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TO EXPLORE THE IMPACT OF MERCURY DETOXIFICATION MECHANISMS ON THE *LENS CULINARIS* PLANT, A LABORATORY INVESTIGATION WAS CONDUCTED.

Dr Shilpa Varma, Ms Shruti Dhingra
Assistant professor; Assistant professor
Dr B lal institute of biotechnology, Malviya industrial area, Jaipur

Abstract:
The present study investigated the effects of mercury (Hg) on morphological aspects and the activity of antioxidant enzymes, as well as nitrate reductase activity, at five different concentrations (C, 0.001, 0.01, 0.1, 1 mM) of the metal. A comparative analysis was conducted between the aerial and radicle parts to correlate enzyme accumulation with mercury concentration effectively. The activities of antioxidative enzymes, including glutathione (GSH), ascorbate peroxidase (APX), and total thiols, demonstrated an enhanced detoxification mechanism in the aerial part compared to the radicle. Similarly, nitrogen assimilation enzymes, namely Nitrate Reductase (NR), Glutamate dehydrogenase (GDH), and Glutamate synthase (GS), exhibited similar patterns. The results of the present study suggest two significant pathways involved in Hg stress and tolerance in Lens culinaris, providing insight into the natural defensive mechanisms in plants under heavy metal stress.

Key words: Ascorbate peroxidase, GDH, GOGAT, Lentil, Mercury, Nitrate Reductase,
Abbreviations: Hg (mercury), GSH (glutathione), APX (ascorbate peroxidase), NR (nitrate reductase), GDH (glutamate dehydrogenase), GS (glutamate synthase).

Introduction:

Bioaccumaltion of heavy metal by higher plants is a serious point of discussion by many researchers one of the toxic heavy metal is mercury; causes gene toxicity and phyto-toxicity (Clarkson, 2003). Higher plants provide a useful model for determine heavy metal pollution level in soil and monitoring environmental pollutants Nitrogen assimilation is the formation of organic nitrogen compounds like amino nitrogen compounds present in the environment (Chinenye et al, 2003; Archana et al, 2016) . The product of nitrate assimilation Antioxidants are important species which possess the ability of protecting organisms from damage caused by free radical-induced oxidative stress. Level of H2O2is regulated by a wide range of enzymes, the most important being catalase and peroxidase. Catalase inactivates H2O2 to oxygen and water. (Ali et al, 2013; Del longo et al, 1993; Sarwar et al, 2017)

Lentils are exposed to heavy metals in their natural environment as a result of various human activities, including burning of fossil fuel, fertilizers, pesticides and the use of pigments and batteries. Briefly knowledge of the detoxification pathways is needed to understand phytoremediation. This research gives an overview of the different enzymatic and physiological clues for better detoxification mechanism.

To fulfill the gap or to achieve the desired aim the present work was planned with following objective:
(1) To study the effect of mercury on enzymes i.e., Nitrate reductase, Glutamate dehydrogenase and Glutamate synthase of nitrogen assimilation for elucidating the possible mechanism;
(2) To work out the physiological (protein) and morphological (root length, shoot length, seed germination (percentage and decline in period) impact on various part of lentil plant due to mercury;
(3) To determine the effect of mercury on antioxidant glutathione, glutamate synthase, ascorbate peroxidase and total thiols for elucidating the possible detoxification mechanism;

Materials and methods

Plant Materials

Plant materials (seeds) of Lens culinaris RLG-5 are collected from the Rajasthan Research Institute (RARI), Durgapura, and Jaipur

Mercury Metal treatment

Seeds were grown in pots with different concentrations of $HgCl_2$. Sand is treated with four different concentration of mercury with 1mm, 0.1mm, 0.01mm, and 0.001mm, for 24 hours respectively. Control sets were also prepared. One fourth Hoagland media is used for watering and mercury treatment Ammarah et al, 2022.

Seeds casting

Take 20 lentil seeds for each pot and wash them with a 1% $HgCl_2$ solution for 30 sec. After the washing with a 1% $HgCl_2$ solution, wash the seeds with distilled water (2–3 times). Now, place the seeds in each pot

Morphological assay: After a week seedlings were harvested then root and shoot were separated for measurements. Seed germination was also counted. All values counted in three replicates of experiment (Ling et al., 2010; Sharma et al., 2009).

Physiological and antioxidant assay: For this study, first excised bean leaf (0.25 g) were cut into small pieces treated with different concentration of one fourth Hoagland for 24 h incubation in continuous light inside "Indosan growth chamber" BOD for 24 h. The leaf is then used in estimation of protein, Enzymatic and antioxidant assay.

Physiological assay:

Protein:

Total protein was determined by Lowry et al. (1951). The protein in the enzyme solution was precipitated out by 10% TCA followed by solubilization with NaOH. Absorbance was measured at 660 nm using double beam spectrophotometer

