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Ameliorative effects of indole acetic acid and naphthalene acetic acid on some biochemical parameters of soybean exposed to lead toxicity.

Efectos de mejora del ácido indol acético y del ácido naftaleno acético sobre algunos parámetros bioquímicos de la soja expuesta a la toxicidad del plomo.

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ABSTRACT

Lead (Pb) is a potential pollutant that readily accumulates in soils. Indole acetic acid (IAA) and Napthalene acetic acid (NAA) belongs to plant hormone auxin that promote plants growth. This work aims at investigating possible ameliorative effects of Indole acid IAA and NAA on some biochemical parameters of soybean exposed to Pb toxicity. Seeds of soybean (TGX 1835-10E) were sown in 5kg bags of soil containing 1000mg/L of lead nitrate($PbNO_3$). 40mg/L, 80 mg/L and 120mg/L each of IAA and NAA were prepared and applied by foliar method after 5 weeks of planting in a screen house. Photosynthetic pigments, mineral elements(including Pb^{2+}) and antioxidant enzymes were determined on the root, stem and leaf after 7 weeks of planting. Data were analysed using anova at 5% level of significance. The results showed that the level of chlorophyll a, b and carotenoid, mineral elements (Calcium, magnessium, potassium, phosphorus, Iron, Sodium and Zinc) and antioxidant enzymes significantly increased in the root, stem and leaf of soybean treated with different concentration of IAA and NAA as compared with the control (C^{+ve}). All bioregulators (IAA and NAA) concentrations significantly decreased in the level of Pb^{2+} (except 40mg/L IAA in the root and leaf while all concentration had significant effects in the stem) and also all concentrations of (IAA and NAA) significantly increased all biochemical parameters to different extent as compared to control(C^{+ve}). These results showed that Indole acetic acid (IAA) and Naphthalene acetic acid (NAA) could alleviate Pb toxicity in plants and therefore could be of relevance to agricultural producers .

Keywords: lead , Indole acetic acid, Napthalene acetic acid

RESUMEN

El plomo (Pb) es un contaminante potencial que se acumula fácilmente en los suelos. El ácido indol acético (IAA) y el ácido naftaleno acético (NAA) pertenecen a las auxinas de hormonas vegetales que promueven el crecimiento de las plantas. Este trabajo tiene como objetivo investigar posibles efectos de mejora del ácido indol IAA y NAA sobre algunos parámetros bioquímicos de la soja expuesta a la toxicidad del Pb. Se sembraron semillas de soja (TGX 1835-10E) en bolsas de 5 kg de tierra que contenían 1000 mg/L de nitrato de plomo (PbNO3). Se prepararon y aplicaron 40 mg/L, 80 mg/L y 120 mg/L cada uno de IAA y NAA por método foliar después de 5 semanas de plantación en una casa de malla. Se determinaron pigmentos fotosintéticos, elementos minerales (incluido Pb2+) y enzimas antioxidantes en la raíz, el tallo y la hoja después de 7 semanas de siembra. Los datos se analizaron utilizando anova con un nivel de significancia del 5%. Los resultados mostraron que el nivel de clorofila a, by carotenoides, elementos minerales (calcio, magnesio, potasio, fósforo, hierro, sodio y zinc) y enzimas antioxidantes aumentaron significativamente en la raíz, tallo y hoja de soja tratada con diferentes concentraciones de IAA y NAA en comparación con el control (C+ve). Todas las concentraciones de biorreguladores (IAA y NAA) disminuyeron significativamente en el nivel de Pb2+ (excepto 40 mg/L de IAA en la raíz y la hoja, mientras que todas las concentraciones tuvieron efectos significativos en el tallo) y también todas las concentraciones de (IAA y NAA) aumentaron significativamente todos los niveles bioquímicos. parámetros en diferente medida en comparación con el control (C+ve). Estos resultados mostraron que el ácido indolacético (IAA) y el ácido naftalenoacético (NAA) podrían aliviar la toxicidad del Pb en las plantas y, por lo tanto, podrían ser de utilidad.

Keywords: lead , Indole acetic acid, Napthalene acetic acid

INTRODUCTION

Soybean is a pea-like leguminous vegetable that grows in tropical, subtropical, and temperate climates. Soybeans are a globally important crop that produces oil and protein. Soybean was domesticated around northeast China in the 11th century BC. It is thought that Chinese traders along Africa's east coast introduced it to the continent in the nineteenth century. Many leguminous crops contain protein, but soybean is the only crop that provides a low-cost, high-quality source of protein comparable to meat, poultry, and eggs. Pb is one of the most toxic and regularly encountered among pollutants that affect plants and therefore generally considered as a potent environmental pollutant. Apart from the natural weathering processes, Pb contamination of the environment has resulted from human activities like mining and smelting activities, paints, gasoline and explosives. Plants are the target of a wide range of pollutants that vary in concentration, speciation, and toxicity (Zeeshanur and Ved 2019). However, decrease in plant growth under stress conditions might be as a result of altered hormonal balance. Also, toxicity from Pb is known to negatively affect some important process in plant like seed germination rate, seedling

