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The effect of rhizosphere growth promoter bacteria on enzymes activities of *Hordeum vulgare* and *Brassica napus* plants in polluted soil with Pb

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Abstract

Soils and accumulated materials are resources of heavy metals, which are available for absorbance by plants. One of the potential methods for eliminating heavy metals from polluted places includes usage of resistance creature to metals which are able to accumulate and absorb high amount of material. Present study was carried out in a greenhouse format in completely accidental plots with 4 times repetition and 2 plants of *Hordeum vulgare* and *Brassica napus*. This experiment was done in 2011 in Islamic Azad University, Karaj branch. Variance analysis results showed significant effect of pb levels, growth promoters bacteria and interaction effects of them on capabilities of Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPX) enzymes ($P<0.01$).

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Introduction

Soils are the sources of heavy metals which are available for plants to absorb them. To resurrect polluted environments, some solutions are designed that include chemical and physical methods or biological techniques which need interference of biological factors (Gianfreda and Rao, 2004; Boularbah *et al*., 2006). Refining of polluted soils to heavy metals is a necessary and inevitable issue. Among different heavy metals, Pb is considered as a polluted material which accumulates rapidly in soils (Sharma and Dubey, 2005) and is one of the heavy metals that has lots of disadvantages for human being (Lin *et al*., 2009; Estrella – Gomez *et al*., 2009; Brunet *et al*., 2008).

Antioxidant enzymes are considered as defendant systems for plants to light against oxidize stress that is result of metals (Ali *et al*., 2003). Anti oxidant system includes some enzymes such as (SOD), (CAT) and (GPOD).

Produced super oxide radicals have transformed to H_2O_2 by SOD function and Ascorbate peroxidase (APX), CAT, G-POD, GPX enzymes activity prevent from H_2O_2 accumulation; therefore balance between production of ROS and its elimination guarantees system's survival (Khatun *et al*., 2008). These enzymes cooperate in a set of biological processes that are require for grow and development (Gaetke and Chow, 2003), and preserve animals from oxidation damages (Garnczarska and Ratajczak, 2000).

Rhizosphere bacteria that have settled selectively in one of the three fold sections of rhizosphere, have negative impacts on plants' function. These bacteria are called Deleterious Rhizobacteria (DRB). Harmful effects of this group of bacteria are the result of metabolites accumulation such as aliphatic, phenolic acid, sulfuric acid and mostly Hydrocyanic acid (Saleh Rastin, 2002). Consequently those risobacteria that lead to enhancement of plants' growth and improvement of agronomical plants' functions are called Plant Growth Promoters Rhizobacteria (PGPR) (Vessey *et al*., 2003).

The aim of this study is effect of Pb heavy metal and growth promoter bacteria on activities of *Hordeum vulgare* and *Brassica napus* antioxidant enzymes.

Method and materials

Present study was carried out as a greenhouse study in 2011 in Islamic Azad University, Karaj branch. The geographical coordinate of study location were 35° and 55// latitude, 50° and 54// (longitude) and 1313 m height from sea level. The relative moisture of greenhouse 57% and the lowest temperature was 15.5°c and the highest temperature was 30°c. The experimental soil was loamy and sandy with pH = 7.6, its conductivity was 2.9 $ds.m^{-1}$ and pb amount was 2 $mg.kg^{-1}$ of soil. Preferable Pb concentration was provided regarding capacity of 5 kg plots, and 2 months before cultivation plots' soils become polluted with Pb heavy metal by spray.

This experiment was in factorial and completely accidental format with 4 times repetition. This experiment included 40 treatments in 160 experimental plots with 2 plants of *Hordeum vulgare* and *Brassica napus*.

Experimental factors included

Growth promoter bacteria in 5 levels:

1st level: control (without insemination of bacteria), 2nd level: insemination with *Azosprillium crococum* bacteria, 3rd level: insemination with *Azotobacter*, 4th level: insemination with *P. putida*, 5th level: insemination with *Azosprillum crococum*, *Azotobacter*, *P. putida* bacteria; and second factor included Pb levels in 4 level (0, 250, 500 and 750 mg/kg).