Enzymatic

1. **Nitrate reductase assay**: The activity of nitrate reductase in the treated material was estimated by in vivo (Srivastava, 1974) method with slight modification. About 0.25 g of leaf material were incubated with 10 ml of incubation medium consisting of 0.1 M sodium phosphate buffer (pH 7.2), 0.2 M KNO_3, and 25% isopropanol in dark vial of 20 ml capacity. The whole set was incubated in dark for 30 min at 30ºC. Nitrite released in the incubation mixture due to enzyme activity was measured by color development by the formation of diazo compound with sulfanilamide and nitrate coupled with NED to give a red dye. The absorbance was read at 540 nm after 20 min by using UV- spectrophotometer. In intact seedlings nitrate reductase activity was measured only in the leaves as they are believed to be major nitrate reducing organic in most plants.
2. **Glutamate Synthase**: The GOGATE was estimated by Puranik & Shrivastava, 1990. Add 250 mg of leaves were extracted with 4 ml of extraction medium with a pinch of acid, 0.2M phosphate buffer (pH 7.5), 50 mM KCl, 2 mM EDTA, 0.1% Mercaptoethanol, and 0.5% triton X-100. The homogenate was centrifuged at 20000 rpm for 20 min at 4°C. Take 0.4 ml of enzyme from the supernatant and mix it with 0.025M phosphate buffer (pH 7.5) containing 1 mM EDTA, 50 mM KCl 1.2 ml, 1 mM NADH 0.6 ml, 5 mM 2-oxoketoglutarate 0.4 ml, and 20 mM glutamine 0.4 ml. The control sets contained all the ingredients except glutamine. The enzyme activity was measured at 340 nm. The amount of NADH oxidized was calculated in number of moles NADH oxidised per min.

3. Glutamate dehydrogenase (GDH):

The GDH was estimated by Singh and Shrivastava, 1983, take 250 mg of leaf tissue and extracted with pinch of acid wash sand in prechilled mortar and pestle. The extraction medium containing 0.05M phosphate buffer (7.4), 0.5% triton X-100, 0.1% mercaptoethanol, 1mM calcium choride and 2.0 mM EDTA (each dissolved in buffer). The ratio of plant tissue to medium was kept at1:4 (W/V). The crude extract was centrifuged at 16,000g for 20 min in 4oC the supernatant was used further for enzyme isolation. Assay mixture contains 0.1 M phosphate buffer (pH8.1) 2.0ml, 1mM NADH 0.4ml, enzyme 0.2ml, 1.5 M ammonium sulfate 0.2ml and 0.2 M 2-oxoglutarate 0.2 ml. control tubes contain all the contents except substrate ammonium sulfate and 2-oxoglutarate. Reaction was started by addition of 2-oxoglutarate. Decrease in absorbance was due to oxidation of NADH was linear for 5 min at least. The amount of NADH oxidized was calculated in no. of moles NADH oxidized per min

Antioxidant:

1. Ascorbate Peroxidase

Add 2.7 ml of 100 mM sodium phosphate buffer (pH 7), 0.1 ml of 15 mM L-ascorbate with 50 µl of enzyme, and then 0.15 ml of 10 mM of H2O2. The control set contained all the ingredients except the enzyme. After 2 min of incubation, the enzyme activity was measured at 290 nm. Praveen, 2022

2. **Reduced Glutathione (GSH):**

Crush 0.25 mg of the sample with 3 ml of 5% Sulphosalicylic acid in a pre-cooled mortar and pestle. The GSH content in leaves was determined by the method of Anderson (1985) as mentioned by Mohd Isar (2006) with slightly modification Incubate the sample at 4°C for at least 1 hour before centrifuging it at 10,000 rpm for 40 minutes. 0.5 ml of supernatant was taken in test tubes to which 0.5 ml of reaction buffer was added. The reaction buffer includes 0.1 M phosphate buffer (7.2); 0.5 mM EDTA, and 50 µl of 3 mM DTNB. After 5 minutes, the yellow colour was developed and read immediately at 412 nm

3. Total Thiols:

Excised plant root samples were homogenised with 0.02M EDTA (5 ml per gram of plant sample), grind with mortar and pestle under chilled conditions. The solution was centrifuged for 10 min at 12000 rpm at 4°C. The supernatant was analysed as follows:

a. 5 ml aliquots of the supernatant were mixed with 1.5 ml of 0.2M Tris buffer (pH 8.2) and 0.1 ml of 0.01M DTNB in a 15 ml plastic tube.

b. Add 7.9 ml of methanol to the mixture.

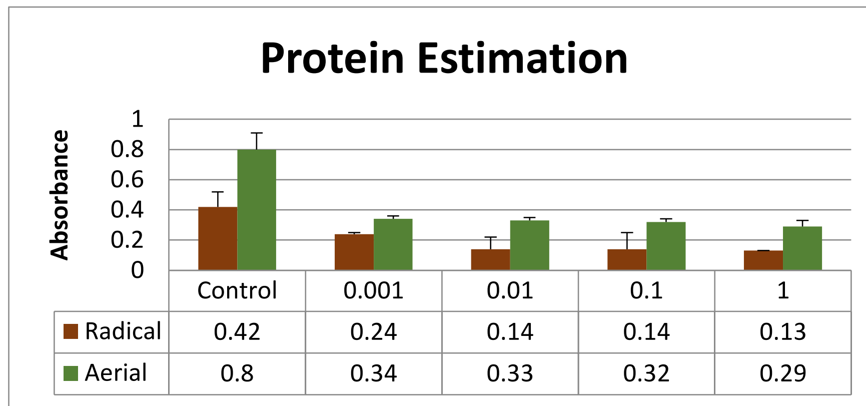
The reaction was allowed to develop at room temperature (20°C) for 10 min before absorbance was measured at 412 nm. Concentrations of thiols were calculated by using an Ellmans (1959)

Results:

Effect on Protein:

In examining the impact of Mercury treatment on lentils, it was observed that an increase in mercury concentration led to a reduction in the estimated protein content in the radical part. Conversely, in the aerial part, an enhanced effect was noted with rising concentrations of mercury. The decreased levels of photosynthetic pigments may be attributed to mercury-induced inhibition of chlorophyll and protein biosynthesis, potentially caused by nutrient deficiencies such as manganese (Mn), copper (Cu), iron (Fe), and phosphorus (P) (Rama Devi and Prasad, 1998; Sobrino et al, 2009). Similar outcomes were also reported in Mentha arvensis, Medicago sativa, and Bacopa monnieri under mercury stress (Manikandan, 2015; Zhou et al., 2007; Sinha et al., 1996).