growth, dry mass of roots and shoots, photosynthesis, plant water status, mineral nutrition, and enzymatic activities although the effects are more pronounced at higher concentrations. However, the range of these effects varies and depends on the lead concentration tested, the duration of exposure, the intensity of plant stress, the stage of plant development, and the particular part of the plant under study (Ghanati *et al.,* 2005). Oxidative stress in growing plant parts due to enhanced production of reactive oxygen species (ROS) is one of the phytotoxic effects of Pb. Plants in turn poses some internal detoxification mechanisms and species to deal with metal toxicity that includes selective metal uptake, excretion, complexation by specific ligands, and compartmentalization to cope with metal toxicity. In plant cells, the antioxidant defense system is essentially constituted by superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione (GSH), ascorbate (vitamin C), tocopherol (vitamin E), and carotenoids among others. Although at very high concentration of these toxicants (Pb and any other heavy metals), these antioxidants are lowered and plant becomes susceptible (Sonia and Brono, 2012). Therefore, to combat the toxic effects of Pb on crops, bioregulators may serve as strategies as they have been reported to be a possible tools for plant defense, growth and developments (Olaiya *et al*., 2013). Indole acetic acid and Naphthalene acetic acid are natural or synthetic chemicals that affect the expression of biological responses in plant tissues. They include auxins, gibberellins, cytokinnis, ethylene and abscic acid (Simon and Petrasek, 2011). Application of naphthalene acetic acid has been reported to promote growth and yield of cowpea *Vigna Unguiculata* (Jafar et al., 2007). Also, application of indole acetic acid and naphthalene acetic acid has been reported to increase yield and morphological traits in pigeon pea *Cajanus caja* (Udensi et al.,2013). Saleha et al. (2020) had also reported amelioration of lead toxicity due to combined effect glutamate zerovalent iron nanoparticles with indole acetic acid in maize (*Zea mays*). Bushra et al.(2019) had reported potential of indole-3-acetic acid-producing rhizobacteria to resist Pb toxicity in polluted soil. *Chandra et al.* (2011) had reported the alleviating effects of IAA on effect of toxic heavy metal (Pb, Cr and Cd) on wheat plant due to stimulatory effect of antioxidant enzyme like Superoxide dismutase, Catalase and Glutathione reductase. Hence, on the present study on the ameliorative effects of Indole acetic acid and Naphthalene acetic acid on some biochemical parameters: photosynthetic pigments, mineral elements and antioxidant enzymes of soybean exposed to Pb toxicity.

MATERIAL AND METHODS

The study was carried in a screen house at the Department of biochemistry, University of Ibadan Oyo State Nigeria

Plant Materials: Seeds of Soybean genotype (TGX 1835-10E) were obtained from International Institute of Tropical Agriculture Ibadan.

Preparation of lead nitrate: One gram of lead nitrate was dissolve in a little quantity of distilled water and was later transfer to 1 litre volumetric flask and was made up to the mark with distilled water to make 1000mg/L

Application of lead nitrate to the Soil: 500 ml of 1000 mg/L of lead nitrate solution was applied to each 5kg of soil in the polythene bag. It was left for 14 days for proper equilibration of soil and lead nitrate.

Preparation of Indole and Naphthalene acetic acid: This was done according to the method of Heydecker and Coolbear (1977). 120mg/L, 80mg/L and 40 mg/L of IAA and NAA were prepared respectively.

Planting: The seeds were sown on the soil containing lead nitrate in the polythene bags, IAA and NAA was applied at the 5^{th} and 10 weeks of planting by foliar method while only distilled water was applied on the controls (C^{+ve} soybean grown on Pb only and C^{-ve} soybean grown with distilled water only). The plants were harvested after 49 days and were divided into root, stem and leaf for the following analysis: mineral elements, photosynthetic pigments and antioxidant enzyme activities.

Estimation of photosynthetic pigments: The photosynthetic pigments estimated (chlorophyll a, chlorophyll b and carotenoids) were estimated by spectrophotometric procedure of (Hadeer and Aisha 2018). The concentrations of chlorophyll a, chlorophyll b, and total carotenoids were determined using Arnon's equation (1949).

Determination of mineral elements and lead: Preparation of Extract for Element content Analysis: Mineral elements were determined according to the method of (A.O.A.C 2005). The extracts were later analysed for magnessium, iron, zinc and lead using Atomic Absorption Spectrophotometer while flame photometer was used to determine sodium, potassium and calcium

Determination of antioxidant enzymes: Sample Preparation for Enzyme Assay: 1g of root, stem and leaf were grinded in 10ml solution containing 0.1M Potassium phosphate buffer, pH 7.5 containing 0.5mM Ethylenediamine tetraacetic acid (EDTA). The extracts were centrifuged for 20 minutes at 15000 rpm and the supernatant were collected for enzymes assays.

Determination of Catalase: Catalase was determined according to the methods of Beers and Sizer (1952) in which the disappearance of peroxide is followed spectrophotometrically at 240nm. One unit decomposes one micromole of H_2O_2 per minute at 25^oC and pH 7.0 under specified conditions.

Determination of Superoxide Dismutase: Superoxide dismutase (SOD) accelerate the dismutation of the toxic superoxide radical (O_2*), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen.

