Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 57-64 https://dx.doi.org/10.4314/sa.v20i1.5
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

LIPID PEROXIDATION AND ACTIVITY OF SOME ANTIOXIDATIVE ENZYMES IN THE ROOT OF MAIZE (*ZEA MAYS*) CULTIVATED ON CADMIUM CONTAMINATION SOIL

[1]Ugbeni, O.C., [2]Dania, O. E and [1]Eruotor, H

[1]University of Benin, Benin City, Nigeria
[2]Covenant University, Ota , Ogun State.
Correspondence: osezele.ugbeni@uniben.edu

Received: 02-02-2021
Accepted: 12-03-2021

ABSTRACT

In this study, we examined the tolerance capacity of *Zea mays* to cadmium pollution. Soil was treated with varied concentrations of Cadmium; 5 mg, 10 mg, 20 mg and 30 mg/kg soil and *Zea mays* planted. Root samples were collected in weeks 3, 4, 5 and 6. Activities of Peroxidase, catalase superoxide dismutase, and lipid peroxidation were investigated. Decrease in peroxidase activity was extremely significant ($p < 0.05$) in weeks 4 and 5 while that of week 6 was not significantly ($p > 0.05$) different from normal. The decrease correlated with increase in Cadmium concentration. However, at the highest concentration of 30 mg/kg of soil the trend was not significant. Increase in the activity of catalase was recorded in weeks 3 and 6. This increase didn't follow a particular trend but at higher concentration of Cd and long term exposure, it became apparent. There was a negative correlation between catalase activity and lipid peroxidation. In week 3, catalase activity was not significant ($p > 0.05$) and lipid peroxidation was significant ($p < 0.05$) while at week 4, catalase activity was significant ($p < 0.05$) and lipid peroxidation was not significant ($p = 0.8432$). Catalase activity was not significant ($p = 0.2753$) at week 5 and lipid peroxidation was significant ($p = 0.0030$). At week 6 when catalase activity became extremely significant ($p < 0.05$), lipid peroxidation had a p value of 0.0128. Generally no significant activity ($p > 0.05$) was observed for superoxide dismutase. A significant increase in absorption of cadmium ($p = 0.0374$) at 30mg/kg soil was observed between weeks 5 and 6. It was also observed that cadmium had no significant effect ($p > 0.05$) on the root weight during the period of study. It's suggestive therefore Cadmium contamination of soil could affect growth of maize and induce oxidative stress.

Key words: cadmium, reactive oxygen species, Thiobarbituric acid reactive species.

INTRODUCTION.

Cadmium is a highly toxic element which affects plant antioxidant defenses, generates oxidative stress and lipid peroxidation (Andersen and Kupper, 2013). Cadmium is not an essential element for plant growth, but it is readily absorbed by the roots of plants growing in soil containing it, though some species restrict cadmium transport from roots to the stems and grains (Trejo *et al*., 2016). Although it has been shown that cadmium ion may have a positive effect on the plant growth at low concentrations in some plants (Liu *et al*., 2008), it is widely recognized as an element which induces toxicity in plants, the critical level varying with species. Absorption of cadmium depends on the cadmium concentration in soil as well as the amount of cadmium ion available for absorption by plants (Hegedus *et al*., 2001). *Zea mays* is the world's third

leading cereal crop after wheat and rice. It belongs to the family Poaceae and is a tall annual herb with an extensive fibrous root system (Parle and Dhamija, 2013). Plants usually respond to heavy metal stresses by activating the reactive oxygen species system (He *et al*., 2015).To counteract the toxicity of ROS, ROS produced are scavenged easily by the action of antioxidative defense system such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Shakeel *et al*., 2015).

