International Journal of Scientific Reports
Oke OG et al. Int J Sci Rep. 2022 Oct;8(10):274-283
http://www.sci-rep.com
pISSN 2454-2156 | eISSN 2454-2164
DOI: https://dx.doi.org/10.18203/issn.2454-2156.IntJSciRep20222392

Original Research Article

Growth response and antioxidant enzyme capability of *Helianthus annuus* L. in lead contaminated soil under organic and urea fertilizer applications

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Received: 18 July 2022
Accepted: 20 August 2022

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ABSTRACT

Background: Indiscriminate dumping of wastes, including heavy metals such as lead (Pb) on Nigerian soils is increasing. *Helianthus annuus* has been found to have ability to clean up contaminated soils, but with paucity of information on the effect of Pb stress on the plant' antioxidant enzymes activities when fertilizers are applied as soil enhancers, hence this study.
Methods: The experiment consisted of four levels (0, 400, 800, 1200 mg Pb kg^{-1}) using [Pb $(CH_3COO)_2.3H_2O$], three rates (0, 5 and 10 t ha^{-1}) of organic and two rates (0 and 2 t ha^{-1}) of urea fertilizers, and each treatment was replicated thrice to give a total of 72 experimental units in pot culture. Each pot contained 10 kg of sieved topsoil and arranged in a complete randomized design. The *H. annuus* seeds were sown, fertilizers were applied and stands of *H. annuus* were collected for antioxidant enzymes (SOD, CAT, POX and APX) activities determination in the roots and shoots using standard methods.
Results: Soil pH was slightly acidic and soil texture was loamy sand. Biomass yield of *H. annuus* increased with increase in organic fertilizer, but decreased with increase in Pb contamination. There was significant ($p<0.05$) increase in detoxification responses in the shoots than the roots of *H. annuus* against oxidative stress caused by Pb toxicity when organic fertilizer was applied to soil.
Conclusions: The study concluded that addition of organic fertilizer increased biomass yield and had superior enhancing detoxification responses on *H. annuus* against oxidative stress by Pb toxicity.

Keywords: *Helianthus annuus*, Antioxidant enzymes, Detoxification, Reactive oxidative species, Fertilizers, Pb contaminated soil

INTRODUCTION

Contamination of soils, though not limited to the following, could include: incessant disposal of municipal wastes and industrial effluents into soil environment as well as improper application of fertilizers to crops for enhanced yield. Most of these soil contaminants include heavy metals such as lead (Pb). Lead accumulates on the soil surface and reduces in concentration with soil depth.[1] Lead is one of the highly toxic elements and the response of plants to Pb stress depends on their physiological and genotype features.[2] This is because different plants respond differently to soil polluted environment. Plants have efficient enzymatic defense systems to protect themselves against oxidative damage.[3] *Helianthus annuus* is a hyperaccumulator of heavy metals and hydrocarbon due to its high biomass and fast growth rate.[4] This plant possesses the possible traits to tolerate and detoxify metals accumulated in the shoots, even at higher concentrations.[5]

Helianthus annuus belongs to family *Asteraceae* and it is a very important edible oil crop. It also has high potential in removing metal contaminants in soils, particularly when EDTA, organomineral fertilizers and organic fertilizers were used.[2,6,7] These additives lower the soil pH, thus prevent cell wall retention of Pb by translocating it to aerial parts of the plants.[8] Once this barrier is broken, more Pb is localized in the shoot than root.[7,8] In addition to *Helianthus annuus* as pollutants removal from soil, *Brassica juncea*, *Solenostemon monostachyus*, *Calopogonium mucunoides*, have been used at different occasions.[9-11] These plants use their detoxification system to defend themselves from oxidative stress caused by the creation of reactive oxygen species (ROS) by increasing antioxidant enzymes activities.[12] In order to avoid oxidative impairment due to increased ROS synthesis, plants possess antioxidant enzymes system that hunt ROS present in compartments of several cells.[13] These antioxidant enzymes involved in the detoxification of ROS include superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), peroxidase (POX) and ascorbate peroxidase (APX).[12]

The type and species of plants, and experimental conditions during remediation are key factors which Pb-induced inhibition of antioxidant enzymes depend.[14] There is however paucity of information on the effects of Pb concentrations on the growth response and antioxidant enzymes activities on *H. annuus* in the presence of organic and urea fertilizers as soil enhancers. This study was therefore designed to investigate the growth response of *H. annuus*, and the effects of Pb on antioxidant enzymes (SOD, CAT, POX and APX) activities in the roots and shoots of *H. annuus* when organic and urea fertilizers are used either singly or co-applied to enhance soil fertility.