Seeds were disinfected with sodium hypochlorite (1.5%), and sterilized with water. Growth promoter bacteria were used in mixing the seed format. Bacteria were provided from microbe bank of soil's biological study section of Karaj soil and water research institute.

7 seeds of *Brassica napus* and 20 seeds of *Hordeum vulgare* were used which 10 mil/lit bacteria

suspension was added to each seed (each mil lit of inoculum had 107 live and active cells of each type of bacteria).

Measurement of activity amount of SOD enzyme

Some young leaves were separated and put in an icebox which its bottom was covered with ice then they were transferred to laboratory. Then activity amount of SOD enzymes was measured by Misra and Fridorich (1979) method.

Measurement of GPX activity amount

Transferred leaves were washed by distilled water. Immediately after this phase they were entered to phosphate buffer 0.16 m and its pH was 7.2 then they were crushed and homogenized). Then in presence of similar volume of that buffer included Digitonin and digestive enzymes, digestive process of cell walls was done. Finally, 0.5 mlit of homogeneous solution was used to measure protein in terms of mgr/milt. Then in the rest of extracted solution activity amount of GPX enzymes was measured by Paglia and Valentine (1987) method.

Measurement of CAT activity amount

By means of Paglia and Valentine (1987) math CAT changes amount was determined.

Variance analysis was done with SAS software. In addition to compare means, Duncan test was used. Moreover, graphs were drawn by Excel software.

Results

SOD enzyme's activity of Hordeum vulgare

The results of variance analysis (table 1) show that there is a significant effect of Pb levels, growth promoter bacteria and their interaction effect on SOD enzyme's capability (P<0.01). The mixture of growth promoter bacteria and Pb leads to significant increase of SOD amount.

Table 1. Result of variance analysis on characteristics measured of *Hordeum vulgare*.

		MS		
S.O.V	d.f	SOD	CAT	GPX
A (growth promoter bacteria)	4	1371478/8**	93716**	13006/62**
B (Pb level)	3	740425/7**	61951/4**	8326/8**
A*B (Interaction)	12	54107/44**	2706/1**	509/48**
Error	57			
c.v.		3/62	4/59	3/97

Note: * and ** indicate significant difference at 5% and 1% probability level, respectively ns is not significant.

According to figure 1, it is observed that the treatment of mixture of growth promoter bacteria lead to enhancement of SOD amount. Actually, in this relation, when Pb amount of soil is lower the effect of bacteria is more significant. When we had mixture of bacteria along with 750 mgr/kg Pb, 804.5 SOD was secreted in the plant, while in the same level of bacteria along with 250 and 500 mg/kg Pb the amount of SOD enhanced to 1436 and 1236 u/mg. pr.

SOD's activity in Brassica napus

The results of variance analysis (table 2) show that there is a significant effect of Pb levels, growth promoter bacteria and their interaction effect on SOD enzyme's capability (P<0.01). The mixture of growth promoter bacteria and Pb leads to significant increase of SOD amount.

According to figure 2, it is observed that the treatment of mixture of growth promoter bacteria lead to enhancement of SOD amount. Actually, in this relation, when Pb amount of soil is lower the effect of bacteria is more significant. When we had mixture of bacteria along with 750 mgr/kg Pb, 1565.5 SOD was secreted in the plant, while in the same level of bacteria along with 250 and 500 mg/kg Pb the amount of SOD enhanced to 2110.25 and 1843.5 u/mg. pr.