Figure 1: The decrease in protein content is a consequence of Hg toxicity at higher concentrations.



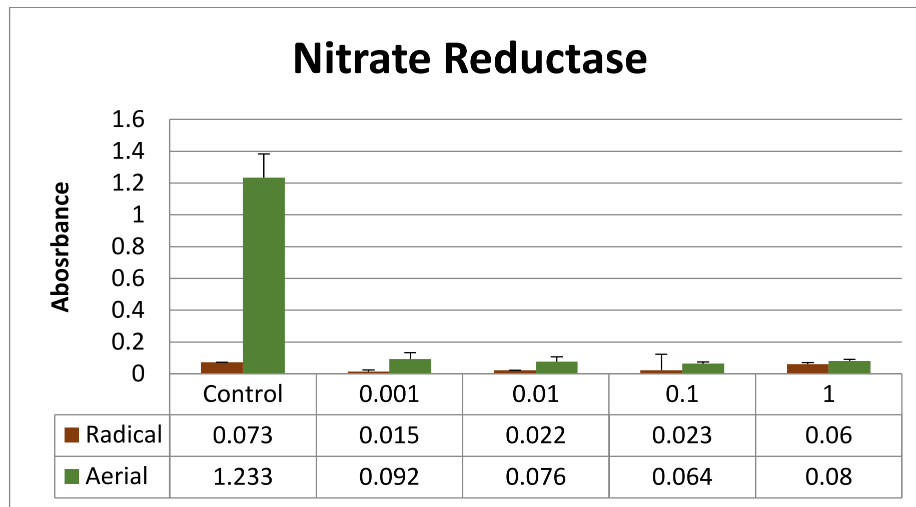
Effect on Nitrate Reductase:

The enzymatic study of Nitrate Reductase suggested that, there is decrease in enzymatic reduction with increase concentration of mercury uptake in radical part it shows more pronounced effect at 0.001 mM. Whereas in aerial part (Stems and Leaves) was shown enhanced activity with increase metal uptake. one of the most hazardous pollutant is Nitrate (Awasthi and Rai; 2005). NR is an enzyme that is being used to help clean up the environment, by conversion of nitrate to nitrite and has great potential to be part of the solution to the global problem of excess nitrate and related nitrogen nutrients in water sources (Campbell and Campbell, 1998). The study elucidated genotype-specific changes in key enzyme genes and enzyme activity in the potato N metabolism pathway under varying nitrogen levels, establishing a foundation for further exploration of gene functions and offering a theoretical basis for optimal nitrogen application. Lu et al, 2022.

In the context of algae growth and protein production, the pivotal factor influencing these processes is the activity of Nitrate Reductase (NR), as noted by Lau et al. in 1998. Their findings underscored that NR activity serves as the limiting factor in these biological activities.

Additionally, insights from Madhu et al. in 2008 revealed that the impact of lead toxicity on NR manifests diverse responses in mung beans. Specifically, the study highlighted distinct alterations in NR activity, shedding light on the intricate relationship between environmental stressors, enzymatic processes, and plant physiology. This dual perspective emphasizes the multifaceted role of NR, both as a determinant of algae performance and as a responsive element in the face of environmental challenges in plants like mung beans. Our result also showed same pattern mercury show more inhibition in radicle part as compared to aerial part. Hence detoxification mechanism occur from roots to stem as shown in fig 2

Figure 2: The decrease in *Nitrate Reductase* content is a consequence of Hg toxicity at higher concentrations



Effect on Glutamate Dehydrogenase (GDH):

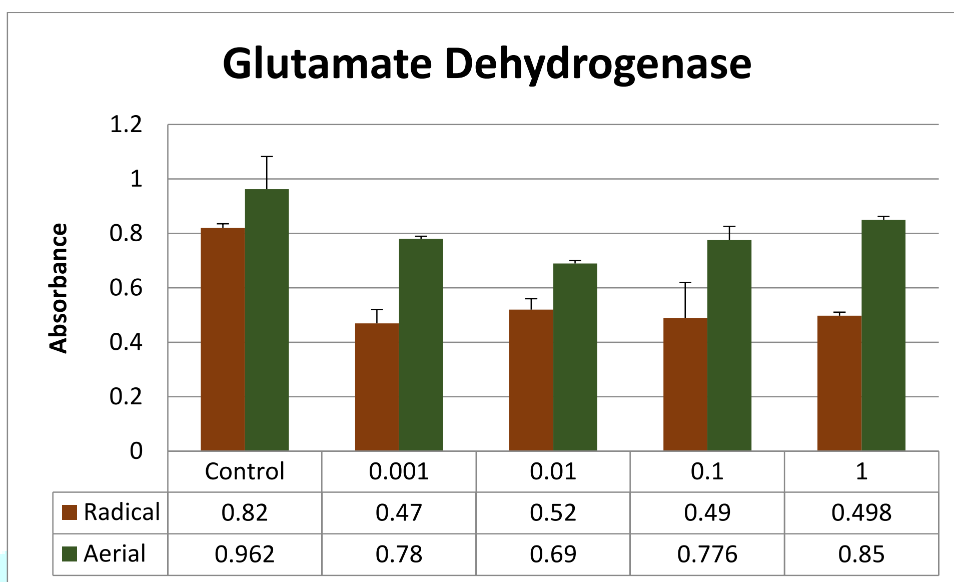
When lentil plants were subjected to mercury supply, an intriguing pattern emerged in their response, particularly in the radicle and aerial parts. The radicle exhibited a notable coping mechanism with mercury at a concentration of 0.1 mM, while the aerial part demonstrated a more pronounced effect at 1 mM. This differential sensitivity suggests a nuanced interaction between mercury exposure and different plant organs. (Fig 3)