METHODS

Experimental design, agronomic practices and harvesting of H. annuus

The study was carried out in the screenhouse of the faculty of pharmacy, Obafemi Awolowo university (OAU), Ile-Ife, Nigeria. Organic and urea fertilizers purchased from an open market in Ibadan, Nigeria were used to enhance the soil fertility. Lead acetate [$Pb(CH_3COO)_23H_2O$] was used as the soil contaminant at variable concentrations. Seeds of *H. annuus* were obtained from the Department of Environmental Health Sciences, University of Ibadan, Ibadan, Nigeria. Ten kg of sieved topsoil was filled into each polythene bag perforated at the bottom. The experiment consisted of four levels (0, 400, 800, 1200 mg kg^{-1}) of Pb concentration, three rates (0, 5 and 10 t ha^{-1}) of organic and two rates (0 and 2 t ha^{-1}) of urea fertilizers. It was a factorial experiment arranged in a complete randomized design. Each treatment was replicated thrice to give a total of 72 experimental units. The pots were wetted with the prepared standard solutions of Pb acetate to attain moisture capacity and allowed to incubate for two weeks. During the period of incubation, the prepared standards were also added to keep the soil moist till they were exhausted.

The *Helianthus annuus* seeds were sown at six seeds and thinned to four stands per pot at 2 weeks after sowing (WAS). Organic fertilizer was applied at planting and urea fertilizer was applied at 2 WAS using 'dump and bury' method. During the growth period, all the pots were maintained weed-free and distilled water added as the need arose. At 4 and 8 WAS respectively, the plants were carefully collected, one stand per pot and rinsed under running tap water to remove the soil debris from them for the determination of antioxidants enzymes (SOD, CAT, POX as well as the APX) activities in the root and shoot samples of *Helianthus annuus* using standard methods. Also, at the twelve WAS the experiment was terminated for the determination of the total biomass weight of the plant.

Soil and organic fertilizer analyses

Sieved soil and organic fertilizer samples were analyzed using standard methods.[15] The soil pH was determined in 1:1 soil-water suspension using a glass electrode pH meter. Total nitrogen of the soil and organic fertilizer were determined by the macro-Kjeldahl method. Available phosphorus in the soil and organic fertilizer were extracted using Bray P1 method and P in the extracts was determined by colorimeter. The organic carbon in soil and organic fertilizer was determined using Walkley-Black wet oxidation method.

Exchangeable cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) of the soil were determined using 1M Ammonium acetate buffered at pH 7.0 as extractant. The K^+ and Na^+ concentrations in the extract were read using flame photometer, while Ca^{2+} and Mg^{2+} were read using atomic absorption spectrophotometer (AAS), Perkin-Elmer model 403. The summation of these gave cation exchange capacity. The soil and organic fertilizer micronutrients (Zn, Cu and Fe) were extracted with 0.1M HCl and their concentrations were read on AAS. Also, mixture (5 ml) of conc. HNO_3 and conc. $HClO_4$ in the ratio 2:1 with 5 ml of conc. H_2SO_4 were used to digest 0.5 g of each soil and organic fertilizer sample for 2 h at 150ºC. The digests were allowed to cool and each was made up to 25 ml with distilled water. Concentration of Pb in the soil and organic fertilizer extracts were read on AAS.

Estimation of growth traits

The harvested and air-dried *Helianthus annuus* plant were separated into roots and shoots using a clean razor blade, weighed and each was separately put inside a brown envelope and oven-dried to constant weight at 70ºC. The dried roots and shoots samples were allowed to cool before taking the dry weight measurement.

Enzymatic antioxidants assays

The root and shoot tissues, 1 g each of *Helianthus annuus* seedlings were homogenized separately with pestle under ice-cold situations in a pre-chilled mortar with 10 ml of extraction buffer [50 mM phosphate buffer (pH 7.5), 0.5 mM ascorbate and 1 mM EDTA]. For 15 minutes, the homogenate was centrifuged at 10,000 rpm. The activities of the antioxidant enzymes SOD, CAT, POX and APX were estimated from the supernatant obtained.

Superoxide dismutase (SOD)

The superoxide dismutase (SOD) activity was quantified according to the method of Marklund and Marklund.[16] The reaction mixture contained 1.4 ml buffer [0.1M Tris-HCl buffer pH 8.0 containing 2 mM ethylene diammine teraacetic acid EDTA), homogenate (0.05 ml) and 0.05 ml Pyrogallol (4.5 mM in 10 mM HCl). The absorbance readings of the mixture were recorded at 420 nm at 30 sec interval for a period of 2.5 min spectrophotometrically. Blank contained all reagents except the homogenate which was replaced with distilled water. The rate of auto-oxidation of pyrogallol was measured by changes in absorbance/ min at 420 nm, and one unit of enzyme inhibited it by 50%.

Calculation

Increase in absorbance per minute=As-Ao/ 2.5

Where Ao=Absorbance after 30 seconds, As=Absorbance after 150 seconds.