Fortress method (kit) employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (I.N.T) to form a red formation dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes 50% inhibition of the rate of reduction of INT under the conditions of the assay. The SOD activity is then measured by the degree of inhibition of this reaction.

Determination of Glutathione Peroxidase: Glutathione peroxidase (GPx) is an enzyme found in cytoplasmic and mitochondrial of cells. GPx catalyses the reduction of hydrogen peroxide and hydroperoxides formed from fatty acids, thus effectively removing toxic peroxides from living cells. It plays the important role of protecting cells from potential damage from free radicals formed by peroxide decomposition.

Fortress kit was used for the quantitative determination of total Glutathione peroxidase(GPx). GPx catalyses the oxidation of Glutathione (GSH) by cumene hydroperoxide. The oxidised glutathione reductase and NADPH is oxidised to $NADP^+$ Simultaneously. The decrease in absorbance at 340nm is then measured.

Statistical analysis: The data were analysed using student independent T-test to compare mean difference between control (C^{+ve}) and $PbNO_3$(C^{-ve}). Anova was then used to compare between the six groups of treatments used. Bonferroni multiple range test was used to determine the level of significant ($P \leq 0.05\%$) among the six groups of treatment.

RESULTS AND DISCUSSION

The results in Tables 4-6 show that Pb uptake occurs in the order root> stem> leaf. This could be attributed to Pb moving into the root apoplast and then accumulating in the endodermis. The endodermis, in turn, serves as a temporal barrier for Pb transport from the root to the shoot. This explains why roots have a higher Pb accumulation than stem and leaf. Verma and Dubey (2003) also reported that when rice (*Oryza sativa*) seedlings were grown in sand cultures for 10 and 20 days in nutrient medium containing 500 M and 1000 M Pb(NO3)2, the localization of absorbed Pb was 1.7 to 3.3 times higher in roots than in shoots

Moreover, the result in Figure 1, 2 and 3 shows that application of IAA and NAA increase chlorophyll a, b and carotenoids in the root, stem and leaf . In the root, 120mg/L IAA significantly increase all the photosynthetic pigments (chlorophyll a, b and carotenoid), 80mg/L IAA and 80mg/L NAA significantly increased carotenoid only. In the stem, 120mg/L NAA significantly increased the level of chlorophyll a ,b and carotenoid. 80mg/L IAA, 80mg/L and 40mg/l NAA significantly increased the level of carotenoid only. In the leaf, 120mg/L IAA and 120mg/l NAA significantly increased the level of chlorophyll a, b and carotenoid 80mg/L IAA , 40mg/L IAA and 80mg/L NAA significantly increased the level of carotenoid only,. 40mg/l NAA significantly increased the level of chlorophyll a

and carotenoid. This is due to effectiveness of this concentration to alleviate Pb toxicity so able to promote uptake of mineral elements (magnessium and iron) necessary for chlorophyll formation.

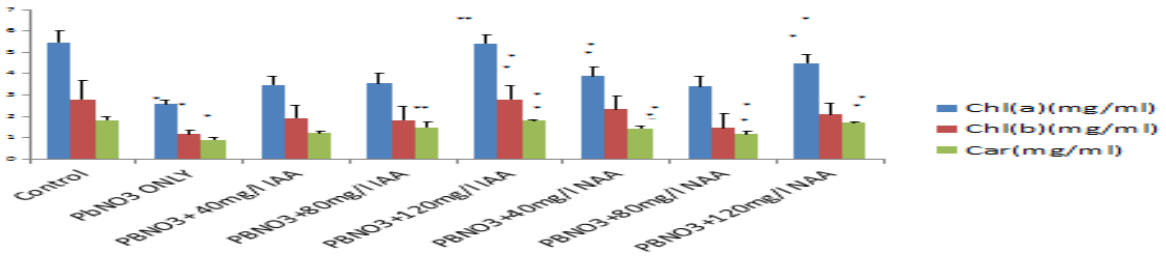

Fig 1: Effect of Indole acetic acid and Naphthalene acetic acid on the Photosynthetic Pigment of Root. Mean with * are significantly different from the control while mean with ** are significantly different from PbNO3 only Group

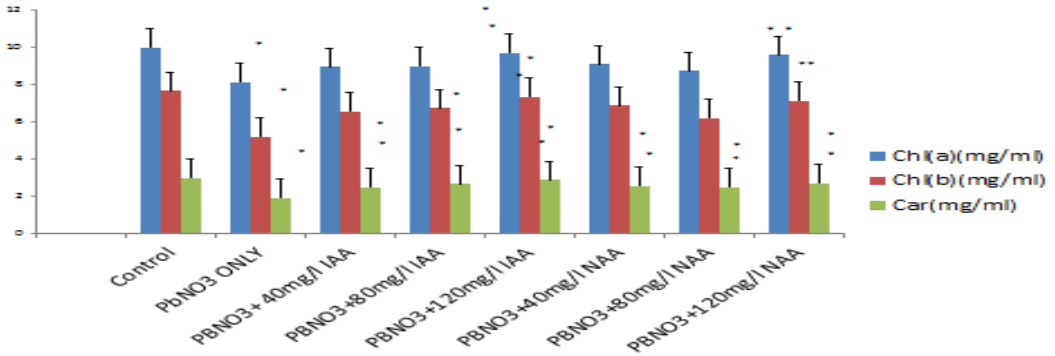

Fig 2: Effect of Indole acetic acid and Naphthalene acetic acid on the Photosynthetic Pigment of Stem. Mean with * are significantly different from the control while mean with ** are significantly different from PbNO3 only Group

