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Role of enzymes catalase, peroxidase and amino acid (proline) in *Raphanus sativus* and *Lepidium sativus* in exposure levels different water pollution of ion lead

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Abstract

Radish (*Raphanus sativus* L.) and (*Lepidium sativus*) is commonly grown in urban and suburban areas where the soil may be polluted with heavy metal such as lead. In this study, short exposure of radish and cress plantlets to (0,10,20,30,50)ppm lead in nutrient solution (three months) in growth chamber conditions elicited an antioxidative response, measured in terms of lipid peroxidation, protein and proline accumulation and peroxidase and Catalase activity. Longer exposure to lead when radish and cress was grown outdoors for 90 days in pots filled with field soil with different lead content also resulted in higher lipid peroxidation and proline accumulation and altered protein content and enzyme activity. The results also showed significant decrease antioxidant enzymes activity with the decreased oxidative stress. Also, the antioxidant enzymes activity (CAT and POD) were more activity after 30 days because of resistant for (50 ppm ,30 ppm) . Besides, at high level, the response of the antioxidant enzymes activity (CAT and POD) and proline accumulation were similar to that observed in control treatment. The highest proline content (81and 96 µmole/g) were recorded by control treatment after 30days radish and cress, respectively. The maximum average increase in peroxidase activity (43.76 and 68.38 unit/ml) were though control level treatment after 30 days, respectively. Maximum results were found in treatments (56.25 and 56.52 unit/ml) after 30 days in radish and cress, respectively.

Keywords: Catalase, peroxidase, water pollution**,** lead, leaf, oxidative stress, proline, *Raphanus sativus* L., *Lepidium sativus*.

دور الإنزيمات الكتاليزو البيروكسيدز و الحامض الاميني البر ولين في نبات الفجل والرشاد عند تعرضهم لمستويات مختلفة من المياة الملوثة بالرصاص

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الخلاصة

الفجل *Raphanus sativus* L والرشاد *Lepidium sativus* يزرع عادة في المناطق الحضرية والضواحي حيث يمكن أن تلوث التربة مع المعادن الثقيلة مثل الرصاص. في هذه الدراسة، والتعرض قصيرة من الفجل والرشاد شتلات الرصاص في المحلول المغذي (ثلاثة أشهر) في ظروف الغرفة نمو أثارت رد فعل مضادات الأكسدة، وتقاس من حيث بيرو كسيد الدهون والبروتين و تراكم

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البرولين والبيروكسيديز وفعالية الكتاليز . التعرض الطويل لقيادة عندما كان يزرع الفجل والرشاد في الهواء الطلق لمدة 90 يوما في الأواني مليئة التربة الميدان مع محتوى الرصاص مختلف أدى أيضا إلى ارتفاع بيرو كسيد الدهون وتراكم البرولين والبروتين المتغير ونشاط إنزيم. كما أظهرت النتائج انخفاض ملحوظ بنشاط الإنزيمات المضادة للأكسدة بسبب الإجهاد التاكسدي انخفض. كان نشاط الإنزيمات المضادة للأكسدة (الكتاليز,بيروكسديز) تزيد من النشاط بعد 30 يوما بسبب مقاومة (50ppm ، 30 ppm). الى جانب ذلك، على مستوى عال، واستجابة للمضادات الأكسدة الإنزيمات النشاط (الكتاليز,بيروكسديز) وتراكم البرولين كانت مشابهة لتلك التي لوحظت في معاملة السيطرة. وسجلت أعلى نسبة البرولين (81و96 مايكرو مول على مل) من خلال معاملة السيطرة بعد 30يوم في نبات الفجل والرشاد، على التوالي. وكان متوسط زيادة الحد الأقصى في النشاط البيروكسيديز (43.76 و 68.38 وحدة / مل) على الرغم من العلاج مستوى المعاملة بعد 30 يوما على التوالي. وجد أقصى قدر من النتائج في المعاملات (56.25 و 56.52 وحدة / مل) في الفجل والرشاد بعد 30 يوما على التوالي.

