

Assessment of Antioxidant Enzymes and Vitamins on Lettuce(*Lactuca Sativa*) Obtained From Cement Company of Northern Nigeria (Sokoto Cement) and Kwalkwalawa Area.

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ASSESSMENT OF ANTIOXIDANT ENZYMES AND VITAMINS ON LETTUCE(*LACTUCA SATIVA*) OBTAINED FROM CEMENT COMPANY OF NORTHERN NIGERIA (SOKOTO CEMENT) AND KWALKWALAWA AREA.

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ABSTRACT

*Environmental pollution is a major issue which confronts industry and business in today's world on daily basis. Industrial activities are the leading cause of metals emission, often associated with soil and plant metal concentration in adjacent regions. Cement industry is one of the 17 most polluting industries listed by the central pollution control board (CPCB). Impact of dust deposition from Cement Company of Northern Nigeria on the antioxidant enzymes and antioxidant vitamins concentrations of lettuce (*Lactuca sativa*) was studied. A comparative study of vitamins concentration and enzymes of *Lactuca sativa* samples from Kalambaina (Industrial area) and Kwalkwalawa (Non-Industrial area) were estimated using spectrophotometric method and standard analytical procedures respectively. Antioxidant enzymes (CAT and SOD) were found to be significantly higher ($P < 0.05$) in lettuce sample obtained from Kalambaina compared to sample obtained from Kwalkwalawa, CAT (881.22 ± 87.735 units/ml), SOD (2.030 ± 0.017 units/ml) and GSH (2025 ± 115.47 mg/dl), and value of antioxidant vitamins Vit A (219.48 ± 10.970 mg/dl), Vit C (4095.28 ± 54.843 mmol/L) and Vit E (33.5 ± 1.782 mg/dl) were found to be significantly higher in *Lactuca sativa* collected from Kalambaina ($P < 0.05$) when compared to*

sample collected from Kwalkwalawa. This might be as a result of activities in the cement industry. Conclusively, plants grown at cement industries might not be safe for consumption.

Keywords: Antioxidant enzyme; catalase; peroxidase; Glutathione peroxidase; lettuce; Superoxide dismutase. CAT = Catalase, GSH = Glutathione peroxidase, SOD = Superoxide dismutase.

1.0 INTRODUCTION

In the course of normal metabolic functions, highly reactive compounds called free radicals can be generated in the body in the form of reactive oxygen species (ROS). Most of the environmental toxicants also are potential source of free radicals. These molecules are unstable because they have lone pair of electrons and therefore become highly reactive species. They easily react with cellular molecules such as proteins, lipids, carbohydrates and nucleic acids, and denature them. As a result of these reactions, those components lose their ability to function normally and ultimately resulting in various pathological conditions in living organisms [1]. Due to ROS are natural by-products of ongoing biochemical reactions, they are involved in the cell growth, differentiation, progression, and death. The concentration levels of ROS in the body are very important because low concentrations of ROS may be beneficial or even necessary in processes such as intracellular signaling and defense against micro-organisms; however, overproduction of ROS play a role in the aging process as well as in a number of human disease states, including cancer, ischemia, and failures in immunity and endocrine functions. As a safeguard against the accumulation of ROS, living organisms possess antioxidant defense systems including several non-enzymatic and enzymatic antioxidant components. Therefore, when ROS overproduction arises as a consequence of a pathologic event, antioxidant defense system promotes the regulation and expression of these (Mates *et al.*, 1999). Antioxidant enzymes are capable of stabilizing or deactivating free radicals before they oxidize the components of cells. There are various mechanisms for antioxidant enzyme preservation; they

could reduce the energy of the free radicals or give up some of their electrons for their use, thereby causing them to become stable. Moreover, they may also interrupt the oxidizing chain reaction to minimize the damage caused by free radicals. Our body's potential of overcoming free radicals related health problems could be made more substantial by reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements. Consequently, antioxidant enzymes play a critical role for maintaining optimal cellular and systemic health and wellbeing [1]. In this connection, antioxidant enzyme rich fruits and vegetables are effective preservers of various diseases[2]. Plants have the effective enzymatic antioxidant defense system including catalase (CAT), superoxide dismutase (SOD), and Glutathione (GSH) enzymes. This system allow for scavenging of reactive oxygen species (ROS) leading to protection of plant cells from oxidative [2]. Superoxide dismutase (EC 1.15.1.1) is the antioxidant enzyme that responsible for the disutation of the highly reactive superoxide anion to O₂ and to the less reactive species H₂O₂. The released hydrogen peroxide can be reduced to water by catalase (EC 1.11.1.6) or glutathione peroxidase (EC 1.11.1.9) enzymatic reactions [3,4,5]. Polyphenol oxidase (EC1.10.3.1) is a copper containing metallo enzyme that oxidizes phenolic compounds to quinone's and eventually the Quinone's are polymerized (nonenzyme-catalyzed) to melanin pigments [6].