Percentage inhibition= Increase in absorbance for homogenate×100/ Increase in absorbance for blank

Pb SOD Activity (min g^{-1}fr.wt)=% inhibition of Pyrogallol autoxidation/ 50%

One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of pyrogallol. 1 unit=150

Catalase (CAT)

The catalase activity in the plant (root and shoot) homogenate was determined by using the method of Aebi, though with a little modification. The reaction mixture consisted 750 µl of 0.1 M phosphate buffer (pH 7.0), 750 µl of 70 mM hydrogen peroxide, 1500 µl of distilled water as well as 150 µl of the homogenate and at wavelength of 240 nm at the intervals of thirty secs for two min, the mixture absorbance was taken.[17] The one µmol of hydrogen peroxide degraded per minute was defined as one unit of catalase activity which was expressed as units per min g^{-1}fr. wt. Catalase activity was calculated as:

Catalase (units)=ΔA/min×Df ×3.15/ Sv×0.0436

where: ΔA/min=change in absorbance per minute, Df=dilution factor, Sv=sample volume

Peroxidase (POX)

Peroxidase activity was determined by the method of Kumar and Khan.[18] The assay mixture consisted 1 ml of 0.1 M phosphate buffer (pH=6.8), 0.5 ml of 0.01 M pyrogallol, 0.5 ml of 0.005 M H_2O_2, and 0.25 ml of homogenate extract. The reaction was terminated by adding 0.5 ml of 2.5 N H_2SO_4 to the solution after it had been incubated for 5 minutes at 25°C. Quantifying the absorbance at 420 nm against a blank made by adding 2.5 N H_2SO_4 at zero time produced the amount of purpurogallin created.

The activity was measured in minutes per gram of body weight (min g^{-1}fr.wt).

One unit is defined as a 0.1 min g^{-1}fr.wt change in absorbance.

Calculation=(r $A_{420nm/20secsample}$ − r $A_{420nm/20sec\ Blank}$)×Tv×Df / Extinction coefficient×Sv

Where; Tv= Total volume of assay, Df=dilution factor, extinction coefficient of 1 mg/ml of purpurogallin at 420 nm= $2.8*10^{-3}M^{-1}cm^{-1}$, Sv=sample volume

Ascorbate peroxidase (APX)

Ascorbate peroxidase activity was estimated using Nakano and Asada method.[19] The reaction mixture contains 1.5 ml of 50 mM phosphate buffer (pH 7.0), 0.3 ml of 2 mM ascorbic acid, 0.6 ml of 2 mM hydrogen peroxide, and 0.6 ml of homogenate, totaling 3.0 ml. The reaction was instigated through the addition of H_2O_2 and the absorbance was recorded at 290 nm with the aid of spectrophotometer for 2 min at 30 sec intervals. One unit of ascorbate peroxidase was defined as the amount of enzyme oxidizing 1µmol of ascorbic acid per minute.

Activity (min/g. fr.wt)=ΔA/min×Tv×Df / $2.8\times10^{-3}\times1.0\times$Sv

Where: ΔA/min=change in absorbance/ minute, Tv=total assay volume, Df=dilution factor, 2.8×10^{-3} $M^{-1}cm^{-1}$, 1.0=light path length (cm), and Sv=sample volume.

The specific activity of ascorbate peroxidase was expressed as min g^{-1}fr.wt.

Statistical analysis

Microsoft excel version 2010 was employed to analyze for mean values of properties of soil and organic fertilizer used, and biomass yield with their standard errors. One-way analysis of variance (ANOVA) was carried out using GraphPad Prism 5.0 at $p<0.05$ to test for difference in the mean values of antioxidants enzymes (SOD, CAT, POX

and APX) activities. Differences in the treatment means were compared using Turkey's multiple range test at a significant level of $p<0.05$.

RESULTS

Growth response of H. annuus, soil and organic fertilizer properties

Table 1 shows the properties of soil and organic fertilizer used for this experiment. The soil pH in 1:1 soil to water suspension was 6.29 indicating a slightly acidic soil condition. Soil organic carbon and total nitrogen were 14.90 and 1.70 g kg^{-1} respectively. Organic fertilizer has total organic carbon, 62.00 g kg^{-1} and total nitrogen, 12.10 g kg^{-1} respectively with C:N ratio of 5.12. Plant biomass, wet and dry weights of *H. annuus* measured is given in Table 2.

Table 1: Properties of pre-cropped soil and organic fertilizer applied.

Variables	Value	
	Soil	**Organic fertilizer**
pH (1:1 soil-water)	6.29±0.02	-
Organic carbon (g kg^{-1})	14.90±0.30	62.00±0.20
Total nitrogen (g kg^{-1})	1.70±0.20	12.10±0.30
Available phosphorus (mg kg^{-1})	1.27±0.01	0.13±0.01
Cations exchange capacity (cmol kg^{-1})	9.26±0.07	-
Selected micronutrients (mg kg^{-1})		
Zn	4.41±0.01	100.27±0.07
Cu	2.43±0.02	5.83±0.19
Fe	1139.30±0.33	55.58±0.40
Heavy metals (mg kg^{-1})		
Pb	1.25±0.03	7.05±0.04
Particle size (g kg^{-1})		
Sand	813.30±3.30	-
Silt	100.00±0.03	-
Clay	86.70±0.33	-
Textural class	Loamy sand	-