Introduction

Amongst many a biotic stresses influencing plant growth and development, heavy metal toxicity is very important, especially if crop species are grown in the vicinity of sites of heavy industry, particularly in developing countries [1, 2]. The term "heavy metal" is generally used to refer to metals and semi-metals associated with pollution and toxicity, but the term also includes some elements which in low concentrations are essential nutrients for cells [3].Although lead is not an essential element for plants, it is easily absorbed and accumulated in different parts of the plant, and its phytotoxicity can lead to inhibition of enzyme activity, disturbed mineral nutrition, water imbalance, changes in hormonal status and alteration of membrane permeability [4]. Lead also acts as aROS-promoting heavy metal, inducing antioxidative responses in plant roots where it is mostly accumulated, as well as in leaves. Heavy metal intoxications, especially toxicity caused by lead (Pb) cadmium (Cd) arsenic (As) and mercury (Hg) constitute serious threat to human health. Reactive oxygen species (ROS) including the superoxide radical (O^{2-}) and hydrogen peroxide which are inevitably generated via a number of metabolic pathways [5], are constant risk for organisms with aerobic metabolism. Malondialdehyde (MDA) one of the decomposition products of polyunsaturated fatty acids of membrane, is considered as one of the reliable indicators of oxidative stress [6]. Moreover, plant cells are equipped with enzymatic mechanisms to eliminate or reduce the oxidative damage. These protective enzymes include SOD, CAT,APX, GPX and GR. SOD works as the first line of defense, converting superoxide radical to hydrogen peroxide, which is then reduced to water and oxygen either by APX in ascorbate-glutathione cycle or by GPX and CAT in cytoplasm and other cellular compartments. GR is another important enzymatic constituent of this orchestrated antioxidative defense system that helps in maintaining a high GSH/GSSG ratio [7]. However, anthropogenic inputs associated with agricultural practices, mineral exploration, industrial processes and solid waste management play a significant role in contamination of terrestrial ecosystem through heavy metals [8, 9]. Removal of hydrogen peroxide is therefore a protective mechanism for the preservation of biological membranes when lead and other metals accumulate in the symplast of the cell [10]. Proline is probably the most widespread and is considered to be an indicator of environmental stress [11]. Accumulation of proline has been shown to protect plants against damage by ROS (Reactive oxygen species) [12], acting as a very effective singlet-oxygen quencher, binding to redox-active metal ions and also activating and protecting enzymes such catalase, peroxidase and polyphenol oxidase [13]. Acting as a very effective singlet-oxygen quencher, binding to redox-active metal ions and also activating and protecting enzymes such catalase, peroxidase and polyphenol oxidase [14]. The present investigations were aimed to study role of enzymes catalase, peroxidase and amino acid (proline) in *Raphanus sativus* and *Lepidium sativus* in exposure levels different water pollution of ion lead.

Materials and Methods

Two *R. sativus* and *L. sativus* seeds were used in this study. State Board redistricted the cultivars for Seed Testing and Certification, Ministry of Agriculture, Iraq. The cultivars were kindly provided by Seed Technology Center, Ministry of Science and Technology and by State Board for Seed Testing

and Certification, Ministry of Agriculture. Field experiment was conducted during 2015-2016 in silt loam soil at the research field of the Department of Biology, College of Science, Baghdad University, Baghdad, Iraq. The chemical and physical characteristics of field soil were measured in laboratory of soil department, college of agriculture, Baghdad University. Chemical fertilizers used were urea (46% N) at 200 kg ha-1 and triple super phosphate (46% P_2O_5) at 100kg ha-1. All phosphorus fertilizer was applied at planting during seedbed preparation, while urea was divided into two equal amounts. The first amount was added during the land preparation prior to planting, the second was added 30 days after sowing (during the early tillering stage) and the final amount was added at panicle initiation. Seeds of radish (*R. sativus*, *L.sativus*) were sown in plug plates filled with commercial substrate and grown for 3 months in glasshouse conditions, until the 4–5-leaf stage. The uniform plantlets were selected and uprooted from the substrate with water, causing minimal damage to the roots. After washing thoroughly with running deionized water they were planted on perforated polystyrene fasteners containing Hoagland nutrient solution. The experiment was carried out in three replicates (of four plants each. The pots were kept for 3 months in a growth chamber and rotated there every day.