2.0 Materials and Methods

2. 1 Reagents

Chemicals used are laboratory or analytical grade reagent obtained from reputable companies. Some of the common laboratory reagents used is shown in the table below.

Table 2.1: Reagents Used

Name	Formula	Manufacturer
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Alloxan	$C_4H_2N_2O_4$	Qualikemfinechem.pvt.ltdindia
Ethanol	CH_3CH_2OH	Jinhuada china
Pyrogallol	$C_6H_6O_3$	England
Betaphenantholine		England
Buffer	$C_{14}H_{27}N_3O_{11}$	
Distilled water	H_2O	Biochemistry Lab UDUS
Potassium hydroxide	KOH	England
Sodium hydroxide	NaOH	BDH
Metaphosphoric acid	HPO_3	England
Phosphotungstate reagent	$H_3PW_{12}O_{40}$	England
Xylene	C_8H_{10}	England
Sulphuric acid (vi)	H_2SO_4	M&B
Pet ether	C_7H_{16}	BDH
Oxalic acid	$C_2H_2O_4$	M&B
Anhydrous ethanol	C_2H_6O	BDH
Ferric chloride	$FeCl_3$	M&B

2. 1.2 Instruments

For the conduct of this research, routine laboratory apparatus were used. In addition, the instruments listed in table (1) were also employed for various analyses.

Table 2.1.2: Instruments

Instrument	Model	Manufacturer
Spectrophotometer	Erma	Great Britain
Weighing Balance	Mettler Balance DT100	Mettler Switzerland
Beaker	Pyrex	Great Britain
Centrifuge Machine	800D/Metal	Ltd/England
Sieve	20 μ m	England
Mortar and Pestle	Ceramic	England
Flat botton flask	Pyrex 1000 _{ml}	Great Britain
Pipette	Rubber	England
Filter paper	Erma	England

2.2 Sample Collection

The fresh lettuce samples were collected from each sample location (MarmaronGantsare Garden which is behind Sokoto cement factory and Kwalkwalawa area, Sokoto) using clean plastic bags. The collected samples were washed separately under running tap water before cut down into smaller pieces using knife and dried at room temperature 20-27⁰C for about two weeks. The samples were grounded using a mortar and pestle into fine powder, sieved through 2mm sieve and stored in plastic jar labeled for analysis.the samples were identified and authenticated by Botanical unit of the UsmanuDanfodiyo University Sokoto.

2.3 Extractions of plant samples.

Five (5) grams of the dried samples were put in a 250ml conical flask and 100ml of distilled water was added and was covered with Aluminum foil. It was allowed to stand for 72hours and filtered using whatman No. 1 filter paper in a separate 250ml conical flask. The filtrate was used for the analysis.

2.4 Determination of Antioxidant Enzymes

2.4.1 Catalase

Two test-tube was labeled as test and blank into which the following was pipette. 2.9ml of reagent A was pipette into the test-tube labeled as blank, and 2.9ml of reagent B (Substrate or analyzed sample) was pipette into the test-tube labeled as test. At 25°C, the absorbance was monitored at A₂₄₀ unit constant and was recorded using spectrophotometer. Then 0.1ml of reagent C (catalase solution) was added, the solution was mixed immediately by inversion and the required for the A₂₄₀ to decrease from 0.45 to 0.40 absorbance units was recorded.

The activity of catalase was calculated using the formula below.