Superoxide dismutase production is considered as primary step of defense at cellular level (Noctor and Foyer, 1998). SOD belongs to the group of metalloenzymes and that catalyzes the dismutation of superoxide anion to oxygen and hydrogen peroxide. Catalase is a ubiquitous tetrameric heme-containing enzyme that catalyzes the dismutation of two molecules of hydrogen peroxide into water and oxygen. It has high specificity for hydrogen peroxide, but weak activity against organic peroxides (Corpas *et al*., 2008). Peroxidase is widely distributed in higher plants and protects cells against the destructive influence of hydrogen peroxide by catalysing it's decomposition through oxidation of prejudice and economic cosubstrates (Fabio *et al.*, 2004).These enzymes are reported to be involved in plant hormone regulation and, defense mechanisms (Almagro *et al.*, 2009). Thiobarbituric acid reactive substances (TBARS) are formed as a by-product of the oxidation of fat cells i.e. Lipid peroxidation and can be detected by TBARS assay. Because reactive oxygen species have extremely short half-lives, they are difficult to measure directly. Instead, malondialdehyde MDA could be used as an important indicator of physiological status (Wenwen *et al.*, 2016). Due to the increasing world population and technological advancement, the environment is asulted with some noxious chemicals such cadmium that have advance effect on food production. To guarantee food security, a study of the effect of these chemicals on plant growth is imperative. The research was therefore designed to evaluate the possible effects of cadmium ion the activity of some antioxidative enzymes and monitor the presence of lipid peroxidation in the root of *Zea mays*.

MATERIALS AND METHODS.

Maize (*Zea mays*) seeds were obtained from Ring road market in Benin City. Uncontaminated Soil was obtained from the University of Benin. Cadmium used in contaminating the soil was obtained in the form of $CdCl_2$. 21/2 H_2O. All chemicals used were purchased from Sigma Aldrich Steinheim, Germany.

Experimental design.

20bags each containing 5 kg of soil were used. The bags were divided into five groups labeled 1 to 5. Each group contained 4 bags; Group 1 was untreated and served as Control. Group 2 were treated with 5 mg cd/kg soil, Group 3, 10 mg cd/kg soil, Group 4, 20 mg cd/kg soil and group 5, 30 mg cd/kg soil respectively. 10 seeds of maize (*Zea mays*) were planted in each bag. The bags were housed in the green house, department of biochemistry, University of Benin and tended to throughout the duration of the study. Plants were harvested at weeks 3, 4, 5 and 6 for analysis. The roots were pulled carefully, cut, rinsed thoroughly in running water to remove soil and dabbed in Whatman filter paper to remove excess

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 57-64 https://dx.doi.org/10.4314/sa.v20i1.5
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

water then weighed. It was homogenized using mortar and pestle and 8 ml phosphate buffer pH 7.4. The homogenized samples were placed in sample bottles and centrifuged at 1500×g for 10 minutes. The supernatant was then used for the different enzyme assays.

Enzyme assays: SOD activity was assayed by the method of Misra and Fridovich (1989) and the activity computed and expressed as described by Baum and Scandalios (1981) in which one unit represents the amount of the enzyme required for 50% inhibition of adrenaline. Catalase activity was assayed by the method of Cohen *et al.*, (1970). Each catalase unit specifies the relative logarithmic disappearance of hydrogen peroxide per minute and is expressed as $Kmin^{-1}$ (Asagba and Obi, 2005). For peroxidase assay the method of Chancee and Maehly (1955) was adopted; Five milliliters of the assay mixture for the peroxidase activity comprised: 125 µmoles of phosphate buffer, pH 6.8, 50 µmoles of pyrogallol, 50 µmoles of H_20_2, and 1 ml of the enzyme extract. This was incubated for 5 min at 25 ^{0}C after which the reaction was stopped by adding 0.5 ml of 5% (v/v) H_2SO_4. The amount of purpurogallin formed was determined by taking the absorbance at 420 nm. The amount of thiobarbituric acid reactive substances (TBARS) which are indicators of lipid peroxidation was assayed by the method of Buege and Aust (1978). Values for TBARS were quantitated using a molar extinction coefficient of 1.56 × 105 M/cm and expressed in terms of malondialdehyde (MDA) units per gram tissue (Asagba and Obi, 2005). The cadmium concentrations in the roots were measured using 210VGP atomic absorption spectrophotometer.

RESULTS

Table 3.1: Effect of Cadmium on thiobarbituric acid reactive substances (TBARS/MDA) in root of *Zea mays*.