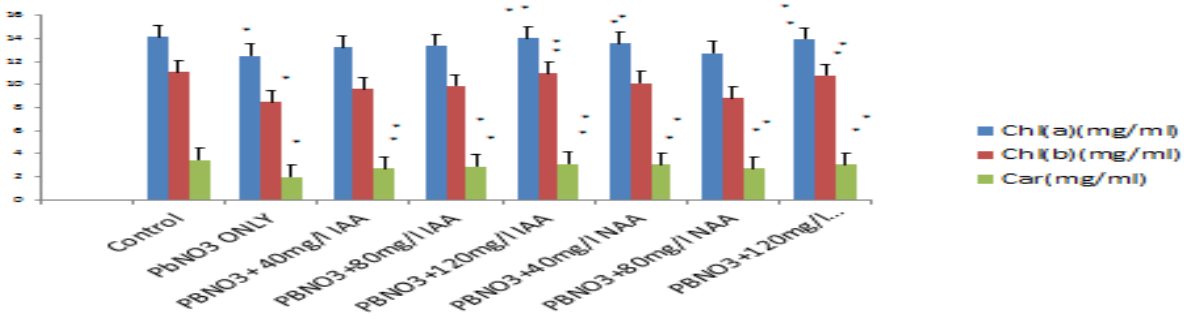

Fig 3: Effect of Indole acetic acid and Naphthalene acetic acid on the Photosynthetic Pigment of Leaf. Mean with * are significantly different from the control while mean with ** are significantly different from PbNO3 only Group

In addition, the result in Tables 1, 2 and 3 shows that in the root, 120mg/L and 80 mg/L IAA significantly increased the level of SOD, GPX , CAT , POX , 40mg/l IAA significantly increased the level of SOD, CAT, POX . In the root, 120 mg/L NAA significantly increased the level of SOD, GPX, CAT and POX. 80mg/l NAA significantly increased the level of SOD , 40mg/L NAA significantly increased the level of SOD and CAT. In the stem, 120mg/L and 80 mg/L IAA significantly increased the level of SOD, GPX ,CAT ,POX, 40mg/L IAA significantly increased the level of SOD. 120 mg/L NAA significantly increased the level of SOD, GPX, CAT and POX. 80mg/L NAA significantly increased the level of SOD, GPX, CAT , 40mg/L NAA significantly increased the level of SOD and CAT. In the leaf, 120mg/L and 80 mg/L IAA significantly increased the level of SOD, GPX ,CAT , POX, 40mg/L IAA gave no significant increased on all the level of enzyme. 120 mg/l and 80mg/l NAA significantly increased the level of SOD, GPX, CAT and POX, 40mg/l NAA significantly increased the level of SOD, CAT and POX . In the Figure 4, 5, 6, 7, 8 and 9 there is increase in activity of LOX observed in all (root, stem and leaf) except 40mg/L IAA in the stem. Significant increased in the level of these enzymes suggest stimulatory effects of IAA and NAA which then results in removal of oxidants and lowering of ROS-mediated injury. This also in accordance with the work of Chandra et al.(2011) on alleviating effects of indole acetic acid on adverse effect of toxic heavy metal (Pb, Cd, Cr) due to the increased production of antioxidant enzyme like SOD, CAT and GR . Olaiya (2013) also reported inducing effects of IAA, NAA and IBA on LOX and POX activity of tomato. Gaied et al.(2013) also reported inducing effect of NAA on SOD and Ascorbate peroxidase level of tomato. Sibgha (2010) also reported increase in SOD, catalase and POX in sunflower on exogenous application of salicylic acid.

