# Effect of Cadmium and Lead Exposure on Tissue Specific Antioxidant Response in *Spodoptera litura*

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## ABSTRACT

**Introduction:** Oxidative stress in insects may result from an imbalance of oxidants and antioxidants under a significant impact of metals. Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radical, are generated from normal metabolic processes in all oxygen-utilizing organisms. In the present study was designed to the impact of heavy metal (Cadmium and Lead) toxicity and its free radical scavenging enzymes in *Spodoptera litura* larvae. **Methods:** Heavy metals were administered using artificial diet method having different concentrations. The metal toxicity was observed after 24 and 48 hrs post treatment. In antioxidant parameters were SOD, CAT, POX, GST, LPO, ACP and AKP were assessed. In additionally metal accumulation analysis using ICP-MS. **Results:** In particular, after exposure to diets containing environmentally relevant concentration (Cd 0.3 mg, Cd 0. 44 mg, Cd 0.50 mg or Pb 0.48 mg, Pb 4.8 mg, Pb 9.6 mg) at 48 hrs changes in the activity of antioxidant enzymes were measured in larvae. The results showed that compared with control, the based on concentration significantly increased of antioxidant enzymes activities. In ICP-MS analysis results shows based on the concentration Pb 9.6 mg, 325.61 ppm/mg$^{-1}$ and Cd 0.44 mg, 36.5 ppm/mg$^{-1}$ will be increased. **Conclusion:** In conclusion antioxidant systems of *S. litura* were altered by Cd and Pb exposure as determined by measuring in the enzymes activities. All of Cd and Pb result in strong oxidative stress and toxicity to *S. litura* larvae. Based on the results we suggest that metal toxicity and metal accumulation of insects influenced by Cadmium chloride and Lead nitrate in *S. litura* larvae.

**Key words:** Metal toxicity, *Spodoptera litura*, Antioxidant enzymes, SOD, CAT, GST, ICP-MS.

## INTRODUCTION

The metal which has a relatively high density and toxic at low quantity is referred as 'heavy metal', e.g., arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), thallium (Tl), etc. Some 'trace elements' are also known as heavy metals, e.g., copper (Cu), selenium (Se) and zinc (Zn). They are essential to maintain the body metabolism, but they are toxic at higher concentrations. The heavy metals can enter the bodies to a small extent via food, drinking water and air. The heavy metals concerned with the environmental science chiefly include Pb, Hg, Cd, Cr, Cu, Zn, manganese (Mn), nickel (Ni), silver (Ag), etc. The excess quantities of heavy metals are detrimental as these destabilize the eco systems because of their bioaccumulation in organisms, and elicit toxic effects on biota and even death in most living organisms.[1]

Natural activities including volcanic eruptions, erosion, and spring water, and human activities such as exploration, mining, agriculture, and the search for fossil fuels form an accumulation of heavy metals in the soil and implicit toxicity to plants, animals, and humans. It has been proven by,[2] that there exists an accumulation of heavy metals in insects that feed on plants containing one, two or all three of the heavy metals: cadmium (Cd), copper (Cu), and zinc (Zn). Some heavy metals are essential, such as cooper and zinc.[3] Heavy metals in insects have a clear effect on growth,[4] mortality,[5] and physiology.[6] Zinc and copper connect to the cytosol metalothionein in the midgut of many organisms and are essential elements, but at high concentrations can be toxic.[3] Cadmium is highly toxic, even at a low concentration, and

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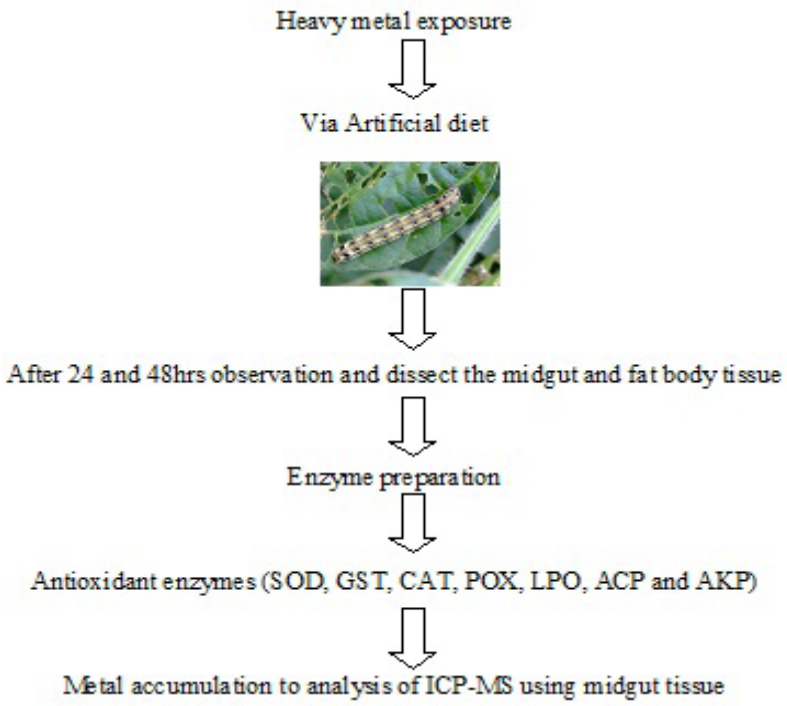


**Graphical Abstract**

can create mutations in the organism.[7] For example, the effects of environmental factors on feeding intake can take on indicators of nutritional influence.[8] The effect of heavy metals on an index such as RGR is quite various. For example, lipids, and proteins act as energy sources and have a great effect on insect populations the effect of a high concentration of nickel in *Spodoptera litura* reduced RGR but a low concentration of nickel increased RGR.[9] Intermediary metabolism includes multiple pathways in insects and the energy is stored as carbohydrates, lipids, and proteins for energy production through degradation or synthesis.[10]