Robinson et al. (1992), Aubert et al. (2001), and Miflin and Habash (2002) collectively concluded that Glutamate Dehydrogenase (GDH) plays a crucial role in the supply of 2-oxoglutarate, particularly when carbon availability becomes limiting, rather than being directly involved in ammonium assimilation. However, the comprehensive physiological role of GDH within the entire plant system remains speculative, as recent findings by Dubois et al. (2003) highlighted that a significant portion of GDH protein resides in the mitochondria of companion cells.

GDH, identified in all examined higher plants, tends to be abundant in senescing and root tissues (Loyolevargas and Jiminez, 1984). Notably, the enzyme exhibits tight regulation, being the first mitochondrial protein identified with potential modulation through ADP-ribosylation (Andres, 2001). The study observed an increase in GDH activity during growth, peaking at the flowering stage and subsequently decreasing during grain filling (Punesh, 2014).

In contrast to previous research, the current findings highlight the lentil plant's detoxification of mercury, particularly in relation to GDH dynamics observed from roots to stem. This underscores the intricate interplay between mercury stress, GDH activity, and the spatial distribution of the enzyme within the plant, shedding new light on the detoxification mechanisms in lentil plants.

Figure 3: The decrease in *GDH* content is a consequence of Hg toxicity at higher concentrations



	Control	0.001	0.01	0.1	1
Radical	0.82	0.47	0.52	0.49	0.498
Aerial	0.962	0.78	0.69	0.776	0.85

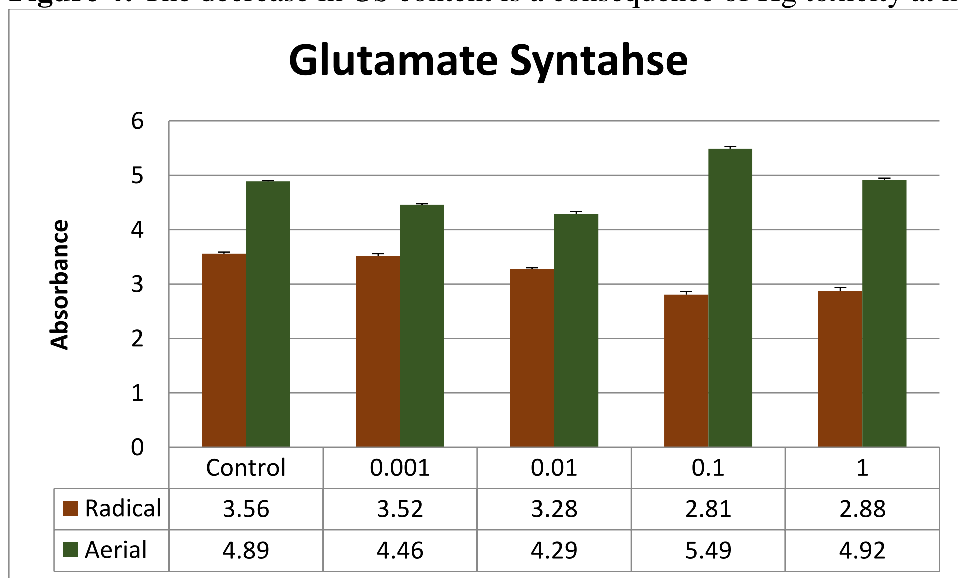
Effect on Glutamate synthase:

In this study, the result shows inhibition in enzyme activity with increase mercury concentration. When mercury induced in sand, activity enhanced with increase in concentration. The enhanced activity was found at 0.001mM and 0.01mM enhanced activity with respect to control in radicle part. In aerial part enhanced enzyme activity was found in 0.1 mM and 1 mM with respect to control. (fig 4)

The enzyme seems to be playing a pivotal role in linking the enzyme activity in plants, but the effect seems to be dependent on the isoform and the plant species analyzed (Puranik and Srivastava, 1994). The key enzyme involved in the de novo synthesis of glutamate is glutamate synthase, also known as glutamine: 2-oxoglutarate aminotransferase. Glutamine is a source of nitrogen for the synthesis of purines, pyrimidines, a number of amino acids, glucosamine and ρ-benzoate, whereas glutamate provides nitrogen for most transaminases.

This pattern was same form NR and GDH activity. Chromium treatment adversely affect nitrogenase, nitrate reductase, nitrite reductase, glutamine synthetase, and glutamate dehydrogenase in various plant organs at different growth stages as specific enzyme activity of these enzymes decreased with an increase in chromium(VI) levels (Dixit, 2002).