Formula:

$$\frac{3.45 \times df}{\text{min} \times 0.1}$$

2.4.2 Glutathione

Three test-tubes were set and labeled as sample, blank and standard 1.0ml 5% metaphosphoric acid was added into the test-tube labeled as blank, 1.0ml standard reagent was added into the test-tube labeled as standard, 1.0ml sample solution was measured into the test-tube labeled as sample and 1.0ml of 0.1M aloxan was added into all the test-tubes respectively. All test-tubes were line up in order and 1.0ml 0.5M phosphate buffer was added to first test-tube, followed

immediately by 1.0ml of equivalent sodium hydroxide solution and mix. In this procedure was repeated with each successful tube at 30sec intervals. After exactly 6min, 1.0ml 1N sodium hydroxide was added to each test-tubes (again adding at 30sec intervals) and mix. This stops the reaction and stabilizes the “305” products for several hours. The concentration of the samples was determined spectrophotometrically at 340nm. The concentration was calculated using the formula.

$$\text{Conc.} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

2.4.3 Superoxide dismutase (SOD)

Two test-tubes were set and labeled as sample and blank, 0.1ml and 0.15ml of buffer (pH = 7) solution was added into the test-tubes respectively, 0.83ml of distilled water was measured and transferred into each of the test-tubes. 0.2ml of analyzed sample was measure and transferred into the test-tube labeled as sample only. The test-tubes contents were mixed and incubated for ten (10) minutes at room temperature. 0.02ml of pyragallol was added to each of the test-tubes and immediately, the change in absorbance was read at 420mm after 3minutes for each.

The percentage inhibition and unit values were calculated using the formula:

$$\% \text{ Inhibition} = \frac{\frac{\text{CDA}}{\text{minute}} \text{ of blank} - \text{DA minute of sample}}{\frac{\text{DA}}{\text{minute}} \text{ of blank}} \times 100$$

$$\text{units} = \frac{\% \text{ Inhibition}}{(100 - \% \text{ Inhibition})}$$

2.5 Determination of Antioxidant Vitamins

2.5. 1 Vitamin A

Three test-tubes were labeled as test, standard and blanks 1.0ml of sample was pipette into the test-tube labeled as test, 1.0ml ethanol was pipette into all test-tube respectively, 1.0ml standard working reagent was pipette into the test-tube labeled as standard, 3.0ml Pet ether was pipette

into all test-tubes respectively and 1.0ml of distilled water was added into all test-tubes respectively. The test-tubes were shaken thoroughly and centrifuged at 3000rpm for 5mins. The supernatant was used to measure the absorbance at 450nm against reagent blank. The concentration was calculated using this formula.

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

2.5.2 Vitamin C (Rotkowskiet al. 1998)

1ml of the analyzed liquid was measured into the centrifugal test-tube and 1ml of the Phosphotungstain Reagent was added, the solution was mixed thoroughly and left in a room temperature for 30 minutes. The solution was centrifuged and the whole of the separated supernatant was collected with a pipette which was used for spectrophotometry measurements. The standard sample was prepared as above (using 1ml of the standard solution instead of the analysed liquid) without centrifugation. The absorbance of sample (A_x) and of standard (A_s) was measured at 700nm against the mixture PR: 50mm solution of oxalic acid = 1:1 (v/v) as a reference sample.

The concentration (C_x) of Vitamin C (um) in the analysed liquid was calculated using the formula:

$$C_x = \frac{A_x}{A_s} \times C_s$$

2.5.3 Vitamin E (Rolkowskiet al., 2005)

0.5ml of the analyzed fluid was measured into the test-tube 1 and 0.5ml of anhydrous ethanol was added, the solution was shaken vigorously for 1 minutes. 3ml of xylene was added and shaken vigorously for another 1minutes the test-tube (solution) was centrifuged for ten minutes. Simultaneously, 0.25ml solution of betaphenanthroline was measured into a separate test-tube labeled as test-tube II. 1.5ml of the extract upper layer was collected and transferred into the test-tube II, the content was mixed. 0.25ml FeCl_3 solution was added to the test-tube II, mixed and of

H₃PO₄ solution was added and the content (solution) was mixed again. This way, the test sample was obtained for spectrophotometry. The standard sample was prepared (0.5ml of the standard solution, instead of the analyse liquid) using Trolox-prepared as the test sample. The standard sample was not centrifuged.