Cadmium pollution has increased for decades due to industrial, agricultural and municipal wastes.[11] Because cadmium is a non-degradable metal, it may be accumulated in animal tissues and may disturb their physiological functions.[12] A contaminated ecosystem may exert strong selection on populations, resulting in their higher metal tolerance. This phenomenon was found for individual autumnal moths (*Epirrita autumnata*) that inhabited an area locally polluted with heavy metals.[13] Some herbivorous insects are strong accumulators of cadmium, e.g., *Locusta migratoria*, *Oxya chinensis*, *Acrida chinensis* (Orthoptera), *Eligma narcissus* or *Lymantria dispar* (Lepidoptera) larvae).[14] Cadmium is a persistent environmental pro-oxidant, which causes a wide variety of detrimental effects in organisms that eventually lead to higher mortality. Thus, the evolved tolerance to metals may be due to an increasingly effective anti-oxidant defence.[15] Pro-oxidants, which accelerate the generation of reactive oxygen substances (ROS) instead of their degradation, increase oxidative stress. ROS interact with vast number of biomolecules and degrade them. These detrimental effects can be indicated by the elevation of carbonyl groups of proteins.[16]

Exposure of Pb can cause many side effects depending on level and duration of Pb. The developing fetus and infant are more sensitive than the adult. Mostly, the bulk of Pb is received from food; however, other sources may be more important like water in areas with Pb piping and plumb solvent water, air near point of source emissions, soil, dust and paint flakes in old houses or contaminated land. In environment, the Pb comes from both natural and anthropogenic sources. The high levels of Pb may result in toxic effects in humans which in turn cause problems in the synthesis of hemoglobin (Hb), effects on kidneys, gastrointestinal tract (GIT), joints and reproductive system, and acute or chronic damage to nervous system.

Risks associated with polluted soils are contamination of the food chain. They are closely related to the bioavailability of toxic elements and primarily to the phyto availability. Plants are essential components of natural ecosystems and agro ecosystems, and are the first compartment of the terrestrial food chain. Plants may also have their growth sharply reduced by high levels of toxic elements in their tissues, causing a decrease in crop yields and further economic damage to farmers, as can be observed near metal smelters or mine spoils.

On the other hand, some elements, toxic when present at high concentration in tissues, are also essential to plants, and their deficiency induces loss in biomass production and physiological disorders in plants. It is necessary to determine the pathways of transfer of trace elements from soil to plants in order to properly manage polluted soils. The risks of heavy metal transfer into the food chain are dependent on the mobility of the heavy metal species and their availability in the soil.[17] Different kinds of extract ants are used for the extraction of the mobile forms of heavy metals. 1.0 M mineral acids extract most heavy metals and the species extracted are considered to represent a pool closely related to the total concentration, which can be mobilized potentially.[18] Heavy metals extracted by an acetate-ammonium buffer solution characterize these mobile pools.[19] The biochemical defence may facilitate the successful adaptation of the organism in metal contaminated environment.[12] Thus, in addition to the assessment of well-known anti-oxidants, it is important to investigate total anti-oxidant capacity (TAC), which reflects a crude measurement of all antioxidant processes. One radical that is suitable for such measurements is 2, 20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), ABTS.[20] After exposure to Cd, increased metal concentration was found in the haemolymph.[21] There is also the evidence that ROS production in haemolymph is promoted by Cd.[22] This is important because Cd may be transferred to other internal tissues via the haemolymph. Additionally, the haemolymph helps to protect the other organismal systems, through immunological and anti-oxidant defences.[23]

As a model animal, we have chosen to use the beet armyworm *Spodoptera litura* (Lepidoptera: Noctuidae), a well-known polyphagous pest of many vegetable crops, widely distributed across India, Asia, America, Africa and Australia.[24] In the present study was to investigated that the effects of metal (Cadmium and Lead) exposure on Lepidopteran insects antioxidant enzyme system and to analyses the metal accumulation in midgut tissue of *Spodoptera litura* larvae using ICP-MS analysis.

## MATERIALS AND METHODS

### Insects

*Spodoptera litura* (Lepidoptera) eggs were obtained from the National bureau of Agricultural important insect (NBAII), Bangalore. Larvae fed on castor leaf and adult were separated in to different plastic caps (20×20×30 cm), fed with 10% honey solution, the insect maintained at (10:14 L:D) 28 ±10°C, 70 ± 10% Relative Humidity(RH).

### Chemicals

Cadmium chloride and Lead nitrate were purchased form (Hi media) laboratory India.

### Treatment of insects

3rd instar larvae of *Spodoptera litura* were treated with heavy metal using artificial diet,[25,26] various dosage of concentration on cadmium chloride 0.3 mg, 0.44 mg, 0.50 mg and lead nitrate 0.48 mg, 4.8 mg, 9.6 mg. Each treatment were 20 larvae released after 24 h, 48 h observation were assessed in mortality data recorded.