Figure 4: The decrease in *GS* content is a consequence of Hg toxicity at higher concentrations



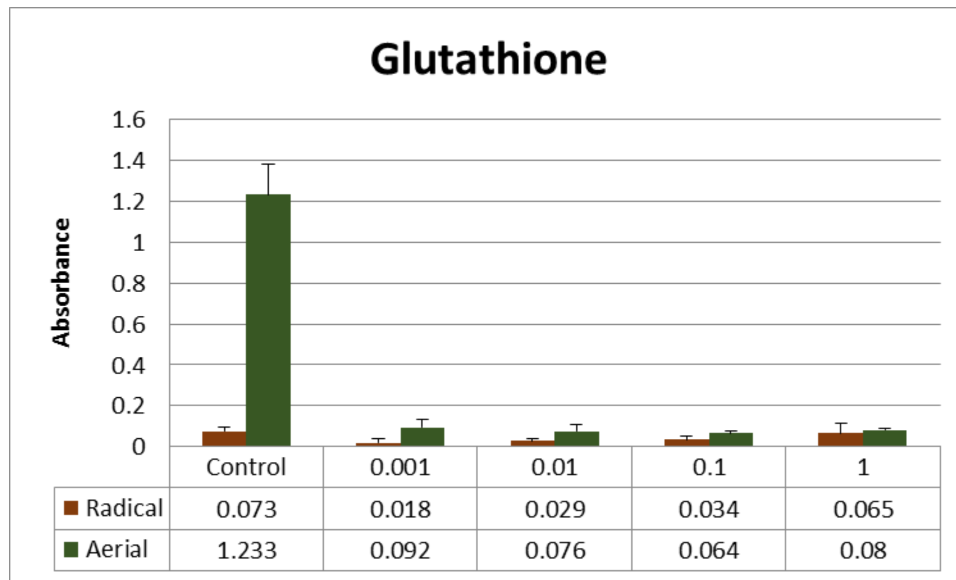
	Control	0.001	0.01	0.1	1
Radical	3.56	3.52	3.28	2.81	2.88
Aerial	4.89	4.46	4.29	5.49	4.92

Effect on Antioxidant Enzyme:

1. Glutathione Reductase (GR)

In the sand treatment, a noteworthy pattern emerged in the radicle part, where enzyme activity exhibited an enhancement at concentrations of 0.1 mM and 1 mM compared to the control. Conversely, a slight decrease in activity was observed at lower concentrations of 0.001 mM and 0.01 mM. This trend was consistently reflected in the aerial part, as illustrated in Figure 5. The figure demonstrates that, similar to the radicle part, both 0.1 mM and 1 mM concentrations led to enhanced enzyme activity, while the lower concentrations of 0.001 mM and 0.01 mM resulted in a slight decrease in activity compared to the control.

Figure 5: The decrease in *Glutathione* content is a consequence of Hg toxicity at higher concentrations

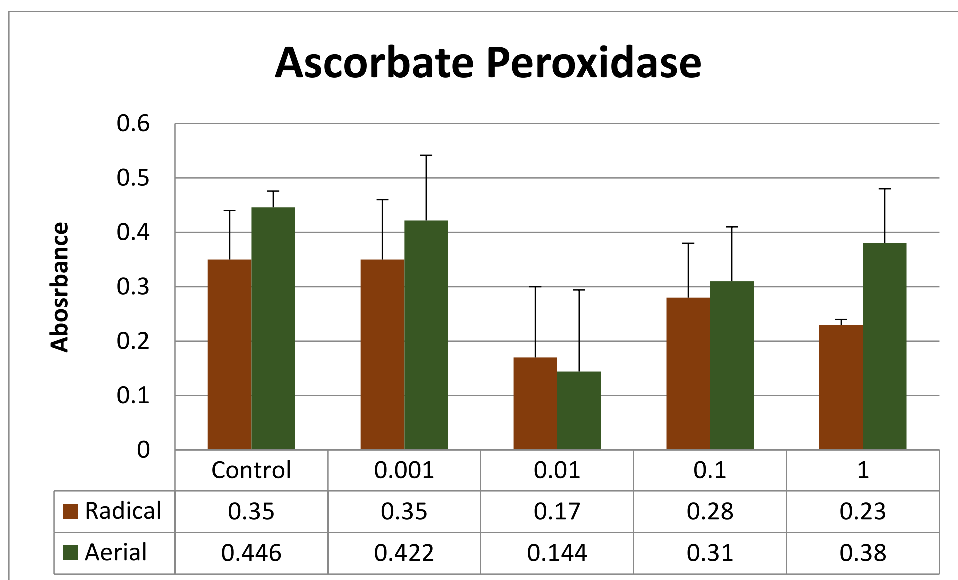


	Control	0.001	0.01	0.1	1
Radical	0.073	0.018	0.029	0.034	0.065
Aerial	1.233	0.092	0.076	0.064	0.08

2. **Ascorbate peroxidase**:

In the sand treatment, enhanced enzyme activity was observed at concentrations of 0.001 mM, 0.1 mM, and 1 mM in comparison to the control. Notably, this pattern held true for both the radicle and aerial parts, as depicted in Fig. 6. However, a slight decrease in activity was noted at the 0.01 mM concentration in the radicle part. This consistent trend underscores the concentration-dependent impact of the sand treatment on enzyme activity in both plant segments.

Figure 6: The decrease in *Ascorbate peroxidase* content is a consequence of Hg toxicity at higher concentrations