The absorbance of Test (A_x) and standard (A_s) was measured at 539nm against blank test (prepare as the test sample but using distilled water instead of the analyzed liquid)

Concentration (C_x) of Vitamin E is calculated using the formula:

$$C_x = \frac{A_x}{A_s} \times C_s$$

3.0 RESULTS

Table 4.1: Concentration of antioxidant enzymes activity in lettuce

ENZYMES (units/ml)	KALANBAINA	KWALKWALAWA
Catalase	881.22 ± 87.735 ^c	564.34 ± 56.322
Glutathione reductase	2009.21 ± 173.21 ^b	1909.21 ± 155.47
Superoxide dismutase	1.326 ± 0.173 ^a	4.882 ± 0.462

Values were expressed as the average mean± standard deviation (n=3) of the measurements. Significance in comparison to control (p<0.05).

TABLE 3.2 : Concentration of antioxidant vitamins in lettuce.

VITAMINS (mg/dl)	KALANBAINA	KWALKWALAWA
A	219.48 ± 10.970*	28.2 ± 4.619*
C	4095.28 ± 54.843*	2731.23 ± 54.843*
E	33.5 ± 1.782*	23.8 ± 2.309*

Values were expressed as the average mean ± standard deviation (n=3) of the measurements. Significance in comparison to control (p<0.05).

4.0 DISCUSSION AND CONCLUSION

Previous researchers have pointed out that cement dust has a detrimental effect on the growth of most plants compared with non-cement-dusted plants [15]. Shafiq and Igbal also found a reduction in the number of species around heavily polluted industrial cement units in Karachi; it is clear from these results that cement dust pollution is an operative ecological factor causing differentiation of plant diversity in an ecosystem. Therefore, to investigate the oxidative response of the plants exposed to cement dust pollution may provide important knowledge. In the present study, the antioxidant enzymes and antioxidant vitamins activities (concentration) of the plant grow within cement factory (Sokoto Cement Company) sampling area were compared with plant from pollution free environment (Kwalkwalawa Area) in order to determine their antioxidant response to environment factors. For this reason, the plant collected from Kalambaina Area is regarded as test while kwalkwalawa sample is regarded as control group. Catalase (CAT) activity

was found to be significantly ($P < 0.05$) higher (881.22 Unit/ml) in the sample grown in Sokoto Cement factory sample area, compared to the control sample (564.34 Unit/ml). It is known that CAT detoxifies H_2O_2 by breaking it down directly to form water and oxygen (Siedlecka and Kropa, 2002). If CAT activity is reduced, H_2O_2 which is toxic to living cells can accumulate in cell compartments which can result in cell damage (Siedlecka and Kropa, 2002). Vitamin C (Ascorbic acid) concentration was very high (4095.28 mmol/l) also in lettuce obtained from Kalandaina while that of Kwakwalawa is (2731.23 mmol/l). The reason is this vitamin C is a monosaccharide, oxidation-reduction (redox) catalyst found in both animals and plants, and thereby neutralizes, reactive oxygen species such as hydrogen peroxide (Padayatty, A *et al.*, 2003). In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the redox enzyme ascorbate peroxidase, functions particularly important in stress resistance in plants (Shigeoka M *et al.*, 2002). Ascorbic acid is present at high levels in all parts of plant and can reach concentrations of 20 millimolar in chloroplasts (Smirnoff and Wheeler 2000). Vitamin A concentrations of lettuce sample obtained from Kalandaina was (219.49 mg/dl) and that of control area (Kwakwalawa) was (28.2 mg/dl). While Vitamin E concentration was (33.5 mg/dl) in test sample and (23.8 mg/dl). The pollutants interfered with the biosynthesis of the antioxidant vitamins which result in increase of their respective concentration in various plants under study.

4.1 CONCLUSION

In this research, it is observed that cement dust is one of the environmental pollutants which have detrimental effect on plants. Cement dust aggravate the antioxidant enzymes which result in increases in their concentration in the plants. The increase in concentration of antioxidant enzymes is due to their crucial role in subduing the Reactive Oxygen Species (ROS) from causing damage to the plant. Another aspect of observation on the antioxidant vitamins is the interference of the pollutants with their concentration. Our results showed that *Lactuca sativa*.

Has a good antioxidant potential. Based on the results, it can be concluded that the plant is a promising source of natural antioxidants and might be used in the treatment of diseases associated with oxidative stress.

Cement dust has a dangerous effect on organisms which has to be control in order to avoid further (future) catastrophic effect.

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Appendix I



Figure 2: Map of the study area.

Map data ©2017 Google