Experiment

A field experiment was conducted during 2015 in silt loam soil at the research field of Baghdad, Iraq. The chemical and physical characteristics of field soil were measured in laboratory of soil department, college of agriculture, University of Baghdad.

Proline determination

Free Proline content of *R. sativus* and *L.* leaves was determined following the method of [15]. Samples of 0.5 g of fresh weight of leaves from each treatment were homogenized in sulphosalicylic (3% w/v H_2O), then centrifuged at 3000 rpm for 5 minutes. Samples of 2 ml from the supernatant were added to 2 ml of each of ninhydrin and glacial acetic acid and incubated at 100ºC for 1 hour in water bath. The reaction was arrested in an iced bath and the chromophore was extracted with 4 ml toluene and its absorbance at 520 nm was determined in spectrophotometer (Varian Australia PTY LTD). Proline concentration was determined using a calibration standard curve prepared with authentic Proline and calculated its amount on fresh weight basis using the following formula.

µmoles of Proline/g of fresh material = [(µg Proline / ml × ml toluene) / 115.5 µg /µmoles] / [(g sample (0.5)/5]

Catalase Activity (CAT)

Determination of Catalase (CAT) Activity

The activity of catalase was determined according to [16] in 3ml, of reaction solution, which contained: 2ml of phosphate buffer pH 7.0 and 0.3ml of hydrogen peroxide solution (3%) in a test tube, then 0.2ml of extract containing enzyme (supernatant) was added .The blank was composed from: 2.3 ml of phosphate buffer pH 7.0 and 0.2 ml of extract containing enzyme (supernatant). After 1min the absorbance was measured at wave length of 240 nm using UV-Vis spectrophotometer for activity test and blank tubes .The activity of catalase was calculated as shown below:

$$\text{Enzyme activity (unit/ml)}=\frac{\Delta Ab(Abt-Abb)\times dilution factor(DF)\times Reaction\ volume(V)}{Extinction\ Cofficient(EC)\times \text{Volume of enzyme(EV)}}$$

Abt = absorbance of test tube/min
Abb = absorbance of blank tube/min
DF= dilution of supernatant
V= Volume of reaction
EC= 40
EV= Volume of enzyme (0.2 ml).

Peroxidase activity (POD)

Determination of peroxidase activity (POD)

1.5 ml of hydrogen peroxide solution (1.7mM) was mixed with 1.5ml of 4-Aminoantipyrine reagent (2.5mM), then 0.1ml of extract containing enzyme (supernatant) was added to the absorbance which measured at wave length of 510 nm using UV-Vis spectrophotometer. The activity of peroxidase was calculated as shown in below equation:

$$\text{Enzyme activity (unit/ml)} =\frac{\Delta A/min\times RV\times D}{6.58\times EV}$$

Where:
ΔA/min = The change in absorbance at 510nm/minute

RV= Total volume of reaction mixture (3ml)
D = Enzyme dilution factor
6.58 = Extinction coefficient
EV = Volume of enzyme sample (0.1ml) [17, 18].