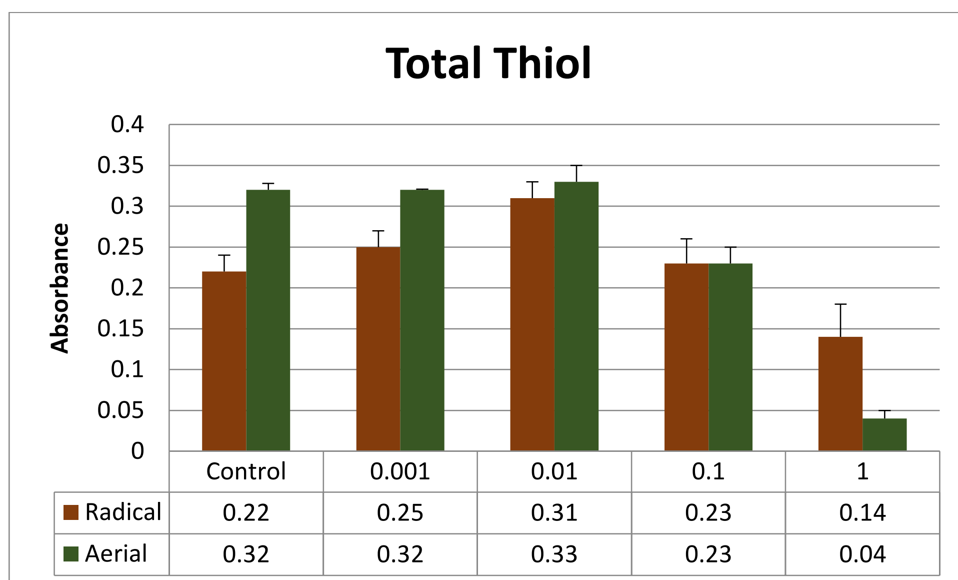
3. **Total Thiol**

Upon supplying Hg to the sand, a noteworthy observation emerged regarding the induction of total thiols. Surprisingly, the 1 mM concentration exhibited the least induction in comparison to the control group. In contrast, concentrations of 0.001 mM, 0.01 mM, and 0.1 mM showcased the maximum induction of total thiols. This intriguing pattern persisted in both the radicle and aerial parts, as illustrated in Fig. 7.

The differential response in total thiol induction suggests a concentration-dependent effect of Hg on this biochemical parameter. The lower concentrations (0.001 mM, 0.01 mM, and 0.1 mM) appear to trigger a more pronounced induction of total thiols, indicative of a potential adaptive or defensive response in the plant system. Conversely, the higher concentration (1 mM) may lead to a reduced induction of total thiols, possibly reflecting a saturation point or an adverse impact on the plant's ability to mount a robust thiol-based response.

These findings shed light on the intricate relationship between Hg concentration and total thiol induction, emphasizing the need for a nuanced understanding of the biochemical dynamics involved in plant responses to heavy metal stress

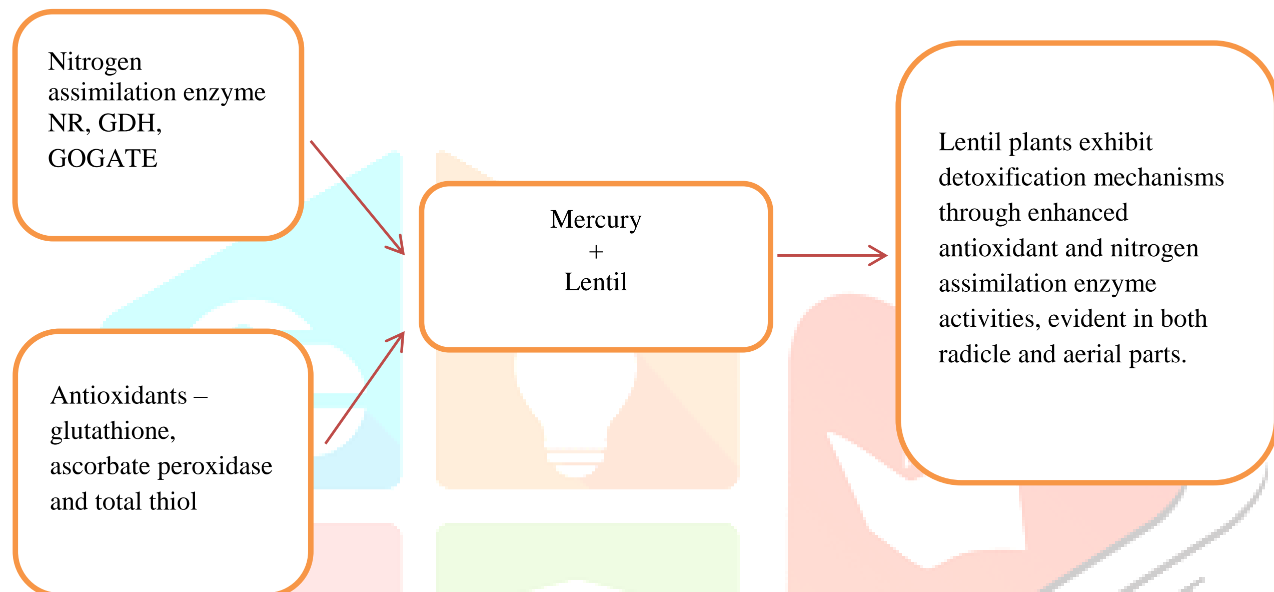
Figure 7: The decrease in *total thiol* content is a consequence of Hg toxicity at higher concentrations



The investigation of antioxidant enzyme activities was undertaken to comprehend the protective mechanisms employed by plants against oxidative stress induced by varying concentrations of mercury. The enzymatic system examined encompassed catalase (CAT), peroxidase (POX), glutathione reductase (GR), and the estimation of glutathione (GSH). These enzymes play pivotal roles, with catalase facilitating the dismutation of superoxide anions into H2O2 and molecular oxygen.
Previous data from various studies have consistently shown an enhanced activity of peroxidase in response to mercury stress, as evidenced in plants such as Alfalfa (Sobrino-Platam et al., 2009), Tomato (Cargnelutti et al., 2006), and Cucumber (Gupta et al., 2009).
Glutathione reductase (GR) assumes a crucial role in maintaining the glutathione pool by catalyzing the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) at the expense of NADPH. In the present study, a substantial alteration in GR activity was observed, particularly at concentrations up to 0.01 mM and 0.1 mM, supporting the aforementioned hypothesis. This observation aligns with previous studies that reported an increase in GR activity with cadmium (Cd) and zinc (Zn) treatments in bean and Brassica juncea (Chaoui et al., 1997; Prasad et al., 1999).
These findings collectively underscore the dynamic nature of antioxidant enzyme responses in plants, highlighting their adaptive strategies in the face of oxidative stress induced by mercury and other heavy metals.