### Enzyme preparation

Heavy metal treated with larvae were mid gut and fat body homogenized on ice in homogenization buffer (0.1 M phosphate buffer, pH 7.2) containing 1 mM EDTA, 1 mM DTT, 1 mM PTU, 1 mM PMSF and 20% glycerol. Insects were chilled on ice before homogenization. Tissues were homogenized in 2 ml buffer, the homogenate was centrifuged at 4°C, 10, 000 rpm for 15 min, and the solid debris and cellular material were discarded. The supernatant was decanted into a clean eppendorf tube, placed on ice and used immediately for Glutathione-S-transferase (GST), Catalase (CAT), Peroxidase (POX), Acid phosphatase (ACP), Alkaline phosphatase (AKP),Lipid peroxidase (LPO), and Super oxide dismutase (SOD). The total protein content was determined by Lowry *et al.*[27]

### Superoxide dismutase assay (SOD)

SOD activity was assayed using the method.[28] Reaction mixtures were prepared in 3-ml glass spectrophotometer cuvettes by adding 2.8 ml of Tris-EDTA (50 mM Tris and 10 mM EDTA, pH 8.2) buffer and 50µl of enzyme supernatant. The content was mixed and the final volume was adjusted to 2.9 ml with Tris-EDTA buffer. Reaction in the cuvette was started with the addition of 100 µl of Pyrogallol (15 mM). The rates of autoxidation were followed at 440 nm in the UV-Visible spectrophotometer (Systronics), and absorbance was measured for 3 min. One unit total SOD activity was calculated as the amount of protein per milligram causing 50% inhibition of pyrogallol autoxidation. The total SOD activity was expressed as units per milligram of protein.

### Glutathione S-transferase Assay

GST activity was estimated using the method of Habig *et al.*[29] with minor modifications. 50 µl of 50 mM 1-chloro-2, 4-dinitrobenzene (CDNB) and 150 µl of 50 mM reduced glutathione (GSH) were added to 2.78 ml of sodium

phosphate buffer (100 mM, pH 6.5). Twenty µl of enzyme stock was then added. The reaction was carried out in duplicate. The contents were shaken gently, incubated 2–3 min at 20°C and then transferred to a cuvette in the sample cuvette slot of a UV-Visual Spectrophotometer (Systronics). Reaction mixture (3 ml) without enzyme was placed in the reference slot for setting zero. Absorbance at 340 nm was recorded for 10-12min employing kinetics (time scan) menu. The GST activity was calculated using the formula: CDNB-GSH conjugate (µM mg protein$^{-1}$ min$^{-1}$).

### Catalase assay

CAT activity was spectrophotometrically measured by the rate of decomposition of $H_2O_2$ by catalase.[30] CAT activity was expressed as µmoles of $H_2O_2$ decomposed /min/mg protein.

### Peroxidase assay

POX activity was determined Reddy *et al*.[31] using UV–Vis spectrophotometer at 430 nm by catalyzing the oxidation in the presence of $H_2O_2$ of a substrate. One unit of POX activity was defined as the amount that catalyses 1 mg substrate per minute per mg protein. POX activity was expressed as U mg$^{-1}$ protein.

### Lipid peroxidation assay

Lipid peroxidation activity was determined by Ohkawa *et al*.[32] The process of lipid peroxidation results in the formation of malondialdehyde (MDA). Thiobarbituric acid reactive substances are formed as a byproduct of lipid peroxidation, which can be detected by the TBARS assay using thiobarbituric acid as a reagent.0.1 ml of the enzyme solution was taken.1.9 ml of 0.1 M sodium phosphate buffer at the pH was added to the enzyme solution. Then the mixture was incubated at 37°C for one hour. This mixture was precipitated with 10% TCA and centrifuged at 5000 rpm for 15 minutes and supernatant was collected. 1 ml of 1% TBA was added to the supernatant collected after centrifugation. The sample was boiled in water bath for 15 minutes. After boiling the supernatant was cooled and absorbance was taken at 532 nm.The MDA level was expressed as nmol/ h /mg of protein.

### Acid and alkaline phosphatase assays

The levels of these two phosphatases in the homogenates of larvae samples were measured following the procedure with slight modifications.[33] The acid phosphatase activity was estimated by mixing 50 µl of larval homogenate with 450 µl of 50 mM sodium acetate buffer at the optimal pH of 4.6 or pH 4.0. For estimation of alkaline phosphatase activity, 20 µl of larval homogenate were made up to 500 µl with 50 mM Tris–HCl buffer at the optimal pH of 8.0, and mixed with an equal volume of the respective buffer containing 12.5 mM p-nitrophenyl phosphate. After incubation for 15 min at 37°C in water bath, the enzymatic reaction was stopped by adding 100 µl of 0.5 N NaOH solution, centrifuged (4000×g; 5 min). The absorbance of the resulting clear supernatants was read at 440 nm.

### Metal Accumulation analysis using ICP-MS

For heavy metals analysis using ICP-MS Hendrickx *et al*.[34] approximately 0.2 g (wet wt) of soft tissues was weighed in a PTFE digestion container. Each sample was added with 2 ml of concentrated nitric acid and left to predigest overnight at 40°C. After cooling, 2 ml of 30% hydrogen peroxide was added. Thereafter the container was covered and placed in a high-pressure stainless steel bomb then put in an oven. The oven temperature was increased to 160°C and kept for 8 h. After cooling, the solution was diluted with Milli-Q water and transferred into PET bottle to 25 g. X series inductively coupled plasma mass spectrometer (ICP–MS) (Thermo Electron Co. Ltd, USA) was used for the determination of metals. All analyses were repeated thrice by internal calibration method. All metal concentration expressed in ppm/mg$^{-1}$of tissue.

### Statistical analysis

All the enzyme assays described above were performed using three replications. The data obtained from enzyme assays were subjected to analysis of variance followed by Dunnett's multiple comparison test using PRISM 5 software (Graph Pad Software Inc, USA). *P*- values <0.05 were considered significant.

## RESULTS

### Bioassay

The Heavy Metal treated larvae was observed in after 48hrs.The mortality rate was obtained from heavy metal against *S. litura* with $LC_{50}$ and $LC_{90}$ values are Pb-80.3250,110.16210,Cd-68.0031,93.16280 ppm respectively (Table 1).