Result and discussion

Effect of lead on proline content of leaves of two *R. sativus* and *L. sativus* under field condition . Results presented in Figure-1 exhibited that average of proline content was for increased by lead concentration. Exposure of plants to 50ppm water stress led to increased proline content of leaves by 29 and 54 % after 30 days after sowing, respectively. Average of proline content was different among lead concentration treatment. The highest proline content (81and 96 µmole/g) were recorded by control treatment after 30days , while treatments other recorded the lowest proline content (11.00 and 16.00 µmole/g),after 30 days *R. sativus* and *L. sativus*, respectively. However, normal and with adding lead concentration, differential response in terms of leaves proline content has been observed. Minimum proline content (11.00µmole/g) was found in *R. sativus* after 30 days.

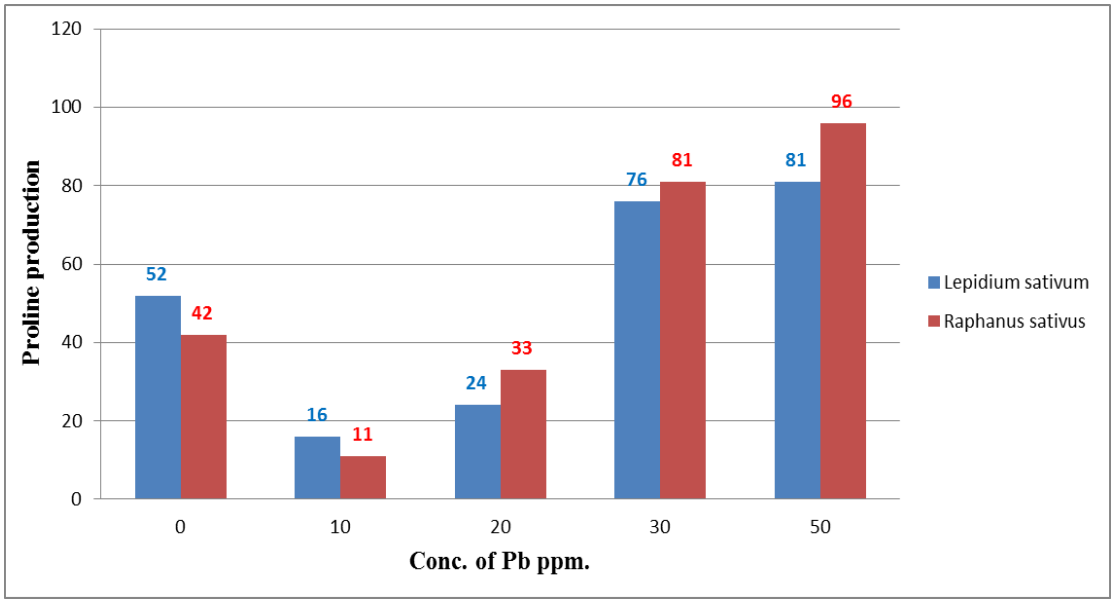

Figure 1- Proline concentration. (µmoles/g) in leaves tissue after 30 days from add different of lead concentration**.**

Effect of lead on Catalase enzymes activity of leaves.

Figure-2 that, showed a decrease in the Catalase production under control (47.01 and 86.18 unit/ml) after 30 days, respectively. Maximum results were found in control treatments (56.25 and 56.52 unit/ml) after 30 days, respectively. Also, there were significantly increases the Catalase content in control treatment (without lead) interaction with lead concentration after 30 days. Although this minimum was much less that (16.87 unit/ml) when *R. sativus* and *L. sativus* treated with lead concentration after 30 days, respectively .Data presented in Figure-2 show the effect lead concentration stress on Catalase activity after 30days of *R. sativus* and *L. sativus* plants grown field capacity. Results presented exhibited that average of Catalase activity was for increased by water deficit stress. Exposure of plants to 50 ppm lead led to increased Catalase activity in leaves by (39.65 and 39.38%) after 30 days after *R. sativus* and *L. sativus*, respectively.