Antioxidant enzymes (SOD, CAT, POX and APX) activities

Effects of Pb contamination and fertilizer applied on SOD activity in the roots and shoots of *Helianthus annuus* at 4, 8 and 12 WAS are presented in Table 3. The SOD activity was more in the roots than shoots at the early stage of the exposure than later stage. There was significant ($p<0.05$) increase in SOD activity with increase in the number of weeks of exposure to Pb stress in the shoots than roots at the early stages of exposure, and organic fertilizer application till 12 WAS when the experiment was terminated. The SOD activity however decreased with increase in Pb contamination. Also, there was *Helianthus annuus* seedlings die-back before 12 WAS when urea fertilizer was applied either singly or in combination with organic fertilizer to enhance soil fertility.

Effects of Pb contamination and fertilizer applied on CAT activity in the roots and shoots of *Helianthus annuus* at 4, 8 and 12 WAS are presented in Table 4. Generally, CAT activity increased significantly ($p<0.05$) with increase in the period of exposure of *Helianthus annuus* to Pb stress. This increase was more in the shoots than roots till 12 WAS, and the CAT activity however decreased with increase in Pb contamination. Effects of Pb contamination and fertilizer applied on POX activity in the roots and shoots of *Helianthus annuus* at 4, 8 and 12 WAS are presented in Table 5. The POX activity increased significantly ($p<0.05$) in the roots and shoots of *Helianthus annuus* with increase in the number of weeks of exposure to Pb stress till 12 WAS when the experiment was terminated. Also, effects of Pb contamination and fertilizer applied on APX activity in the roots and shoots of *Helianthus annuus* at 4, 8 and 12 WAS are presented in Table 6. Also, the APX activity increased significantly ($p<0.05$) in the roots and shoots of *H. annuus* at early stages, but decreased with increase in the number of weeks of exposure to Pb stress till 12 WAS when the experiment was terminated. Both the POX and APX activities increased in *Helianthus annuus* with increase in Pb contamination.

Figure 1: Effect of fertilizer applications (0 t ha^{-1} of urea + 5 t ha^{-1} of organic fertilizer) on growth response of *H. annuus* plant at 8 weeks after sowing in variable Pb contaminated soil.
Legend: A, B, C, D=0, 400, 800, 1200 mg Pb kg^{-1}.

Table 2: Effect of Pb contamination and fertilizer applied on biomass yield of roots and shoots of *H. annuus* at 12 weeks after sowing.

Fr	Pb (mg kg^{-1})	Fresh weight (g/pot)		Dry weight (g/pot)	
		Root	Shoot	Root	Shoot
1	0	14.8±0.2	61.8±0.6	0.8±0.1	4.9±0.1
	400	9.4±0.3	41.4±0.5	0.6±0.2	4.3±0.2
	800	7.7±0.2	40.0±0.4	0.6±0.1	3.2±0.3
	1200	2.4±0.1	12.4±0.3	0.2±0.1	0.8±0.2
2	0	24.0±0.3	81.6±0.5	1.8±0.2	7.6±0.1
	400	22.2±0.4	80.2±0.4	1.6±0.3	5.0±0.2
	800	11.0±0.5	41.0±0.3	0.8±0.3	4.8±0.2
	1200	9.2±0.4	20.2±0.4	0.5±0.2	2.1±0.3
3	0	25.7±0.3	101.8±2.1	2.0±0.2	11.9±0.1
	400	24.2±0.4	100.6±1.2	1.8±0.2	7.8±0.2
	800	17.6±0.2	61.2±0.4	1.2±0.2	4.9±0.4
	1200	12.6±0.2	20.7±0.3	0.6±0.2	3.1±0.3
4	0	*	*	*	*
	400	*	*	*	*
	800	*	*	*	*
	1200	*	*	*	*
5	0	14.5±0.3	30.7±0.4	*	*
	400	11.2±0.4	25.5±0.5	*	*
	800	9.5±0.2	15.8±0.2	*	*
	1200	6.7±0.5	9.3±0.3	*	*
6	0	16.9±0.2	43.5±0.3	*	*
	400	13.4±0.3	29.4±0.2	*	*
	800	9.9±0.2	16.7±0.6	*	*
	1200	3.1±0.5	10.1±0.3	*	*

Legend: Fr=Fertilizer rate; 1, 2, …, 6=Zero fertilizer, 0 t ha^{-1} of urea + 5 t ha^{-1} of organic fertilizer, 0 t ha^{-1} of urea + 10 t ha^{-1} of organic fertilizer, 2 t ha^{-1} of urea, 2 t ha^{-1} of urea + 5 t ha^{-1} of organic fertilizer, 2 t ha^{-1} of urea + 10 t ha^{-1} of organic fertilizer; and*=the plants died before harvesting.

Table 3: Effect of Pb contamination and fertilizer applied on superoxide dismutase activity in the roots and shoots of *H. annuus* at 4, 8 and 12 weeks after sowing.