### Antioxidant enzymes

POX activity of metal treated *S. litura* midgut tissues shows significantly increased activity in Cd 0.50 mg Cd 0.3 mg, Pb 4.8 mg, Pb 0.48 mg, as compared to control. However in cd

**Table 1: Toxicity of two heavy metals against 4th instar *S. litura* larvae**

| Sample | n[a] | $LC_{50}$(LCL-UCL) 95% CL ppm | $LC_{90}$(LCL-UCL) 95% CL ppm | $\chi^2$ | df |
|---|---|---|---|---|---|
| **Pb** | 240 | 80.320 (79.16201-82.8216) | 110.16210 (107.1328-116.1810) | 0.614 | 2 |
| **Cd** | 240 | 68.0031 (66.11628-69.1682) | 93.16280 (89.7286-95.0268) | 0.01 | 2 |

$LC_{50}$-Lethal concentration for 50% killing of the exposure larvae; $LC_{90}$-Lethal concentration for 90% killing of the exposure larvae; C.L.-Confidence Limit (95%); na-number of larvae; $\chi^2$-Chi square; df-Degrees of freedom.

0.44 mg and Pb 9.6 mg, concentration shows significantly decreased as compared to control (Figure 1). In POX activity of metal treated *Spodoptera litura* fat body tissues shows significantly increased activity in Pb 4.8 mg, Pb 0.48 mg, and Pb 9.6 mg as compared to control. However in Cd 0.50 mg, Cd 0.44 mg, Cd 0.3 mg, concentration shows significantly decreased as compared to control (Figure 2).

LPO level of metal treated *S. litura* midgut tissues shows significantly increased activity in Cd 0.50 mg, Pb 4.8 mg, and Pb 9.6 mg as compared to control. However in Cd 0.44 mg, Cd 0.3 mg, Pb 0.48 mg, concentration shows significantly decreased as compared to control (Figure 3). In LPO level of metal treated *S. litura* fat body tissues shows significantly increased activity in Pb 0.48 mg, Cd 0.3 mg, and Cd 0.44 mg as compared to control. However in Cd 0.50 mg, Pb 4.8 mg, Pb 9.6 mg, concentration shows significantly decreased as compared to control (Figure 4).

CAT activity of metal treated *S. litura* midgut tissues shows significantly decreased Cd 0.44 mg, Pb 4.8 mg, Pb 0.48 mg, Cd 0.50 mg, Cd 0.3 mg, Pb 9.6 mg compared to control (Figure 5). In CAT activity of metal treated *S. litura* fat body tissues shows significantly increased Activity in Cd 0.50 mg, Pb 4.8 mg, Cd 0.44 mg, Cd 0.3 mg, as compared to control. However in Pb 0.48 mg, Pb 9.6 mg, concentration shows significantly decreased as compared to control (Figure 6).

ACP activity of metal treated midgut *S. litura* tissues shows significantly increased activity in Cd 0.50 mg, Cd 0.48 mg, Pb 9.6 mg, as compared to control. However in Cd 0.44 mg Cd 0.3 mg, Pb 4.8 mg, concentration shows significantly decreased as compared to control (Figure 7). In ACP activity of metal treated *Spodoptera litura* fat body tissues shows significantly increased activity in Cd 0.50 mg, Cd 0.3 mg, and Pb 0.48 mg as compared to control. However in