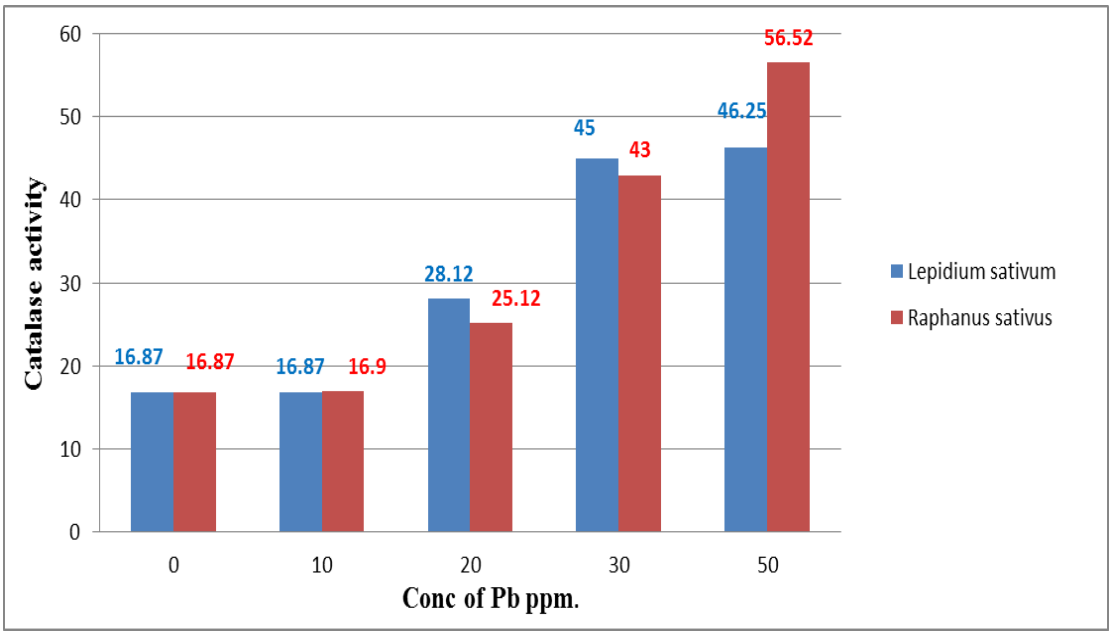

Figure 2- Catalase enzymes activity (unit/ml) in leaves tissue after 30 days from add different of lead concentration**.**

Effect of lead on peroxidase enzymes activity of leaves.

The peroxidase activity Figure-3 at 30 days maximum average increase by (21.47% and 46.09 %) in peroxidase activity *R. sativus* and *L. sativus*, respectively. The activity of this antioxidant enzyme again increased within lead concentration treatments. The maximum average increase in peroxidase activity (43.76 and 68.38 unit/ml) were though control level treatment, while minimum (24.07 and 27.35 unit/ml) were recorded in lead concentration treatments after 30 days, respectively .