CAT activity in Hordeum vulgare

Results of variance analysis (table 1) indicated that there is a significant effect of Pb levels, growth promoter bacteria and their interaction effect on CAT's capacity in *Hordeum vulgare* (P<0.01). Figure 3 shows the changes in CAT's amount in different levels of Pb and bacteria. As it is observable, by increasing Pb concentration in soil, CAT's amount decreases and by using growth promoter bacteria, this amount increases in comparison with control. Application of growth promoter bacteria along with Pb element leads to enhancement of CAT's amount. This procedure is the same as what was happened for SOD activity in *Hordeum vulgare* and *Brassica napus*.

Table 2. Result of variance analysis on characteristics measured of *Brassica napus*.

		MS		
S.O.V	d.f	SOD	CAT	GPX
A (growth promoter bacteria)	4	2305628/5**	32253/03**	17774/9**
B (Pb level)	3	1278124/03**	19320/71**	8405/6**
A*B (Interaction)	12	27579/76**	684/3**	635**
Error	57			
c.v.		3/44	3/92	6/93

Note: * and ** indicate significant difference at 5% and 1% probability level, respectively ns is not significant. In soil contains mixture of growth promoter bacteria in the same level of Pb enzyme's activity will be equal to 123.7 u/mg.pr. 250 mg of Pb lonely can lead to 56.9 u/mg.pr activity in plant. If 250 mg/kg of Pb uses along with growth promoter bacteria, enzyme's activity will reach to 149.82 u/mg.pr (Figure 5).

According to figure 3, it is observed that mixture treatment of growth promoter bacteria leads to enhancement of CAT's amount. But in this relation when Pb amount was lower, the effect of bacteria is more significant. When mixture of bacteria was used along with 750 mg/kg Pb 291 u/mg.pr CAT was secreted, while in the same level of bacteria along with 250 and 500 mg/kg of Pb. CAT's amount enhanced to 423.25 and 394 u/mg.pr orderly, and in when Pb wasn't applied in the soil, this amount reached to 422.5 mg protein per plant.

CAT activity in Brassica napus

Results of variance analysis (table 2) indicated that there is a significant effect of Pb levels, growth promoter bacteria and their interaction effect on CAT's capacity in *Hordeum vulgare* (P<0.01).

According to figure 4, it is observed that mixture treatment of growth promoter bacteria leads to enhancement of CAT's amount. But in this relation when Pb amount was lower, the effect of bacteria is more significant. When mixture of bacteria was used along with 750 mg/kg Pb 296 u/mg.pr CAT was secreted, while in the same level of bacteria along with 250 and 500 mg/kg of Pb. CAT's amount enhanced to 333.5 and 337 u/mg.pr orderly, and when Pb wasn't applied in the soil, this amount reached to 338.75 mg protein per plant.

GPX's activity in Hordeum vulgare

The results of variance analysis showed significant effect of both Pb and bacteria factors and their interaction effect on the activity of GPX (P<0.01) (Table 1).

Figure 5 showed that by increasing Pb concentration, secretion of this enzymes decreases, in contrast by application of growth promoter bacteria GPX's activity increasing. In concentration of 750 mg Pb in soil and control treatment of bacteria, enzymes activity was equal to 78.62 (u/mg.pr). If soil contains mixture of growth promoter bacteria in the same level of Pb, enzymes' activity reaches to 126.02 u/mg.pr.

250 mg of Pb lonely leads to 92.17 u/mg.pr activity in plant. 250 mg/kg of Pb along with application of growth promoter bacteria, enzyme's activity leads to 172.2 (u/mg.pr).

GPX's activity in Brassica napus

The results of variance analysis showed significant effect of both Pb and bacteria factors and their interaction effect on the activity of GPX ($P<0.01$) (Table 2).

Discussion

Under stress condition, active O_2 increases in plant. In such condition, plant uses different mechanism for eliminating different types of active O_2. On the basis of this fact, SOD's activity under Pb and bacteria treatments was considered in 2 plants of *Hordeum vulgare* and *Brassica napus*.