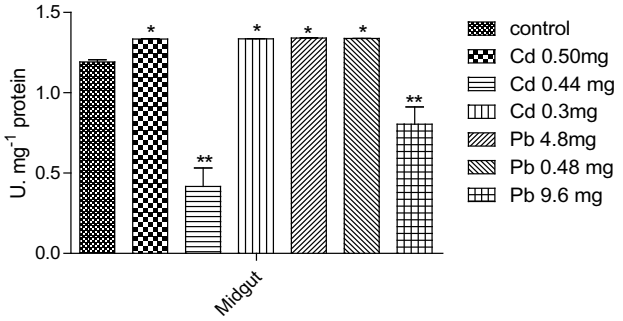


**Figure 1: POX activity in midgut tissue of metal toxicity in *Spodoptera litura***

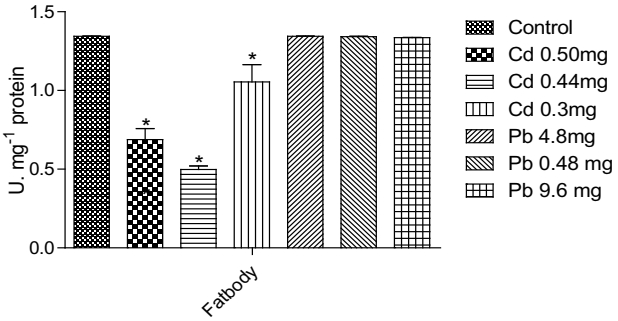


**Figure 2: POX activity in fatbody tissue of metal toxicity in *Spodoptera litura***

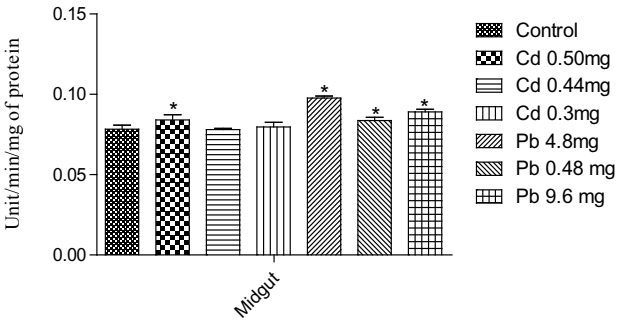


**Figure 3: LPO activity in midgut tissue of metal toxicity in *Spodoptera litura***

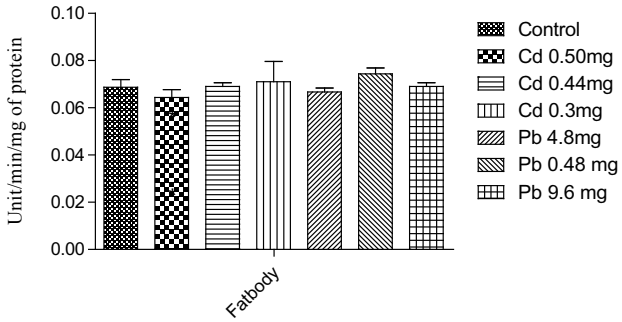


**Figure 4: LPO activity in fatbody tissue of metal toxicity in *Spodoptera litura***

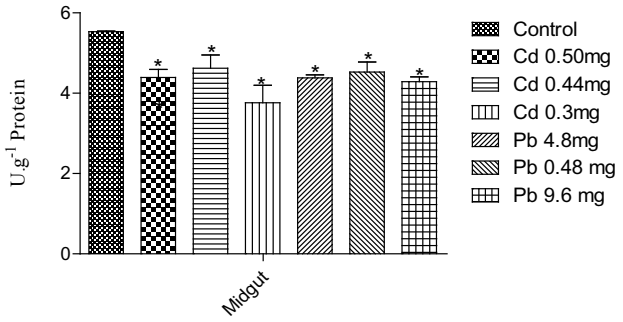


**Figure 5: CAT activity in midgut tissue of metal toxicity in *Spodoptera litura***

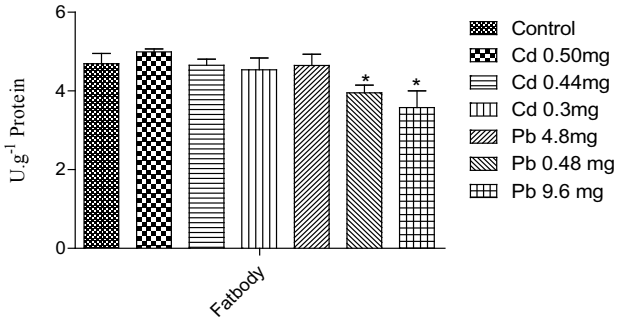


**Figure 6: CAT activity in fatbody tissue of metal toxicity in *Spodoptera litura***

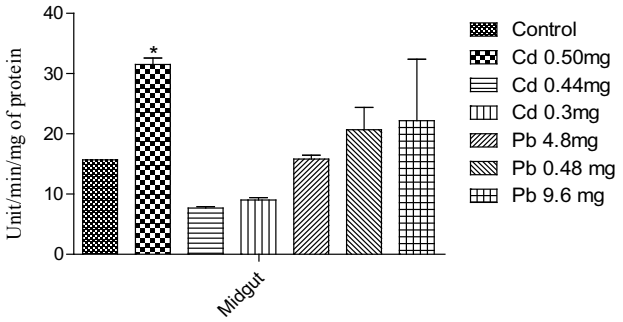


**Figure 7: Acid phosphate activity in midgut tissue of metal toxicity in *Spodoptera litura***

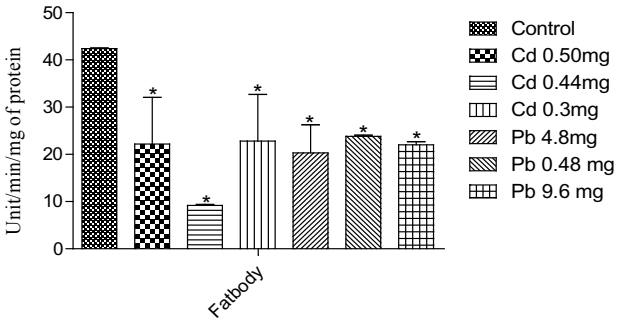


**Figure 8: Acid phosphate activity in fatbody tissue of metal toxicity in *Spodoptera litura***

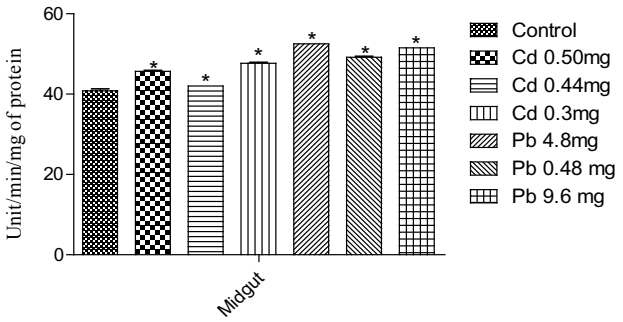


**Figure 9: Alkaline phosphate activity in midgut tissue of metal toxicity in *Spodoptera litura***

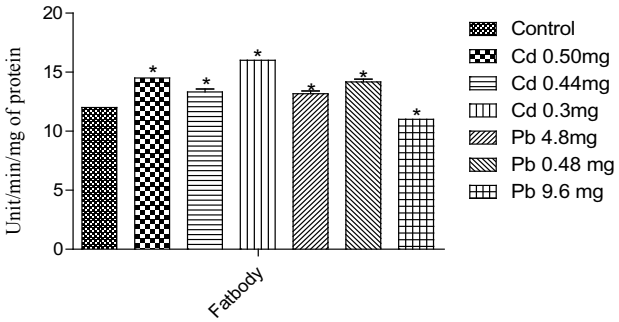


**Figure 10: Alkaline phosphate activity in fatbody tissue of metal toxicity in *Spodoptera litura***

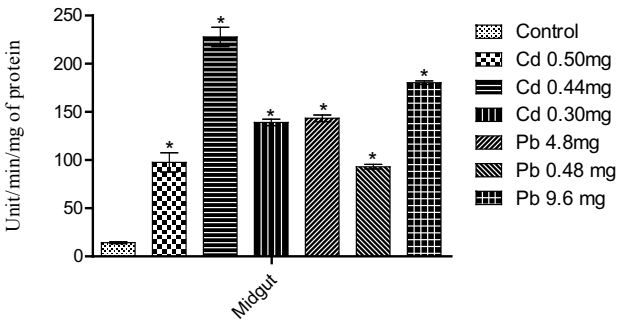


**Figure 11: GST activity in midgut tissue of metal toxicity in *Spodoptera litura***

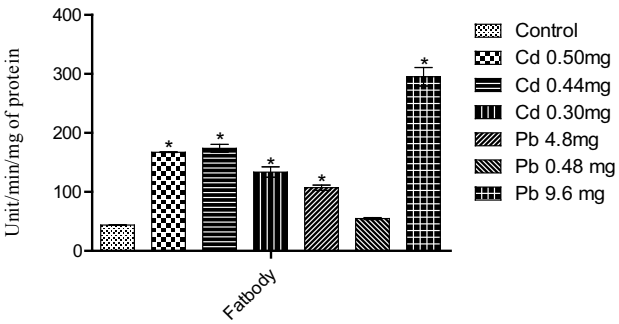


**Figure 12: GST activity in fatbody tissue of metal toxicity in *Spodoptera litura***

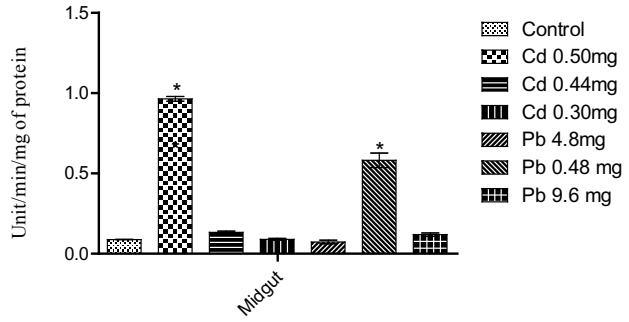


**Figure 13: SOD activity in midgut tissue of metal toxicity in *Spodoptera litura***

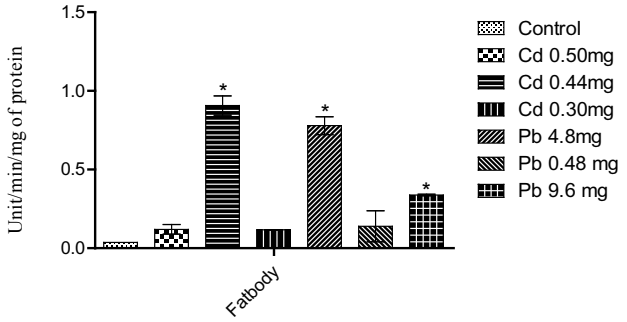


**Figure 14: SOD activity in fatbody tissue of metal toxicity in *Spodoptera litura***

**Table 2: Metal body accumulation in insect midgut tissue of *S. litura* larvae using ICP-MS analysis**

| Sample | (ppm/mg$^{-1}$) of tissue |