Also, the result in Table 5, 6 and 7 shows that in the root, 120mg/L IAA significantly increased the level of Ca^{2+},Mg^{2+},Fe^{2+},Zn^{2+} and phosphorus, 80mg/L IAA significantly increased level of Mg^{2+},K+,Fe^{2+},Zn^{2+}. 120mg/L NAA significantly increased the level of Ca^{2+},Mg^{2+},K^{+},Na^{2+},Fe^{2+},Zn^{2+},P. 80mg/L NAA significantly increased the level of Mg^{2+},K^{+},Fe^{2+},Zn^{2+} and P, 40mg/L NAA significantly increased the level of Mg^{2+},K^{+},Fe^{2+},Zn^{2+},P. All concentration significantly decreased the level of Pb^{2+} except 40mg/L IAA . In the stem, 120mg/L IAA significantly increased the level of Ca^{2+},Mg^{2+},Fe^{2+},K^{+} and P, 80mg/L IAA significantly increased level of Ca^{2+},Mg^{2+},K^{+},Fe^{2+} and Zn^{2+}, 40 mg/L IAA significantly increased the level of Ca^{2+},Mg^{2+},Fe^{2+} and phosphorus. 120mg/L NAA significantly increased the level of Ca^{2+},Mg^{2+},K^{+},Na^{2+}, Fe^{2+}, Zn^{2+} and P. 80mg/L NAA significantly increased the level of Mg^{2+}, Ca^{2+},Fe^{2+} and P, 40mg/L NAA significantly increased the level of Ca^{2+} Mg^{2+},K^{+},Fe^{2+},Zn^{2+},P. All concentration significantly decreased the level of Pb^{2+}. In the leaf, 120mg/L IAA significantly increased the level of Ca^{2+},Mg^{2+},K^{+},Fe^{2+} and Zn^{2+},80mg/l IAA significantly increased level of Ca^{2+},K+,Fe^{2+} and Zn^{2+}, 40 mg/L IAA significantly increased the level of Fe^{2+} and Zn^{2+}. 120mg/L NAA significantly increased the level of Ca^{2+}, Na^{+} ,Mg^{2+},K^{+}, Fe^{2+} and Zn^{2+}. 80mg/L NAA significantly increased the level Fe^{2+} and Zn^{2+}, 40mg/L NAA significantly increased the level of Ca^{2+} Mg^{2+},Fe^{2+} and Zn^{2+}. All concentration significantly decreased the level of Pb^{2+} except 40mg/L IAA. This also follow the work of Chandra *et al.(* 2011) that reported alleviating effects of indole acetic acid on adverse effect of toxic heavy metal (Pb, Cd, Cr) and increase in the nutrient uptake of zinc and Iron in wheat (*Triticum aestivum L*) . Also Rui-Junda *et al.* (2011) reported that application of IAA alleviate Pb toxicity by creating better root system, plant biomass and regulating

the level of nutrient element. Mona et al. (2013) also reported that foliar application of mixture of IAA+NAA promote nutrient element (N, P, K ,Fe, Zn, Mn) in barley. Khan *et al.* (2010) reported that application of 0.5m MSA result in an increase of N,P,K & Ca content by 10.1%, 31.6%,19.3% & 19.1% respectively in mungan bean. Phytohormones in general and IAA in particular, could significantly affect the uptake and the transport of mineral elements in plants by regulating the sink action of developing tissues (Wang *et al*., 2007).

Table 1: Effect of Indole acetic acid and Naphthalene acetic acid on Antioxidant Enzymes of Root

Bioregulators(mg/L)	Superoxide Dismutase (U/mL)	Glutathione Peroxidase (U/L)	Catalase (unit/mg protein/min	Peroxidase(unit/ mg protein/ min
Control	0.7078±0.20	9.81±6.42	0.0014±0.001	0.0004±0.0001
Pb ONLY	1.0591±0.11*	26.64±2.43*	0.1125±0.001*	0.0015±0.0002*
Pb+ 40mg/L IAA	1.435±0.05**	21.03±4.21	0.1186±0.003**	0.0019±0.0001**
Pb+80mg/L IAA	1.58±0.04**	47.67±2.43**	0.1951±0.004**	0.0020±0.0001**
Pb+120mg/L IAA	1.921±0.05**	51.87±2.43**	0.2745±0.003**	0.003±0.0001**
Pb+40mg/L NAA	1.604±0.64**	23.83±2.43	0.2613±0.003**	0.0012±0.0001
Pb+80mg/L NAA	1.468±0.02**	28.04±2.43	0.139±0.004	0.0016±0.0001
Pb+120mg/L NAA	1.935±0.03**	86.92±4.86**	0.2746±0.035**	0.0025±0.0002**

Means with the * are significantly different from the control (C^{-ve})while mean with** are significantly different from Lead Nitrate($PbNO_3$) (C^{+ve}).

Table 2: Effect of Indole acetic acid and Naphthalene acetic acid on Antioxidant Enzymes of Stem:

Bioregulators(mg/L)	Superoxide Dismutase (U/ml)	Glutathione Peroxidase (U/L)	Catalase (unit/mg protein/min	Peroxidase(unit/ mg protein/ min
Control	0.5977±0.01	5.608±2.43	0.0021±0.001	0.0002±.0001
Pb ONLY	0.9927±0.10*	21.031±4.21*	0.0716±0.005*	0.001±0.0001*
Pb+ 40mg/L IAA	1.2687±0.02**	26.638±2.43	0.0703±0.004	0.0008±0.0001
Pb+80mg/L IAA	1.4128±0.03**	33.648±4.23**	0.123±0.001**	0.0017±0.0001**
Pb+120mg/L IAA	1.4344±0.02**	50.472±4.21**	0.1646±0.022**	0.0018±0.0003**
Pb+40mg/L NAA	1.4021±0.04**	28.04±2.43	0.1762±0.010**	0.0009±0.0010
Pb+80mg/L NAA	1.220±0.00**	32.246±2.43**	0.1151±0.004**	0.0011±0.0001
Pb+120mg/L NAA	1.891±0.03**	37.854±4.21**	0.1823±0.010**	0.0022±0.0001**

Means with the * are significantly different from the control (C^{-ve})while mean with** are significantly different from Lead Nitrate($PbNO_3$) (C^{+ve}).