TREATMENT	WEEK 3 (units/g)	WEEK 4 (units/g)	WEEK 5 (units/g)	WEEK 6 (units/g)
Control	1.01 ± 0.19^{d}	2.33 ± 1.33^{a}	1.21 ± 0.68^{a}	4.59 ± 0.64^{e}
5 mg/kg soil	0.82 ± 0.09^{b}	2.60 ± 0.43^{a}	3.16 ± 0.23^{e}	1.48 ± 0.06^{b}
10 mg/kg soil	0.60 ± 0.13^{e}	2.26 ± 0.08^{a}	3.72 ± 0.33^{d}	1.37 ± 0.25^{a}
20 mg/kg soil	0.30 ± 0.06^{c}	1.58 ± 0.16^{a}	1.67 ± 0.32^{b}	2.87 ± 0.84^{c}
30 mg/ kg soil	0.45 ± 0.11^{a}	2.32 ± 0.36^{a}	1.42 ± 0.14^{c}	1.97 ± 0.64^{d}

Results are presented as mean of three determinations± SEM × 10^{-5}Values in the same column carrying different superscripts are significantly greater than expected by chance ($p < 0.05$).

We observed that the level of lipid peroxidation measured by malondialdehyde (MDA) production fluctuated with the fluctuation in activity of catalase. At week 5 when there was a reduction in catalase activity, lipid peroxidation was very significant ($p = 0.0030$) but by week 6 when catalase activity increased significantly ($p = 0.0368$), lipid peroxidation decreased.

Table 3.2: Effect of Cadmium on superoxide dismutase activity in root of *Zea mays.*

TREATMENT	WEEK 3 (units/g)	WEEK 4 (units/g)	WEEK 5 (units/g)	WEEK 6 (units/g)
CONTROL	1.85 ± 1.85^{a}	5.00 ± 0.00^{d}	1.65 ± 0.33^{a}	2.00 ± 0.20^{a}
5 mg/kg soil	0.73 ± 0.57^{a}	4.70 ± 0.40^{b}	1.50 ± 0.10^{a}	1.90 ± 0.40^{a}
10 mg/kg soil	2.14 ± 0.51^{a}	5.10 ± 1.20^{c}	1.96 ± 0.24^{a}	2.30 ± 0.50^{a}
20 mg/kg soil	0.72 ± 0.37^{a}	4.20 ± 0.50^{a}	1.57 ± 0.13^{a}	5.00 ± 0.10^{a}
30 mg/kg soil	0.79 ± 0.23^{a}	1.80 ± 0.20^{e}	1.34 ± 0.08^{a}	3.70 ± 1.30^{a}

Results are presented as mean of three determinations ± SEM× 10^{-2}. Values in the same column carrying different superscripts are significantly greater than expected by chance ($p < 0.05$).

Table 3.3: Effect of Cadmium on catalase activity in root of *Zea mays.*

TREATMENT	Week 3 (units/g)	Week 4 (units/g)	Week 5 (units/g)	Week 6 (units/g)
Control	3.69 ± 0.43^{a}	3.19 ± 0.00^{b}	3.34 ± 0.08^{a}	2.410 ± 0.09^{e}
5 mg/kg soil	3.33 ± 0.14^{a}	3.41 ± 0.07^{d}	3.22 ± 0.01^{a}	3.442 ± 0.07^{a}
10 mg/kg soil	3.44 ± 0.18^{a}	3.35 ± 0.05^{a}	3.24 ± 0.01^{a}	3.383 ± 0.02^{c}
20 mg/kg soil	3.24 ± 0.18^{a}	3.35 ± 0.05^{e}	3.25 ± 0.01^{a}	3.394 ± 0.03^{d}
30 mg/kg soil	3.09 ± 0.01^{a}	3.24 ± 0.04^{c}	3.25 ± 0.01^{a}	3.415 ± 0.01^{b}

Results are presented as mean of three determinations ± SEM. Values in the same column carrying different superscripts are significantly greater than expected by chance ($p < 0.05$).
The activity of catalase varied from week 3 to 6 with the highest activity recorded in week 6 (Table 3.3). At week 3 activities was insignificant ($p > 0.05$) but week 4 was significant ($p < 0.05$). At week 5, activity was not significant ($p > 0.05$) and week 6 was extremely significant ($p < 0.0001$).