|---|---|
| Cd 0.3 mg | 7.17 |
| Cd 0.44 mg | 36.5 |
| Cd 0.50 mg | 1.44 |
| Pb 0.48 mg | 54.08 |
| Pb 4.8 mg | 83.67 |
| Pb 9.6 mg | 325.61 |

Cd 0.44 mg, Pb 4.8 mg, Pb 9.6 mg, concentration shows significantly decreased as compared control (Figure 8).

AKP activity of metal treated *S. litura* midgut tissues shows significantly increased activity in Cd 0.50mg, Cd 0.3 mg, Pb 4.8 mg, Pb 0.48 mg, and Pb 9.6 mg as compared to control. However Pb 0.44 mg, concentration shows significantly decreased as compared to control (Figure 9). In AKP activity of metal treated *S. litura* fat body tissues shows significantly increased activity in Cd 0.50 mg, Cd 0.3 mg, and Pb 0.48 mg as compared to control. However in Cd 0.44 mg, Pb 4.8 mg, Pb 9.6 mg, concentration shows significantly decreased as compared to control (Figure 10).

GST activity of metal treated *S. litura* midgut tissues shows significantly increased activity in Cd 0.44 mg, Pb 9.6 mg, Pb 4.8 mg, as compared to control. However Cd 0.3 mg, Cd 0.50 mg, Pb 0.48 mg, concentration shows significantly decreased as compared to control (Figure 11). In GST activity of metal treated *S. litura* fat body tissues shows significantly increased activity in Cd 0.44 mg, Pb 9.6 mg, Cd 0.3 mg, as compared to control. However in Cd 0.50 mg, Pb 0.48 mg, Pb 4.8 mg, concentration shows significantly decreased as compared to control (Figure 12).

SOD activity of metal treated *S. litura* midgut tissues shows significantly increased activity in Cd 0.50 mg, Pb 0.48 mg as compared to control. However in Cd 0.3 mg, Cd 0.44 mg, Pb 4.8 mg, Pb 9.6 mg, concentration shows significantly decreased as compared control (Figure 13). In SOD activity of metal treated *S. litura* fat body tissues shows significantly increase Activity in Cd 0.44 mg, Pb 4.80 mg, as compared to control. However in Cd 0.3 mg, Cd 0.50 mg, Pb 0.48 mg, Pb 9.6 mg, concentration shows decreased as compared to control (Figure 14).