Table 3 : Effect of Indole acetic acid and Naphthalene acetic acid on Antioxidant Enzymes of Leaf

Bioregulators(mg/L)	Superoxide Dismutase (U/ml)	Glutathione Peroxidase (U/L)	Catalase (unit/mg protein/min	Peroxidase(unit/mg protein/ Min
Control	0.5928±0.01	5.608±2.43	0.0014±0.002	0.0001±0.0001
Pb ONLY	1.0160±0.10*	18.226±2.43*	0.0090±0.002*	0.0006±0.0001*
Pb+ 40mg/L IAA	1.1220±0.02	19.628±2.43	0.0203±0.004	0.0008±0.0001
Pb+80mg/L IAA	1.2388±0.003**	37.854±4.21**	0.0296±0.022**	0.0012±0.0003**
Pb+120mg/L IAA	1.9350±0.03**	54.678±4.21**	0.0731±0.010**	0.0015±0.0001**
Pb+40mg/L NAA	1.224±0.03**	21.030±4.21	0.0627±0.03**	0.0009±0.0001**
Pb+80mg/L NAA	1.0999±0.01	29.442±4.21**	0.016±0.004**	0.001±0.0001**
Pb+120mg/L NAA	1.9202±0.01**	42.060±4.21**	0.1079±0.03**	0.0021±0.001**

Means with the * are significantly different from the control (C^{-ve})while mean with** are significantly different from Lead Nitrate($PbNO_3$) (C^{+ve}).