Table 3.4: Effect of Cadmium on peroxidase activity in root of *Zea mays.*

TREATMENT	WEEK 4 (units/g)	WEEK 5 (units/g)	WEEK 6 (units/g)
Control	23.50 ± 2.12^{d}	123.92 ± 15.12^{b}	99.75 ± 20.31^{a}
5 mg Cd/kg soil	1.75 ± 0.38^{a}	23.75 ± 1.803^{c}	102.91 ± 11.28^{a}
10 mg Cd/kg soil	1.25 ± 0.00^{c}	31.75 ± 7.67^{e}	107.08 ± 11.09^{a}
20 mg Cd/kg soil	2.00 ± 0.38^{b}	20.42 ± 9.22^{d}	51.08 ± 24.84^{a}
30 mg Cd/kg soil	0.83 ± 0.30^{e}	24.58 ± 5.04^{a}	87.5 ± 09.9^{a}

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 57-64 *https://dx.doi.org/10.4314/sa.v20i1.5*
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Results are presented as mean of three determinations ± SEM x 10^{-2}. Values in the same column carrying different superscripts are significantly greater than expected by chance ($p < 0.05$).
Our study shows increase in peroxidase activity under cadmium stress between weeks 4 and 5. Its activity in week 6 was however not significant.

Table 3.5: Effect of Cadmium on root weight of *Zea mays*

TREATMENT	WEEK 3 (units/g)	WEEK 4 (units/g)	WEEK 5 (units/g)	WEEK 6 (units/g)
Control	0.4567 ± 03.71^a	0.7033 ± 06.22^a	1.0767 ± 15.68^a	1.1000 ± 12.22^a
5 mg Cd/kg soil	0.4467 ± 04.66^a	0.7600 ± 12.58^a	0.5567 ± 4.27^a	1.3500 ± 25.79^a
10 mg Cd/kg soil	0.4100 ± 0.0608^a	0.9633 ± 0.2795^a	0.6867 ± 0.1184^a	1.5967 ± 0.4253^a
20 mg Cd/kg soil	0.6467 ± 0.0921^a	0.6967 ± 0.0467^a	0.5067 ± 0.0928^a	0.4100 ± 0.1015^a
30 mg Cd/kg soil	0.4633 ± 0.0549^a	0.5600 ± 0.1206^a	0.7400 ± 0.1528^a	1.0133 ± 0.2967^a

Results are presented as mean of three determinations ± SEM. Values in the same column carrying different superscripts are significantly greater than expected by chance ($p < 0.05$).
Our results didn't show any particular significant correlation of the effect of Cadmium on the root weight of *Zea mays*.

Table 3.6: Cadmium determination in root of *Zea mays*

TREATMENT	WEEK 5	WEEK 6
20 mg Cd/kg soil	1.926 ± 0.023	1.997 ± 0.2455
30 mg Cd/kg soil	2.015 ± 0.065	2.247 ± 0.033

Results are presented as mean of three determinations ± SEM. Values in the same column carrying different superscripts are significantly greater than expected by chance ($p < 0.05$).

Cadmium absorption was measured at weeks 5 and 6 for 20mg/kg and 30mg/kg soil and the results showed that for each of the weeks there was no significant difference in the level of absorption between the plants exposed to 20 mg and 30 mg cadmium ($p = 0.1801$ and $p = 0.6474$ respectively) but when considering individual concentrations in the two weeks and the rate of cadmium uptake, it was discovered that although rate of absorption at 20 mg/kg soil had no significant difference between weeks 5 and 6 ($p = 0.7906$), the case was different for 30 mg/kg soil. There was a significant difference ($p = 0.0374$) in absorption of cadmium at 30 mg/kg soil for weeks 5 and 6.