Conclusion:
The Lentil plant exhibits a detoxification mechanism through the activation of antioxidant and nitrogen assimilation enzymes, as evidenced by a comparative analysis of results in both the radicle and aerial parts.
In the radicle part, the activation of antioxidant enzymes, such as Glutathione and Ascorbate peroxidase (POX), is likely indicative of the plant's effort to combat oxidative stress induced by external factors, possibly including heavy metals like mercury. These enzymes play a crucial role in neutralizing reactive oxygen species, thereby contributing to the detoxification process.
Simultaneously, the results suggest a concerted effort in nitrogen assimilation, as indicated by the activity of enzymes involved in this process. Nitrogen assimilation enzymes, such as NR, GDH and GOGAT likely contribute to the maintenance of the glutathione pool, a key component in detoxification processes. The interplay between antioxidant and nitrogen assimilation enzymes showcases a comprehensive detoxification strategy in the radicle part of the Lentil plant.
Similarly, in the aerial part of the plant, the activation of antioxidant enzymes and nitrogen assimilation enzymes underscores a systemic detoxification response. This dual mechanism suggests that the Lentil plant strategically utilizes both antioxidant defenses and nitrogen assimilation pathways to mitigate the impact of oxidative stress and potentially detoxify harmful substances like mercury.

Overall, the Lentil plant's ability to orchestrate a detoxification mechanism through the concerted action of antioxidant and nitrogen assimilation enzymes highlights its adaptive strategies in responding to environmental stressors and maintaining cellular homeostasis.



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References:

1. Ali H, Khan E, Sajad MA. 2013. Phytoremediation of heavy metals – concepts and applications. Chemosphere 91:869–881. https:// doi. org/ 10. 1016/j. chemo sphere. 2013. 01. 075
2. Ammarah Hasnain, Syed Atif Hasan Naqvi, Syeda Iqra Ayesha , Fatima Khalid, Manahil Ellahi, Shehzad Iqbal, Muhammad Zeeshan Hassan , Aqleem Abbas , Robert Adamski, Dorota Markowska, Alaa Baazeem , Ghulam Mustafa, Mahmoud Moustafa, Mohamed E. Hasan and Mohamed M. A. Abdelhamid, 2022. Plants in vitro propagation with its applications in food, pharmaceuticals and cosmetic industries; current scenario and future approaches. Front. Plant Sci. 13:1009395.
3. Andres herrero-yrcola, Siham Bakhit, Peter Franke, Christoph weise, Manfred schweiger, Dierk jorcke and mathias zieglar. 2001. Regulation of glutamate dehydrogenase by reversible ADP-ribosylation in mitochondria. EMBO journal vol.20;no.of p.p 2404-2412.
4. Archana Shrivastav, Jot Sharma, and Shilpa Shrivastava , 2016. Co-exposure Effects of Selenium and Mercury on *Phaseolus vulgaris* Excised Leaves Segment by Enhancing the NR, Anti-oxidative Enzyme Activity and Detoxification Mechanisms, Adv Tech Biol Med 2016, 4:2,
5. Aubert, Bligny, Douce, Ratcliffe, Roberts. 2001. Contribution of glutamate dehydrogenase to mitochondrial metabolism studied by 13C and 31P nuclear magnetic resonance. J Exp Bot 52: 37–45
6. Awasthi and Rai. 2005. Toxicity of Nickel, Zinc and Cadmium to nitrate uptake in free and immobilized cells of Scenedesmus quadricauda. Ecotox. Environ. Safe., 61, 268-272