Fr	Pb (mg kg^{-1})	4 WAS (min g^{-1} fr. wt)		8 WAS (min g^{-1} fr. wt)		12 WAS (min g^{-1} fr. wt)	
		Root	Shoot	Root	Shoot	Root	Shoot
1	0	3.62±0.21a	0.09±0.01a	0.20±0.04a	0.73±0.13a	1.87±0.01a	4.97±0.13a
	400	2.47±0.18b	0.08±0.01a	0.09±0.02b	0.58±0.12a	1.57±0.06a	2.15±0.10b
	800	0.49±0.13c	0.06±0.01a	0.08±0.01b	0.55±0.16a	1.21±0.08b	1.10±0.03c
	1200	0.44±0.14c	0.04±0.01b	0.01±0.01c	0.03±0.01b	0.90±0.05b	0.35±0.05d
2	0	8.03±0.13a	3.49±0.11a	0.31±0.12a	1.40±0.16a	2.26±0.15a	5.89±0.95a
	400	5.73±0.18b	0.16±0.06b	0.28±0.11a	1.02±0.12a	2.14±0.10a	3.35±0.10b
	800	1.83±0.12c	0.08±0.01b	0.22±0.03a	1.01±0.07a	2.11±0.12a	2.63±0.16b
	1200	0.31±0.01d	0.03±0.01c	0.12±0.02b	0.77±0.12a	2.01±0.01a	2.04±0.09c
3	0	9.19±0.21a	3.60±0.05a	0.69±0.18a	2.12±0.32a	2.31±0.17a	6.47±0.13a
	400	4.14±0.01b	0.09±0.03b	0.32±0.17a	1.60±0.16a	2.24±0.05a	4.33±0.07b
	800	3.06±0.08b	0.08±0.01b	0.29±0.06b	1.27±0.34a	2.16±0.04a	3.30±0.05c
	1200	0.93±0.14c	0.08±0.04b	0.10±0.06b	0.78±0.30b	2.08±0.07a	2.79±0.11c
4	0	0.77±0.21a	0.08±0.01a	*	*	*	*
	400	0.63±0.11a	0.03±0.01a	*	*	*	*
	800	*	*	*	*	*	*
	1200	*	*	*	*	*	*
5	0	4.03±0.16a	1.08±0.13a	0.40±0.01a	2.17±0.13a	*	*
	400	3.90±0.20a	0.06±0.03b	0.17±0.03b	1.17±0.13b	*	*
	800	0.86±0.24b	0.04±0.01b	0.03±0.03b	0.54±0.05b	*	*
	1200	0.67±0.13b	0.02±0.01b	0.01±0.01b	0.01±0.01b	*	*

Continued.

Fr	Pb (mg kg^{-1})	4 WAS (min g^{-1} fr. wt)		8 WAS (min g^{-1} fr. wt)		12 WAS (min g^{-1} fr. wt)	
		Root	Shoot	Root	Shoot	Root	Shoot
6	0	3.08±0.12a	1.42±0.18a	0.73±0.04a	2.03±0.12a	*	*
	400	1.73±0.01a	0.21±0.08b	0.39±0.03b	0.79±0.11b	*	*
	800	1.42±0.07b	0.16±0.03b	*	*	*	*
	1200	1.32±0.03b	0.08±0.01b	*	*	*	*

Means ± SE with the same letters in each column are not significantly different by Tukey's multiple range test at p<0.05. Legend: Fr=Fertilizer rate; 1, 2, …, 6=Zero fertilizer, 0 t ha^{-1} of urea + 5 t ha^{-1} of organic fertilizer, 0 t ha^{-1} of urea + 10 t ha^{-1} of organic fertilizer, 2 t ha^{-1} of urea, 2 t ha^{-1} of urea + 5 t ha^{-1} of organic fertilizer, 2 t ha^{-1} of urea + 10 t ha^{-1} of organic fertilizer; and*=the plants died before harvesting.

Table 4: Effect of Pb contamination and fertilizer applied on catalase activity in the roots and shoots of *H. annuus* at 4, 8 and 12 weeks after sowing.