### Metal Accumulation using ICP-MS analysis

Metal body accumulation in insect midgut tissue of *S. litura* larvae using ICP-MS analysis in based on the concentration Pb 9.6 mg, 325.61 ppm/mg$^{-1}$ and Cd 0.44 mg, 36.5 ppm/mg$^{-1}$ will be increased (Table 2).

## DISCUSSION

Lepidoptera are frequently challenged by several pro-oxidant substances. Present in their food and the nervous system of these insects may be a target for various oxidative stressors.[35] In addition, metals connected with oxidative stress may provide possible disruptions of the insect's nervous system, which has been shown in images of *S. exigua* exposed to the excessive levels of Pb.[36] Generally Cd and Pb metals are toxic on organisms and may cause major hazards to the environmental due to its wide distribution and its extensive. The mechanism of action of Cd has not been clarified completely; however, it is known that its toxic effects are related to the dose, duration of exposure, developmental stage of animals and animals' sensitivity.[37]

The underlying mechanisms involved in the regulation of the enzymes activities in animals after exposure to different doses of heavy metals in several studies have been undertaken to explain the causes of in the enzymes activity induced by pollutants in many invertebrates.[38] Our study was concerned with the measurements of toxicity and antioxidant enzymes activity *S. litura* larvae against Cd and Pb heavy metals exposure. Our results showing CAT activity of metal treated fat body tissues shows significantly increased at Cd 0.50 mg and Pb 4.8 mg.

However Pb 0.48 mg, Pb 9.6 mg, concentration shows significantly decreased as compared to control but metal treated midgut tissues shows significantly decreased in both Cd, Pb as compared to control. Our result suggest that CAT activity was decreased, it is conformed the protection against cadmium. Barat *et al*.[39] reported that increase of CAT activity with intensified lipid peroxidation in caddis fly (*Hydropsyche exocellata*) larvae and a positive correlation of CAT activity with Cd body loads. A role for this enzyme in protection against Cadmium stress was also demonstrated for nymphs of the orthopteran *Oxya chinensis*.[40] Superoxide radicals, generated in the presences of metals, are converted to $H_2O_2$ by SOD. In SOD activity of metal treated *Spodoptera litura* midgut tissues shows significantly increased Cd 0.50 mg, Pb 0.48 mg as compared to control. However Cd 0.3 mg, Cd 0.44 mg, Pb 4.8 mg, Pb 9.6 mg, concentration shows significantly decreased as compared to control to but metal treated *Spodoptera litura* fat body tissues shows significantly increase activity in Cd 0.44 mg, Pb 4.80 mg, as compared to control. However in Cd 0.3 mg, Cd 0.50 mg, Pb 0.48 mg, Pb 9.6 mg, concentration shows decreased activity as compared to control .We detected an increase of SOD activity in both target organs of *S. exigua* larvae following of Cadmium and Lead treatment. A negative correlation between SOD activity and Cd concentration was also reported for *O. chinensis*.[40] The effects may be observed in a non-liner manner in subsequent generations.[41] Similarly report Kafel *et al*.[42] on different effect of Cd (44 and 66 μg/g dry weight of diet) and Zn (200 μg/g dry weight of diet) in fat body of *S. exigua* showing significantly increses at higher concentration. In our results GST activity of metal treated *Spodoptera litura* midgut tissues shows significantly increased activity in Cd 0.44 mg, Pb 9.6 mg, Pb 4.8 mg, as compared to control. However Cd 0.3 mg, Cd 0.50 mg, Pb 0.48 mg, concentration shows significantly decreased as compared to control. Same activity of metal treated *Spodoptera litura* fat body tissues shows significantly increased activity in Cd 0.44 mg, Pb 9.6 mg, Cd 0.3 mg, as compared to control. However in Cd 0.50 mg, Pb 0.48 mg, Pb 4.8 mg, concentration shows significantly decreased as compared to control. GST activity was negatively correlated with metal concentration in the surrounding environment, but no such correlation was found between Cd or Zn body loads.[43]

ACP and AKP are capable of assisting, modulating and accelerating phagocytosis and are also involved in nutrient transport and digestion.[44] In ACP activity of metal treated *Spodoptera litura* midgut tissues shows significantly increased $P<0.001$ at Cd 0.50 mg, Cd 0.48 mg, and Pb 9.6 mg, however in Cd 0.44 mg, Cd 0.3 mg Pb 4.8 mg, concentration shows significantly decreased as compared to control. In AKP activity of metal treated *S. litura* mid gut tissues shows significantly increased activity in Cd 0.50 mg, Cd 0.3 mg, Pb 4.8 mg, Pb 0.48 mg, and Pb 9.6 mg as compared to control. However Pb 0.44 mg, concentration shows significantly decreased as compared to control. ACP and AKP activities in the larval fat body, However Cd 0.50 mg, Cd 0.3 mg, Pb 0.48 mg significantly increased as compared to control than Cd 0.44 mg, Pb 4.8 mg, and Pb 9.6 mg, concentration shows $P<0.05$ significantly decreased as compared to control. The exposure of *G. mellonella* to diets containing 5 μg/g Cr, 5 μg/g Pb or 50 μg/g Pb caused dramatic increases in ACP and AKP activities in the larval hemolymph. However, in the 50 μg/g Cr, 50 μg/g Pb, and 100 μg/g Pb treatment groups, the ACP and AKP activities were significantly decreased with an increasing concentration of heavy metal.[45]