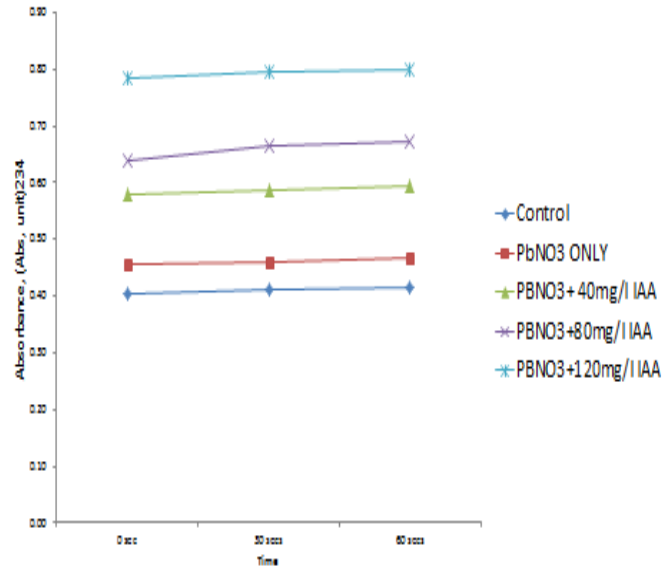

Figure 4:Effect of Indole acetic acid on lipoxygenase activity of Root

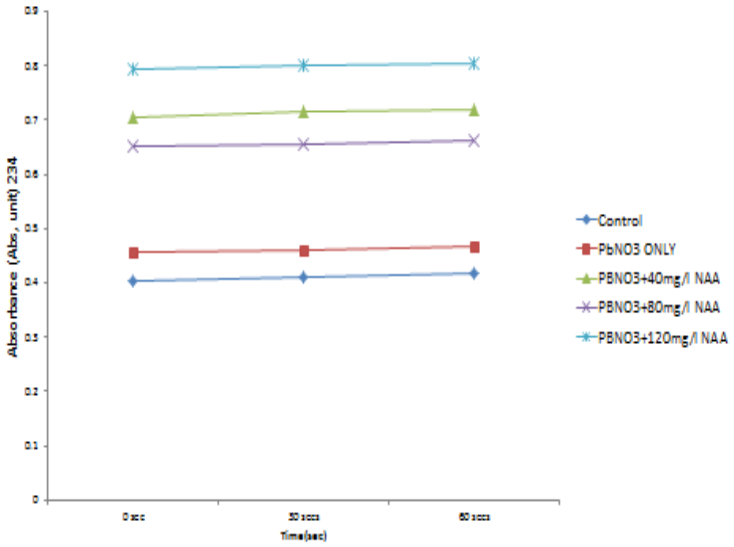

Figure 5:Effect of Naphthalene acetic acid on lipoxygenase activity of Root

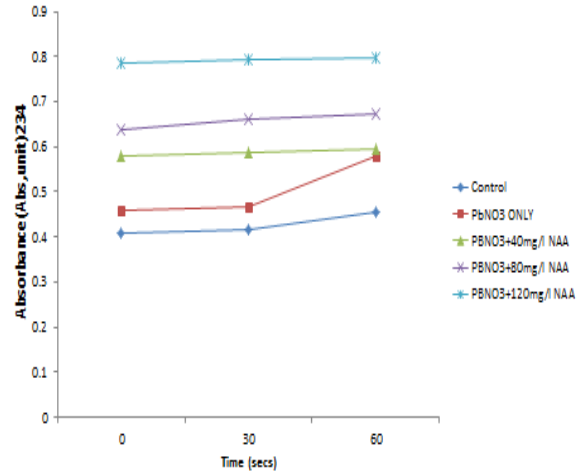

Figure 7 :Effect of Naphthalene acetic acid on lipoxygenase activity of Stem

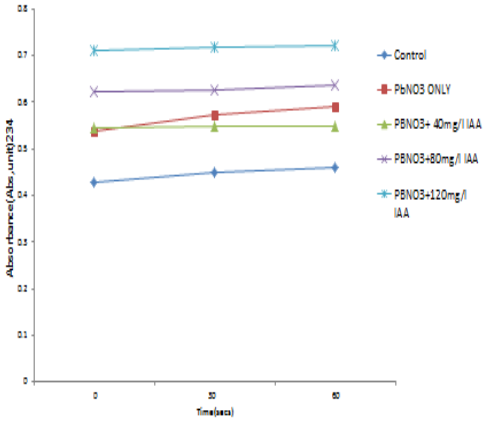

Figure 6 :Effect of Indole acetic acid on lipoxygenase activity of Stem

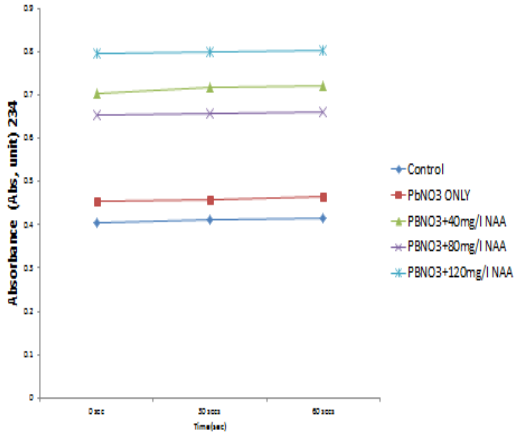

Figure 9: Effect of Naphthalene acetic acid on lipoxygenase activity of Leaf

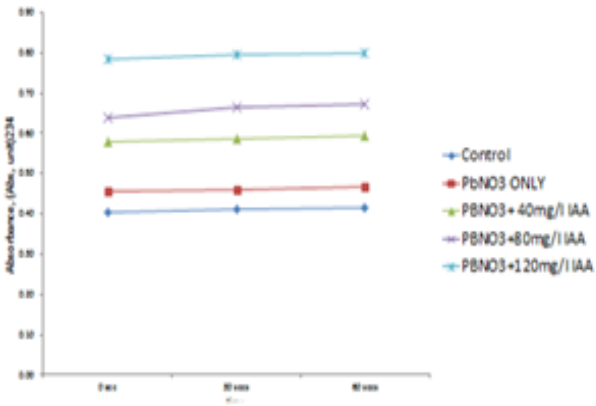

Figure 8: Effect of Indole acetic acid on lipoxygenase activity of leaf

As conclusion, lead is one of the most frequently encountered and highly toxic heavy metals due to the various ways soil is widely polluted through different human activities. Pb could physically block the access of mineral elements to the absorption sites of roots thus causing imbalance within the plant tissues. Phytoremediation of Pb under normal conditions has been challenging because of poor bioavailability a result of its precipitation with carbonates, phosphates and organic matters. Phytohormones therefore serves a possible strategy role for Pb toxicity. Auxins (Indole acetic acid) is the most abundant naturally occurring auxin for its

regulatory function in plant growth as well as synthetic form like Napthalene acetic acid, Indole-3-butyric acid and Salicylic acid. In this study, different concentration of bioregulators used (IAA & NAA) have varying effects in ameliorating the Pb toxicity through increase in the level of mineral elements , antioxidant enzymes and photosynthetic pigments. Therefore, application of IAA and NAA to soybean exposed to Pb toxicity could serve as possible bioremediation to ameliorate the toxic effects by promoting mineral elements, photosynthetic pigments through stimulation of antioxidant enzymes that scavenge the reactive oxygen species produced.

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Table 4: Effect of Indole acetic acid and Naphthalene acetic acid on mineral elements on root

Bioregulator (mg / L)	% Ca	% Mg	% K	% Na	Pb(mg/kg)	Fe(mg/kg)	Zinc (mg/kg)	% P
Control	1.87±0.30	1.16±0.06	5.34±0.10	0.37±0.01	80.00±6.00	5.23±0.56	150.80±4.80	0.16±0.02
$PbNO_3$ ONLY	0.33±0.03*	0.46±0.02*	2.27±0.13*	0.15±0.04*	390.00±14.00*	1.66±0.10*	62.50±2.10*	0.07±0.02*
$PbNO_3$+ 40mg/L IAA	0.43±0.10	0.58±0.05	2.50±0.04	0.17±0.02	371.17±3.13	2.06±0.09	67.00±2.30	0.10±0.03
$PbNO_3$+80mg/L IAA	0.57±0.03	0.64±0.06**	2.76±0.10**	0.23±0.04	290.33±4.3**	2.96±0.53**	84.40±4.40**	0.11±0.02
$PbNO_3$+120mg/L IAA	0.73±0.24**	0.78±0.09**	3.25±0.15**	0.20±0.024	217.09±8.1**	4.38±0.10**	104.57±4.54**	0.16±0.02**
$PbNO_3$+40mg/L NAA	0.59±0.09	1.00±0.05**	3.27±0.33**	0.18±0.01	237.92±9.50**	4.26±0.07**	102.10±2.10**	0.15±0.02**
$PbNO_3$+80mg/L NAA	0.51±0.09	0.57±0.04**	3.18±0.19**	0.15±0.02	233.00±3.00**	3.85±0.46**	86.00±0.65**	0.12±0.11**
$PbNO_3$+120mg/L NAA	1.51±0.25**	1.10±0.04**	4.43±0.10**	0.28±0.04**	140.58±4.78**	5.21±0.15**	107.50±4.04**	0.18±0.03**

Means with the * are significantly different from the control (C^{-ve})while mean with** are significantly different from Lead Nitrate($PbNO_3$) (C^{+ve}).