Fr	Pb (mg kg^{-1})	4 WAS (min g^{-1} fr. wt)		8 WAS (min g^{-1} fr. wt)		12 WAS (min g^{-1} fr. wt)	
		Root	Shoot	Root	Shoot	Root	Shoot
1	0	0.14±0.01a	0.15±0.01a	0.32±0.04a	0.47±0.17a	0.06±0.01a	0.40±0.10a
	400	0.10±0.02a	0.15±0.01a	0.11±0.02b	0.12±0.08b	0.05±0.01a	0.17±0.07b
	800	0.09±0.04a	0.06±0.01b	0.03±0.01c	0.03±0.01c	0.04±0.02a	0.07±0.02b
	1200	0.09±0.06a	0.03±0.01b	0.02±0.01c	0.02±0.02c	0.03±0.01a	0.06±0.02b
2	0	0.20±0.11a	0.40±0.15a	0.15±0.07a	0.37±0.21a	0.30±0.15a	0.38±0.15a
	400	0.16±0.13a	0.17±0.08b	0.11±0.04a	0.34±0.11a	0.07±0.10b	0.30±0.10a
	800	0.07±0.02b	0.15±0.01b	0.08±0.05b	0.04±0.01b	0.06±0.01b	0.24±0.05b
	1200	0.04±0.01b	0.03±0.01c	0.02±0.01b	0.03±0.01b	0.05±0.01b	0.18±0.09b
3	0	0.08±0.02a	1.93±0.01a	0.16±0.05a	0.53±0.32a	0.07±0.13a	1.01±0.11a
	400	0.07±0.03a	0.50±0.01b	0.06±0.01b	0.14±0.16b	0.06±0.01a	0.27±0.03b
	800	0.07±0.03a	0.09±0.03b	0.05±0.01b	0.07±0.34c	0.05±0.01a	0.25±0.02b
	1200	0.06±0.01a	0.08±0.04b	0.04±0.01b	0.04±0.30c	0.02±0.01a	0.09±0.01c
4	0	0.09±0.04a	0.46±0.21a	*	*	*	*
	400	0.08±0.02a	0.14±0.01b	*	*	*	*
	800	*	*	*	*	*	*
	1200	*	*	*	*	*	*
5	0	0.15±0.12a	1.94±0.16a	0.22±0.03a	0.94±0.16a	*	*
	400	0.05±0.02b	1.42±0.05a	0.16±0.04b	0.47±0.23b	*	*
	800	0.04±0.02b	0.06±0.01b	0.02±0.01b	0.03±0.01c	*	*
	1200	0.03±0.03b	0.06±0.04b	0.01±0.01b	0.01±0.01c	*	*
6	0	0.21±0.12a	0.98±0.03a	0.17±0.03a	0.18±0.12a	*	*
	400	0.03±0.01a	0.21±0.08b	0.09±0.03a	0.10±0.11a	*	*
	800	0.03±0.07b	0.14±0.06b	*	*	*	*
	1200	0.02±0.03b	0.06±0.01c	*	*	*	*

Means ± SE with the same letters in each column are not significantly different by Tukey's multiple range test at p<0.05. Legend: Fr=Fertilizer rate; 1, 2, …, 6=Zero fertilizer, 0 t ha^{-1} of urea + 5 t ha^{-1} of organic fertilizer, 0 t ha^{-1} of urea + 10 t ha^{-1} of organic fertilizer, 2 t ha^{-1} of urea, 2 t ha^{-1} of urea + 5 t ha^{-1} of organic fertilizer, 2 t ha^{-1} of urea + 10 t ha^{-1} of organic fertilizer; and*=the plants died before harvesting.

Table 5: Effect of Pb contamination and fertilizer applied on peroxidase activity in the roots and shoots of *H. annuus* at 4, 8 and 12 weeks after sowing

Fr	Pb (mg kg^{-1})	4 WAS (× 10^3 min g^{-1} fr. wt)		8 WAS (× 10^3 min g^{-1} fr. wt)		12 WAS (× 10^3 min g^{-1} fr. wt)	
		Root	Shoot	Root	Shoot	Root	Shoot
1	0	0.22±0.05d	0.99±0.06c	2.25±0.15c	0.54±0.29c	1.79±0.20c	2.47±0.25c
	400	0.48±0.08c	1.43±0.19b	2.75±0.36b	1.35±0.14b	4.32±0.23b	2.91±0.43c
	800	2.97±0.06b	1.65±0.04b	3.28±0.29a	1.94±0.18a	4.70±0.21b	5.72±0.29b
	1200	3.11±0.21a	3.22±0.86a	3.69±0.10a	1.99±0.24a	5.55±0.35a	6.35±0.08a
2	0	0.60±0.13c	1.29±0.08b	3.31±0.06c	0.83±0.15c	4.70±0.11c	5.96±0.06c
	400	1.36±0.19b	1.43±0.20b	3.51±0.28c	2.07±0.86b	5.16±0.41b	6.33±0.04b
	800	1.98±0.16b	1.63±0.12b	3.83±0.16b	2.26±0.41b	5.17±0.11b	6.42±0.03b
	1200	4.17±0.20a	3.71±0.16a	4.02±0.68a	2.66±0.25a	5.84±0.09a	6.94±0.07a

Continued.