DISCUSSION

Plants synthesize numerous antioxidant molecules and enzymes such as superoxide dismutase, catalase and peroxidase as a defense against oxidative stress (Magda *et al.*, 2006). The observed increase in

peroxidase activity under Cadmium stress was corroborated by earlier work done. They reported increases in peroxidase under cadmium stress in barley, sunflower cotyledons and reed (Gallego *et al*., 1999; Hegedus *et al*., 2001; Fediuc and Erdei, 2002). However in this study, there was a decline in peroxidase activity at week 6 this may be due to increased activity of catalase observed or adaptive mechanism of the plant due to long exposure. Variable activity of catalase has been observed under cadmium stress (Tran and Popova, 2013).The increased activity of catalase in week 6 in this study may be due to increased production of hydrogen peroxide since catalase is reported to have a quenching effect on hydrogen peroxide (Khaliq *et al.*, 2015). One of the main mechanisms of metal toxicity in plants is free-oxygen radical generation and oxidative stress. Oxidative stress may occur because of a disruption of detoxification mechanisms for removing free oxygen radicals. This study confirms these observations (Tables 3.3 and 3.1). It has been reported that increase in hydrogen peroxide causes loss of membrane integrity and lipid peroxidation in the presence of heavy metals (Dixit *et al*., 2001; Sanita di Toppi and Gabrielli, 1999). This study is in agreement with such findings, which shows increase in lipid peroxidation due to lon term exposure to Cadmium toxicity. The decrease in the activity Superoxide dismutase (SOD) although not significant, may be attributable to the duration of exposure. This correlates with Ci *et al*, (2009). They reported a decreased in SOD activity under cadmium stress. The plant species and cultivars may also be a factor in determining the impart of Cadmium on the activity of the enzyme. It is suggestive that the duration of exposure to cadmium would increase its possible effect on the root weight because the p values for weeks 5 and 6 were quite lower than those of weeks 3 and 4. A similar result as reported by Yadav, (2010) and Rascio Navari-Izzo, (2011). They posited that Cadmium toxicity could retard the root growth of plants. The duration of Cadmium exposure is a predisposing factor in determining its level of accumulation by Zea mays as shown by this work. Bernal *et al*., (2009) reported similar result. It's reasonable to state therefore that long term exposure of plants to Cadmium toxicity could lead to its accumulation in parts of the plants. Consumption of such plant parts, either for therapeutic or nutritional purposes by humans poses serious danger to health.

Conclusion: this study has shown that cadmium is definitely a toxic metal that increases the generation of reactive oxygen specie particularly hydrogen peroxide in *Zea mays* and also affects antioxidant enzymes in different ways, it could result in lipid peroxidation if the antioxidant enzymes are not effective in combating the reactive oxygen specie generated. *Zea mays* may be able to grow in soil polluted with cadmium at lower concentration.

REFERENCES

Almagro, L., Gomez Ros, L. V., Belchi-Navarro, S., Bru, R., Ros Barcelo, A. & Pedreno, M. A. (2009). Class III peroxidases in plant defence reactions. *Journal of Experimental Botany*.**60:**377-390.

Andresen, E. & Kupper, H. (2013). Cadmium toxicity in plants. *Metal Ions in Life Sciences*. **11**:395-413.

Asagba, S. O. & Obi, F. O. (2005). A comparative evaluation of the biological effects of environmental

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 57-64 https://dx.doi.org/10.4314/sa.v20i1.5
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

cadmium-contaminated control diet and laboratory-cadmium supplemented test diet. *BioMetals.* **18:** 155-161

Baum J. A. & Scadalios J. G. (1981). Isolation and characterization of the cytosolic and mitochondrial superoxide dismutase of maize. *Arch*ives of *Biochemistry* and *Biophys*ics.**206**:249-264.

Bernal, M. P., Clemente, R. & Walker, D. J. (2009). Interaction of heavy metals with soil organic matter in relation to phytoremediation. In: J .P .Navarro-Avino (Ed.) Phytoremediarion : The Green Salvation of the world. Pp: 109-129.

Buege, J. A, & Aust, S. D. (1978). Microsomal lipid peroxidation. *Methods in Enzymology*. **52**:302-310.

Chance B & Maehly, A. C (1955). Assay of catalase and procedures. *Methods in Enzymology*. **11**:764-775.

Cohen, C, Lowry, S., Harrison R.G, Kendrick -Jones, J, & Szent – Gyorgyi A. G. (1970).*Journal of Molecular Biol*ogy.**47**:605.

Das, P, Samantaray, S & Rout, G.R. (1997). A review: Studies on cadmium toxicity in plants. *Environmental Pollution* .**98:** 29–36.

Dixit, V., Pandey V. & Shyam R. (2001). Differential antioxidative responses to cadmium in roots and leaves of pea. *J*ournal of *Experimental Bot*any. **52** (**358**):1101-1109.