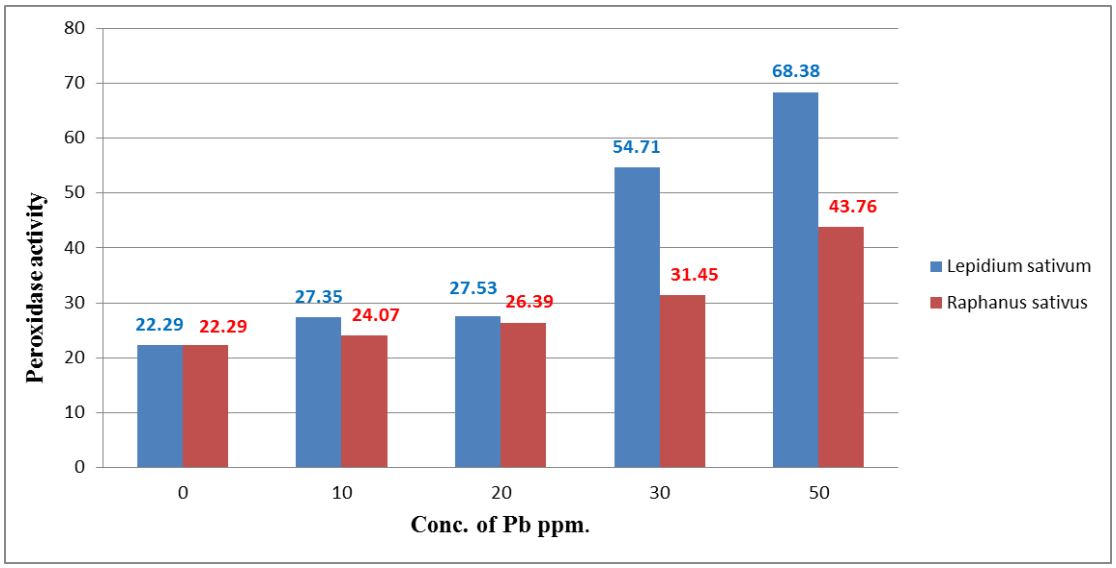

Figure 3- Peroxidase enzymes activity (unit/ml) in leaves tissue after 30days from add different of lead concentration**.**

Peroxidase are widely distributed in living organisms including microorganisms, plants and animals. POD is mainly located in the cell wall [19] and it is one of the key enzymes controlling plant growth and development. It takes place in various cellular processes including construction, rigidification and eventual lignifications of cell walls [20].Removal of two major systems for the enzymatic removal of H_2O_2 and peroxidative damage of cell H_2O_2 is therefore a protective mechanism for the preservation of biological membranes when lead and other metals accumulate in the symplast of the cell [21], proline is probably the most widespread, and is considered to be an indicator of

environmental stress [22]. Accumulation of proline has been shown to protect plants against damage by ROS (Reactive oxygen species) , acting as a very effective singlet-oxygen quencher, binding to redox-active metal ions and also activating and protecting enzymes such catalase, peroxidase and polyphenol oxidase [23]. POD and Catalase are walls is controlled by the potency of antioxidative peroxidase enzyme system [24]. However, the role that peroxidase plays in metabolism is not clear because of the large number of reactions it catalyzes and the considerable number of isoenzymic species [25].It was reported that peroxidase had been used for biotransformation of organic molecules [26-28]. Because of its broader catalytic activity, a wide range of chemicals can be modified using POD. Also, it can be used for the applications such as synthesis of various aromatic compounds, removal of phenolic from waste waters and the removal of peroxides from foodstuffs, beverages and industrial wastes [29]. POD is also related to quality of plant commodities, particularly the flavor, in both raw and processed foods. POD activity is also correlated to fruit ripening as shown in a number of cases and it is also involved in enzymatic browning, either or together with polyphenol oxidase activity. A more precise understanding of the implication of POD in these mechanisms is an essential step towards a more efficient control of these undesirable reactions, particularly in heat-processed products, which frequently contain residual peroxidase activity [30-31]. Adaptation of plants to grow in different habitats requires specific abilities that differ among plant [32] and reduces plant performance through damaging plant parts and cell components such as cell membranes, proteins, lipids, pigments and DNA expression [33, 34].Plants in order to accommodate more tolerance against environmental stresses have developed efficient physiological and biochemical enzymatic response mechanisms such as production of superoxide dismutase, Catalase and peroxidase, and non-enzymatic antioxidant compounds such as phenolic compounds and flavonoids to rid themselves of free radicals [35-42].Thirty four species of *Artemisia* (with English names worm wood and sage brush) are the main and most common prennial species in steppic and semi-steppic ecosystems of Iran . Due to their distinctive features, *Artemisia* plants are highly resistant against extreme environmental conditions and very effective in stabilizing the habitat; have great forage value, are medicinal and exhibit strong antioxidant property via their phenolic compounds and have conservation and aesthetic values .Also both mechanism CAT and POD breakdown free radical release water and oxygen, proline of accumulation in oxidative stress [43, 44] .

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