Fr	Pb (mg kg^{-1})	4 WAS (× 10^3 min g^{-1} fr. wt)		8 WAS (× 10^3 min g^{-1} fr. wt)		12 WAS (× 10^3 min g^{-1} fr. wt)	
		Root	Shoot	Root	Shoot	Root	Shoot
3	0	0.89±0.13d	1.33±0.29c	3.08±0.06b	1.19±0.47c	5.06±0.55c	6.25±0.06b
	400	1.36±0.20c	1.54±0.23c	3.61±0.24a	2.68±0.27b	5.68±0.34b	6.27±0.07b
	800	4.03±0.08b	3.96±0.21b	3.84±0.05a	2.91±0.19b	5.89±0.18b	6.44±0.03b
	1200	6.03±0.33a	4.03±0.53a	3.92±0.15a	3.74±0.40a	6.11±0.21a	7.01±0.06a
4	0	0.98±0.12b	1.77±0.39b	*	*	*	*
	400	1.29±0.09a	3.36±0.02a	*	*	*	*
	800	*	*	*	*	*	*
	1200	*	*	*	*	*	*
5	0	0.42±0.09d	0.94±0.19b	1.31±0.13b	1.64±0.16b	*	*
	400	1.66±0.03c	1.03±0.25b	1.35±0.22b	1.66±0.60b	*	*
	800	3.87±0.06b	1.29±0.25b	1.79±0.89b	1.67±0.35b	*	*
	1200	4.70±0.13a	2.90±0.24a	4.79±0.41a	3.46±0.44a	*	*
6	0	0.85±0.12c	0.87±0.14c	2.43±0.24b	1.39±0.14b	*	*
	400	1.35±0.12c	1.54±0.05b	4.95±0.11a	3.72±0.11a	*	*
	800	4.41±0.32b	1.98±0.20b	*	*	*	*
	1200	4.95±0.10a	3.89±0.63a	*	*	*	*

Means ± SE with the same letters in each column are not significantly different by Tukey's multiple range test at p<0.05. Legend: Fr=Fertilizer rate; 1, 2, …, 6=Zero fertilizer, 0 t ha^{-1} of urea + 5 t ha^{-1} of organic fertilizer, 0 t ha^{-1} of urea + 10 t ha^{-1} of organic fertilizer, 2 t ha^{-1} of urea, 2 t ha^{-1} of urea + 5 t ha^{-1} of organic fertilizer, 2 t ha^{-1} of urea + 10 t ha^{-1} of organic fertilizer; and*=the plants died before harvesting.

Table 6: Effect of Pb contamination and fertilizer applied on ascorbate peroxidase activity in the roots and shoots of *H. annuus* at 4, 8 and 12 weeks after sowing.

Fr	Pb (mg kg^{-1})	4 WAS (× 10^3 min g^{-1} fr. wt)		8 WAS (× 10^3 min g^{-1} fr. wt)		12 WAS (× 10^3 min g^{-1} fr. wt)	
		Root	Shoot	Root	Shoot	Root	Shoot
1	0	0.17±0.02c	0.75±0.07c	0.31±0.03b	0.31±0.01b	0.03±0.00b	0.15±0.04a
	400	0.69±0.16c	0.91±0.26c	0.34±0.08b	0.38±0.02b	0.05±0.00b	0.35±0.02a
	800	1.57±0.37b	1.33±0.26b	0.42±0.09b	0.52±0.03a	0.09±0.00b	0.34±0.02a
	1200	2.24±0.87a	5.14±0.05a	0.98±0.04a	0.76±0.03a	0.15±0.06a	0.79±0.06a
2	0	0.55±0.03b	0.53±0.26b	0.43±0.02b	0.53±0.02a	0.05±0.00a	0.43±0.02b
	400	0.70±0.09b	0.68±0.13b	0.46±0.05b	0.73±0.02a	0.16±0.02a	0.59±0.04b
	800	1.42±0.43a	0.75±0.06b	0.51±0.08b	0.91±0.05a	0.20±0.01a	1.51±0.11a
	1200	1.99±0.42a	1.24±0.57a	6.01±0.29a	1.21±0.23a	0.29±0.02a	1.88±0.13a
3	0	1.07±0.09b	1.41±0.50c	0.54±0.05c	0.34±0.02c	0.43±0.01a	0.56±0.02d
	400	1.29±0.26b	3.93±0.47b	0.90±0.06c	1.41±0.06b	0.30±0.02a	1.45±0.12c
	800	1.63±0.13b	3.93±0.95b	1.74±0.31b	1.60±0.04b	0.43±0.04a	3.71±0.24b
	1200	4.94±0.75a	4.49±0.58a	7.88±0.31a	2.35±0.06a	0.56±0.04a	5.06±0.12a
4	0	2.10±0.13b	3.75±0.90b	*	*	*	*
	400	2.67±0.15a	5.49±0.44a	*	*	*	*
	800	*	*	*	*	*	*
	1200	*	*	*	*	*	*
5	0	0.61±0.32d	1.25±0.20b	0.71±0.06b	0.29±0.13a	*	*
	400	1.78±0.11c	1.36±0.43b	0.99±0.04b	0.32±0.13a	*	*
	800	4.50±0.27b	1.67±0.44b	1.01±0.45b	0.35±0.05a	*	*
	1200	5.84±0.25a	8.97±0.76a	1.69±0.59a	0.48±0.01a	*	*
6	0	1.20±0.06c	1.04±0.42c	0.45±0.03b	0.76±0.02b	*	*
	400	3.17±0.13b	1.22±0.20c	0.89±0.07a	1.30±0.04a	*	*
	800	3.38±0.12b	5.29±0.14b	*	*	*	*
	1200	7.16±0.67a	9.82±0.79a	*	*	*	*