7. Campbell and Campbell. 1998. Determination of nitrate in aqueous matrices using nitrate reductase. In: Current protocols in field analytical chemistry, supplement 1, Chapter 5 "Water Quality Parameters-Anions", John Wiley and Sons, Inc
8. Cargnelutti, Tabaldi, Spanevello. 2006. Mercury toxicity induces oxidative stress in growing cucumber seedlings. Chemosphere, vol. 65, no. 6, pp. 999–1006.
9. Chaoui, Mazhouri, Ghorbal, Ferjani. 1997. Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (Phaseolus vulgaris L.). Plant Sci. 127, 139– 147.
10. Chinenye U, Helen O, Nnabueze D N, Taghi M, 2003 Bioaccumulation for heavy metal removal: a review, SN Applied Sciences Volume 5, article number 125
11. Clarkson TW, Magos L, Myers GJ. 2003 The toxicology of mercury-current exposures and clinical manifestations. New Engl J Med. 349:1731–1737.
12. Del longo, Gonzalez, Pastori, Trippi. 1993. Antioxidant defenses under hyperoxygenic and hyperosmotic conditions in leaves of two lines of maize with differential sensitivity to drought. Plant Cell Physiol. 34, 1023–1028.
13. Dixit, Pandey, and Shyam, "Chromium ions inactivate electron transport and enhance superoxide generation in vivo in pea (Pisum sativum L. cv. Azad) root mitochondria," Plant, Cell and Environment, vol. 25, no. 5, pp. 687–693, 2002.
14. Dubois, Terce, Gonzalez-Moro, Estavillo, Sangwan, Gallais, Hirel. 2003. Glutamate dehydrogenase in plants; is there a new story for an old enzyme? Plant Physiol Biochem 41: 565–576
15. Ellman George. 1959. Tissue sulfhydryl group. Archives of biochemistry and biophysics 82, 70 77.
16. Gupta, Misra, Gaur, Pandey, Chauhan. 2009. Studies of genetic polymorphism in the isolates of Fusarium solani," Australian Journal of Crop Science, vol. 3, no. 2, pp. 101–106.
17. Lau, Tam and Wong. 1998. Effect of carrageenan immobilization on the growth, physiology and nitrate reductase activity of Chlorella vulgaris. Bioresource Technol., 63, 115-121
18. Ling, Fangke, Jun 2010. Effect of mercury to seed germination, coleoptile growth and root elongation of four vegetables. Research Journal of Phytochemistry, 4(4): 225-233, ISSN: 1819-3471.
19. Lowry, Rosebrough, Farr. 1951. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry, 193: 265-275.
20. Loyolevargas, Sanchez de Jimenez. 1984. Differential role of glutamate dehydrogenase in nitrogen metabolism of Maize tissue. Plant Physiology 76: 536-540.
21. Lu, Y., Zhang, J., Han, Z. et al. 2022 Screening of differentially expressed microRNAs and target genes in two potato varieties under nitrogen stress. BMC Plant Biol 22, 478
22. Madhu, Rakesh, Sengar, Kalpana, Reshu. 2008. Effect of lead on seed germination, seedling growth, chlorophyll content and nitrate reductase activity in Mung bean (Vigna radiata). Research Journal of Phytochemistry 2(2):61-68
23. Manikandan, Sahi and Venkatachalam. 2015. Impact Assessment of Mercury Accumulation and Biochemical and Molecular Response of Mentha arvensis: A Potential Hyperaccumulator Plant. Scientific World Journal,Volume 2015, Article ID 715217, 10 pages
24. Miflin, Habash. 2002. The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. J Exp Bot 53: 979–987
25. Mohd Israr, Shivendra Sahi Rupali Datta, Dibyendu Sarkar. 2006. Bioaccumulation and physiological effects of mercury in Sesbania drummondii, Chemosphere 65; 591–598.
26. Prasad, Saradhi, Sharmila. 1999. Concerted action of antioxidant enzymes and curtailed growth under zinc toxicity in Brassica juncea. Environ. Exp. Bot. 42, 1–10.
27. Praveen Kumar. 2022. Measurement of Ascorbate Peroxidase Activity in Sorghum, Bio Protoc. 12(20): e4531
28. Punesh Sangwan,Vinod Kumar and Joshi. 2014. Effect of Chromium(VI) Toxicity on Enzymes of Nitrogen Metabolism in Clusterbean (Cyamopsis tetragonoloba L.). Enzyme Research;Volume 2014, Article ID 784036, 9 pages
29. Puranik and Shrivastava. 1994. Glutamate synthasa. In: Nutration of higher plants. Associated publishing co. pp 229-243
30. Puranik, Shrivastava. 1990. Increase in glutamate synthase activity in bean leaf segments by light. Current sci. 59: 1001-1003.

31. Rama Devi and Prasad. 1998. Copper toxicity in Ceratophyllum demersum L. (Coontail), a floating macrophyte:response of antioxidant enzymes and antioxidants. Plant Science,vol. 138, no. 2, pp. 157–165, 1998
32. Robinson, Stewart and Phillips.1992. Regulation of glutamate dehydrogenase activity in relation to carbon limitation and protein catabolism in carrot cell suspension cultures. Plant Physiol 98: 1190–1195
33. Sarwar N, Imran M, Shaheen MR, Ishaque W, Kamran MA, Matloob A, Rehim A, Hussain S (2017) Phytoremediation strategies for soils contaminated with heavy metals: modifications and future perspectives. Chemosphere 171:710–721. https:// doi. org/ 10. 1016/j. chemo sphere. 2016. 12. 116
34. Sharma, Sharma, Datta. 2009. Morphological and biochemical response of cicer arietinum var.-pusa-256 towards an excess zinc concentration. African Journal of Basic & Applied Sciences, 1(5-6): 105-109, ISSN: 2079-2034.
35. Sigh R.P and Shrivastava H.S, 1983. Regulation of glutamate dehydrogenase activity by amino acids in Maize seedling. Physiol. Plant 57: 549- 554.
36. Sinha, Gupta, Chandra. 1996. Bioaccumulation and biochemical effects of mercury in the plant Bacopa monnieri L. Environ. Toxicol. Water Qual. 11, 105–112.
37. Sobrino-Plata, Ortega-Villasante, Flores-Caceres, Escobar, del Campo, and Hern´andez. 2009. Differential alterations of antioxidant defenses as bioindicators of mercury and cadmium toxicity in Alfalfa. Chemosphere, vol. 77,no. 7, pp.946–954,
38. Srivastava. 1974. In vivo activity of nitrate reductase in maize seedlings. Indian Journal of Biochemistry and Biophysics, 11: 230-232.
39. Suman J, Uhlik O, Viktorova J, Macek T (2018) Phytoextraction of heavy metals: a promising tool for clean-up of polluted environment? Front Plant Sci 9:1476.
40. Zhou, Huang, Guo, Mehta, Zhang and Yang. 2007. Metabolic adaptations to mercury- induced oxidative stress in roots of Medicago sativa L. Journal of Inorganic Biochemistry, 101: 1-9.