Ca: calcium, Mg: Magnessium, K: Potassium, Na: Sodium, Pb : Lead, Fe: Iron, P: Phosphorus

Table 5 : Effects of Indole acetic acid and Naphthalene acetic acid on mineral elements of Stem:

Bioregulator(mg/L)	% Ca	% Mg	% K	% Na	Pb(mg/kg)	Fe(mg/kg)	Zinc(mg/kg)	%P
Control	1.41±0.19	0.47±0.03	4.16±0.10	0.040±0.01	37.17±1.17	414.50±3.50	30.50±3.50	0.37±0.03
$PbNO_3$ ONLY	0.78±0.10*	0.24±0.03*	2.74±0.14*	0.034±0.01*	133.10±3.13*	256.50±10.00*	21.10±1.40*	0.10±0.01*
$PbNO_3$+ 40mg/L IAA	1.23±0.10**	0.36±0.03**	3.03±0.13	0.035±0.01	94.21±2.00**	314.33±7.00**	22.70±1.90	0.23±0.03**
$PbNO_3$+80mg/L IAA	1.18±0.18**	0.40±0.05**	3.24±0.20**	0.039±0.01	81.25±1.25**	389.50±22.10**	23.10±1.10	0.11±0.02
$PbNO_3$+120mg/L IAA	2.04±0.04**	0.44±0.03**	3.29±0.20**	0.041±0.00	65.28±2.00**	398.10±3.10**	26.90±3.10	0.28±0.03**
$PbNO_3$+40mg/L NAA	1.6±0.15**	0.42±0.03**	3.27±0.20**	0.034±0.01	86.67±1.67**	297.67±14.25**	29.10±3.10**	0.29±0.02**
$PbNO_3$+80mg/L NAA	1.39±0.09**	0.37±0.03**	2.92±0.18	0.039±0.01	73.31±1.29**	332.50±10.00**	23.30±0.90	0.24±0.05**
$PbNO_3$+120mg/L NAA	2.3±0.06**	0.45±0.03**	3.74±0.08**	0.04±0.02	47.56±2.53**	410.50±15.00**	30.10±2.90**	0.29±0.03**

Means with the * are significantly different from the control (C^{-ve}) while mean with** are significantly different from Lead Nitrate ($PbNO_3$) (C^{+ve}).

Ca: calcium, Mg: Magnessium, K: Potassium, Na: Sodium, Pb : Lead, Fe: Iron, P: Phosphorus

Table 6 : Effects of Indole acetic acid and Naphthalene acetic acid on mineral elements of Leaf

Bioregulator(mg/L)	% Ca	% Mag	% K	% Na	Pb (mg/kg)	Fe (mg/kg)	Zinc (mg/kg)	% P
Control	4.11±0.11	0.48±0.05	3.59±0.30	0.06±0.01	21.10±2.45	929.60±3.80	101.90±1.90	0.21±0.03

$PbNO_3$ ONLY	0.48±0.08*	0.14±0.03*	1.99±0.30*	0.024±0.00*	36.60±2.60*	165.00±7.00*	35.30±1.70*	0.1±0.03*
$PbNO_3$+ 40mg/L IAA	0.51±0.10	0.16±0.03	2.33±0.13	0.035±0.01	35.50±2.00	452.20±7.02**	44.00±1.90**	0.12±0.03
$PbNO_3$+80mg/L IAA	0.94±0.08**	0.23±0.04	2.77±0.30**	0.041±0.01	26.13±4.10*	506.40±6.40*	60.23±4.61**	0.12±0.03
$PbNO_3$+120mg/L IAA	1.68±0.10**	0.42±0.04**	3.04±0.20**	0.04±0.01	26.48±2.00**	852.50±5.50**	83.00±1.50**	0.16±0.05
$PbNO_3$+40mg/L NAA	1.06±0.12**	0.33±0.04**	2.51±0.17	0.035±0.01	25.75±1.50**	798.70±0.80**	73.70±3.70**	0.14±0.04
$PbNO_3$+80mg/L NAA	0.51±0.04	0.21±0.03	2.08±0.20	0.028±0.01	27.75±1.75**	789.90±8.10**	62.20±1.80**	0.13±0.03
$PbNO_3$+120mg/L NAA	3.27±0.10	0.46±0.06**	3.23±0.23**	0.044±0.01**	24.25±1.25**	896.40±5.60**	84.60±1.60**	0.18±0.05

Means with the * are significantly different from the control (C^{-ve}) while mean with** are significantly different from Lead)Nitrate ($PbNO_3$) (C^{+ve}).

Ca: calcium, Mg: Magnessium, K: Potassium, Na: Sodium, Pb : Lead, Fe: Iron, P: Phosphorus