil resulted in greater performance. Lowest proline value was recorded in maize pre-treated with a higher concentration of proline combined with higher rate of compost. Combination of higher rate of compost and seed pretreatment with exogenous proline and hormone also reduced glycine betaine production in stressed maize compared to contaminated control. Soil amendment with compost and seed pretreatment with proline and hormone either as sole treatment or in combination however reduced the shoot Pb concentration by decreasing the translocation to the shoot and retaining it in the root. The use of high concentration of proline combined with higher rate of compost should, therefore, be encouraged and implemented when growing plants on contaminated or degraded soil so as to increase crop yield and reduce the effect of Pb on maize crop. Seed pretreatments with sole proline, gibberellin (5 mM), IAA (10 mM) and compost (20 t ha^{-1}) induced endogenous production of proline more than control.

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