Fabio, R. C., Jose, T. A. U., Aparecida, S. M., Ricardo, A. V. & Joaquim, A. G. S. (2004). Superoxide dismutase, Catalyze and Peroxidase activities do not confer protection against injustice damage in salt-stressed cowpea leaves. *New Phytologist*. **163**:563-571.

Fediuk, E. & Erdei, L. (2002). Physiological and biochemical aspects of cadmium toxicity and protective mechanisms induced in *Phragmites australis* and *Typha latifolia*. *Journal of Plant Physiology*. **159:** 265–271.

Hart, N. S., Partridge, J. C. & Cuthill, I. C. (1998). Visual pigments.,oil droplets and gene photoreceptor distribution in the European starling (*Sturnus vulgaris*). *Journal of Experimental Biology* **201**:1433-1446.

He, F., Liu, Q., Zheng, L., Cui, Y., Shen, Z & Zheng, L. (2015). RNA. Sequence analysis of rice roots reveals the involvement of post-transcriptional regulation in response to cadmium stress. *Frontiers in Plant Science* **6**:1136.

Hegedüs, A., Erdei, S. & Horváth, G. (2001). Comparative studies of H2O2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. *Plant Science*. **160(6):** 1085-1093

Khaliq, A., Aslam, F., Matloob, A., Hussain, S., Geng, M., Wahid, A. & Rehman, H. (2015). Seed priming with selenium: consequences for emergence, seedling growth and biochemical attributes of rice. Book. Trace Even. Red. Doi :10.1007/s12011-015-0260-4.

Liu, H. J., Zhang, J. L. & Christie, P. (2008). Influence of iron plaque on uptake and accumulation of cadmium by rice (*Oryza sativa* L.) seedlings grown in soil. *Science of the Total Environment*. **394:** 361-368.

Magda, D., Duru M., Huguenot, J & Gleizes, B. (2006). *Grass and Forage Science*. **61**:89-9.

Misra, H. P & Fridovich I. (1978). Inhibition of superoxide dismutases by azide. *Archives of Biochemistry and Biophysics.***189**(**2**):317-22.

Noctor, G. & Foyer, C. H. (1998). Ascorbare and glutathione : keeping active oxygen under control. *Annual Review of Plant Biology*. **49**:249-79

Pal, M., Horvath, E., Janda, T., Paldi, E. & Szalai, G. (2006). Physiological changes and defense mechanisms induced by cadmium stress in maize. *Journal of Plant Nutrition and Soil Science.***169:** 239-246.

Parle, M. & Dhamija, I. (2013). *Zea mays*: A modern craze. *International research journal of pharmacy*. **4(6):** 39-43

Pylor, W. (1991). The antioxidant nutrients and disease prevention- what do we know and what do we need to find out?. *American Journal of Clinical Nutrition*.**53:**391S-393S

Rascio, N & Navari -Izzo, F. (2011). Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting?. *Plant Science*. **180**(**2**):169-181.

Sanita di Toppi, L. & Gabbrielli, R. (1999). Response to Cadmium in Higher Plants. *Environmental and Experimental Botany*. **41(2):**105-130

Scandalios, J. G. (1993) .Oxygen stress and superoxide dismutases. *Plant Physiology*. **101(1):** 7–12.

Trans, T. A & Popova, L. P (2013). Functions and toxicity of cadmium on plants: recent advances and future prospects. *Turkish Journal of Botany*. **37**:1-13.

Trejo, N., Matus, I., del Pozo, A., Walter, I. & Hirzel, J. (2016). Cadmium phytoextraction capacity of white lupine (*Lupinus albus* L.) and narrow-leafed lupine (*Lupinus angustifolius* L.) in three contrasting agroclimatic conditions of Chile. *Chilean Journal of Agricultural Research.* **76**(**2**):228-235.

Wenwen , L., Fei, L., Chu, Z., Jinfeng, Z. & Hailin F., (2016): Non-destructive determination of malondialdehyde (MDA) distribution in oil seed rape leaves by laboratory scale NIR hyperspectral imaging. *Scientific Reports.* **6**:35393

Yadav, S.K (2010). Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal of Botany* .**76:** 167– 179.