POX is an antioxidant enzyme plays an important role in protecting cellular systems from the oxidative damage induced by xenobiotic metabolism.[46] POD activity in the larval hemolymph activities were significantly increased in Cr and Pb concentration, except the 5 μg/Cr. Previous studies that found the inducement effects of heavy metals on antioxidant enzyme activities in aquatic organisms such as goby and mussel.[47] In POX activity of metal treated *Spodoptera litura* midgut tissue shows significantly increased activity in Cd 0.50 mg Cd 0.3 mg, Pb 4.8 mg, Pb 0.48 mg, as compared to control. However in Cd 0.44 mg and Pb 9.6 mg, concentration shows significantly decreased as compared to control, but same activity metal treated fat body tissues shows significantly increased activity in Pb 4.8 mg, Pb 0.48 mg, and Pb 9.6 mg as compared to control. However in Cd 0.50 mg, Cd 0.44 mg, Cd 0.3 mg, concentration shows significantly decreased as compared to control.

In Polyphagous insects, lipid peroxidation is especially harmful, since lipids are not only the components of cell membrane, but also play an important role in development and reproduction physiology of insects. In LPO level of metal treated *S. litura* midgut tissues shows significantly increased activity in Cd 0.50 mg, Pb 4.8 mg, and Pb 9.6 mg, however in Cd 0.44 mg, Cd 0.3 mg, Pb 0.48 mg, concentration shows significantly decreased, and metal treated fat body tissues shows significantly increased in Pb 0.48 mg, Cd 0.3 mg, and Cd 0.44 mg, however in Cd 0.50 mg, Pb 4.8 mg, Pb 9.6 mg, concentration shows significantly decreased as compared to control. The differences between midgut and fat body concentrations were highly metal accumulated for larvae treated with Cd and Pb. This substantially metal contamination of the larave tissues should be important for effective protection against

metal action. In previous studies reported that Tylko *et al*.[48] the effects of metal exposure on *Musca domestica* head and larval. Metal are mostly accumulated in heads and it's found to be higher than abdomens. In these result suggests an imbalance of monovalent cations in the heads; these cations are important for electric potential properties of neurons.

A common mechanism of protection against stress caused by cadmium exposure is the induction of HSP synthesis; however, this is not the most commonly observed effect.[49] Sometimes an observed decrease in HSP levels may be connected with the negative effects of cadmium on the metabolic pathway responsible for synthesis of HSP 70 proteins or on a cellular energy deficiency for the production of these molecules.[50] In the case of the HSP70 response in the terrestrial wood louse *O. asellus,* it has been suggested that the reaction to stress depends on internal metal accumulation followed by increasing toxicity of the metal. During the compensation phase of metal intoxication, researchers observed a Cd dose-dependent increase of heat shock protein levels. During the non-compensation phase, adecline of HSP70 levels occurred due to the high toxicity of Cd.[51] Metal accumulation in insect midgut tissue of *S.litura* larvae using ICP-MS analysis in based on the concentration Pb 9.6 mg, 325.661 ppm/$mg^{-1}$ and Cd 0.44 mg, 36.5 ppm/$mg^{-1}$ will be increased. Similar studies reported that arsenic mediated toxicity induced and its antioxidant potential in thymoquione against wistar rats.[52]

Metal exposure is detrimental to the cellular metabolism by inducing oxidative stress, there by affecting cell survival and death. Many studies have been revealed that the capacity of insect detoxification enzymes to be induced by xenobiotic and the relationship between elevated detoxifying enzyme levels and tolerance to chemical insecticides.[53] Our work demonstrates that Cadmium chloride and Lead nitrate exposure against larval tolerance and antioxidant enzymes. Our results confirm that both Cd and Pb heavy metals induced toxicity and significantly increased antioxidant enzymes. Our results suggest that, anti-oxidant defense system is especially important in wild and semi domesticated polyphagous insects.

## CONCLUSION

In conclusion antioxidant systems of *S. litura* were altered by Cd and Pb exposure as determined by measuring in the enzymes activities. Moreover we found that experimental concentration of Cd and Pb exhibited specific effects on the antioxidant systems in *S. litura*. All of Cd and Pb result in strong oxidative stress and toxicity to *S. litura* larvae. ICP-MS analysis of midgut tissue revealed the maximum accumulation of Pb (325.61 ppm/mg of tissue) at 9.6 mg exposure dose, while for Cd a dose of 0.44 mg leads to 36.5 ppm/mg of tissue accumulation. Based on the results we suggest that metal toxicity and metal accumulation of insects influenced by Cadmium chloride and Lead nitrate in *S. litura* larvae.

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## CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

## ABBREVIATIONS

SOD: Superoxide dismutase
CAT: Catalase
GST: Glutathione-S-Transferase
LPO: Lipid Peroxidase
POX: Peroxidase
ACP: Acid Phosphatase
AKP: Alkaline Phosphatase
ICP-MS: Inductively coupled plasma mass spectrometry (ICP-MS)
Cd: Cadmium chloride
Pb: Lead nitrate

### Highlights of the paper

- Heavy metal pollution can have harmful effects not only on the survival growth, reproduction metabolism of animals but also on the antioxidant system.
- In this work, we analyzed the effects of Cadmium and Lead on antioxidant systems of *S. litura*. In particular, after exposure to diets containing environmentally relevant concentration (Cd 0.3 mg, Cd 0. 44 mg, Cd 0.50 mg or Pb 0.48 mg, Pb 4.8 mg, Pb 9.6 mg) at 48 hrs changes in the activity of antioxidant enzymes were measured in larvae.
- The results showed that compared with control, the based on concentration significantly increased of antioxidant enzymes activities. Whereas the lower concentration significantly decreased of enzymes activities as compared to control.

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