Means ± SE with the same letters in each column are not significantly different by Tukey's multiple range test at p<0.05. Legend: Fr=Fertilizer rate; 1, 2, …, 6=Zero fertilizer, 0 t ha^{-1} of urea + 5 t ha^{-1} of organic fertilizer, 0 t ha^{-1} of urea + 10 t ha^{-1} of organic fertilizer, 2 t ha^{-1} of urea, 2 t ha^{-1} of urea + 5 t ha^{-1} of organic fertilizer, 2 t ha^{-1} of urea + 10 t ha^{-1} of organic fertilizer; and*= the plants died before harvesting.

DISCUSSION

A typical tropical soil environment like this with low native nitrogen and organic carbon for cereals production falls within low fertility class that requires fertilizer additions, hence the need for organic and urea fertilizers application.[20] Plant biomass, wet and dry weights of *H. annuus* was measured as these are good indicators of plant growth under heavy metal toxicity.[21,22] The growth of *H. annuus* was positively and substantially enhanced when organic fertilizer was applied, but with growth reduction when Pb contamination was on the increase (Figure 1, Table 2). Increased plant biomass was also obtained with increase in organic fertilizer addition, thus confirming the positive enhancing effect of organic fertilizer.

Organic fertilizer increased soil organic carbon, N, P, K, and cation exchange capacity, and these invariably enhanced the *H. annuus* growth.[23,24] Co-application of organic and urea fertilizers had similar but lower effects on growth of the plant. However, application of urea alone from 400 mg Pb kg^{-1} resulted to about 100% death of *H. annuus* from 4 WAS, while 50% death of *H. annuus* occurred from 800 mg Pb kg^{-1} with organic + urea application. Impurities such as biuret, tripropylene, and cyanuric acid, and nitrite formed by nitrification, in urea, in addition to the oxidative stress imposed by Pb contaminant at higher concentrations could cause plant seedlings die-back.[25,26]

Plant antioxidant system is a detoxification mechanism against ROS and has the capacity to inhibit, reduce or repair damage cause by ROS to biomolecules.[27] The reactive oxygen species (ROS) are highly reactive chemicals produced when plants are exposed to environmental stress such as Pb contamination.[8,28] There was a significant ($p<0.05$) reduction in the activity of SOD with increase in Pb contamination in the roots and shoots of *H. annuus*, and this was more in the roots than shoots. This decrease could be due to the detrimental effects of over production of H_2O_2 or its derivatives.[29] Also, SOD activity increased with increasing addition of organic fertilizer, but decreased with increase in urea fertilizer addition. The implication of this is that organic fertilizer enhanced SOD detoxification response of *H. annuus* against oxidative damage and Pb-stress resistance.[30] There was a significant ($p < 0.05$) reduction in the activity of CAT with increase in Pb contamination in the roots and shoots of *H. annuus*, and this was more in the shoots than roots. This result contradicts Reza et al that obtained increased CAT activity with increase in Pb soil contamination, though with *Brassica napus* as the test plant.[31] This variation could be due to differences in the physiological and genotype features of the two plants.[2] Also, CAT activity increased with increasing addition of organic fertilizer, but decreased with increase in urea fertilizer addition.

There was a significant ($p<0.05$) increase in the activity of POX with increase in Pb contamination in the roots and shoots of *H. annuus*, and this was more in the shoots than roots at later stage of the plant development. The implication of this is that POX activity was more in the shoots zone of the plant than the roots. The POX activity increased more in the roots and shoots of *H. annuus* with increasing addition of organic fertilizer than when organic and urea fertilizers were combined or urea was used singly. Comparable and significant ($p<0.05$) increase in the activity of APX with increase in Pb contamination in the roots and shoots of *H. annuus* were equally obtained in this study. Organic fertilizer alone also performed optimally than when combined with urea or zero fertilizer for APX activity determination.

CONCLUSION

We concluded that biomass yield of *H. annuus* was positively and substantially enhanced when organic and urea fertilizers, either singly or in combination was applied, but reduced with increase in Pb contamination. The antioxidant enzymes (SOD, CAT, POX and APX) activities enhanced the detoxification responses of *H. annuus* against oxidative stress caused by Pb toxicity, but these reduced with increase in duration of exposure; and that these activities took place, most often, more in the shoot than the root zones of the plant. The enhancement of biomass yield and detoxification responses of *H. annuus* in Pb contaminated soil obtained was in the order: organic>organic + urea > urea > zero fertilizer applications.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

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Cite this article as: Oke OG, Adewole MB, Akinpelu BA. Growth response and antioxidant enzyme capability of *Helianthus annuus* L. in lead contaminated soil under organic and urea fertilizer applications. Int J Sci Rep 2022;8(10):274-83.