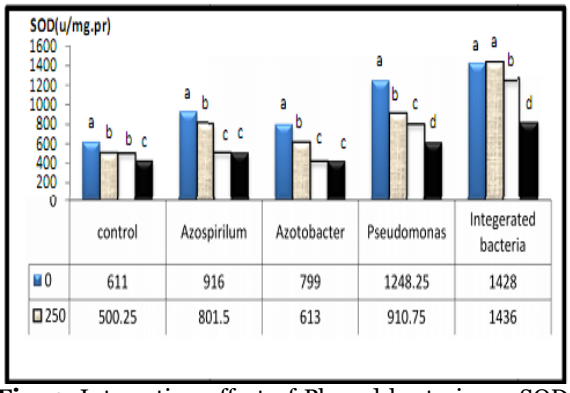

Fig. 1. Interaction effect of Pb and bacteria on SOD activity in *Hordeum vulgare*.

SOD catalyzes reaction of transformation O_2^- to H_2O_2 and O_2 (Lopez *et al.*, 2005). , between these 2 plants showed that *Brassica napus* secreted more SOD's activity in presence of growth promoter bacteria then *Hordeum vulgare*. In present study, by increasing Pb heavy metal in growth environment, activity of SOD, which transforms O_2 to H_2O_2, will decrease.

In maximum amount of Pb in soil, released of SOD in *Brassica napus* was 1028.8 (u/mg.pr) and in control treatment this amount reached to 1615.3 u/mg.pr this amount is 61% more than enzymes' activities of *Hordeum vulgare*. SOD's activity in *Hordeum vulgare* was in medium amount. It seems that activity of SOD is the response to harmful effects of produced oxygen's that are results of application of growth promoter bacteria. The highest amount of activity of these enzymes was related to the lowest concentration of Pb in the soil. Totally, enhancement of Pb concentration in soil of both plant's types led to reduction of SOD's activity. Sharma and Dubey (2005) and Garnczarska and Ratajczak (2000) reported that SOD's activity will increase under the poison level of Pb. Enhancement of SOD and CAT's activity in *Brassica napus* in comparison with *Hordeum vulgare* indicated that enzymes respond to produced free-radicals in plants under the poison level of Pb, because *Brassica napus* absorbed more Pb in its root and aero organs. Therefore, it seems that higher productions and activity of these enzymes is considered as a defendant mechanism against this element which is attributed to resistance and strategy of plant. Probably these enzymes because digesting more O_2^- than *Hordeum vulgare*, and growth promoter bacteria which are effective in absorbing more heavy elements in the root, are also effective in enhancement of enzymes' amount under different level of Pb.

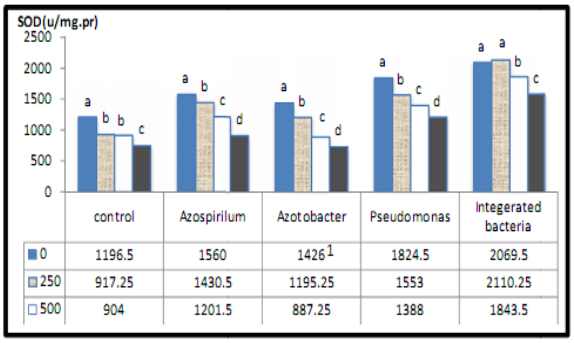

Fig. 2. Interaction effect of Pb and bacteria on SOD activity in *Brassica napus CAT activity in Hordeum vulgare*.

CAT is on enzyme which will be active under oxide stress. This enzyme is enable to digested eliminate H_2O_2 (Khatun *et al.*, 200). A comparison between CAT's activity under poison amount of Pb, growth promoter bacteria treatment indicated that both above treatments had significant increase than control treatment. Although both treatment increased CAT's activity this enhancement wasn't equal for both plant's types. In *Brassica napus* CAT's activity was more than *Hordeum vulgare*.

Hordeum vulgare had the lowest amount of released enzymes. Enhancement of Pb concentration also led to reduction of CAT's activity in both plant's types. CAT had 30% reduction in 750 mg/kg soil comparison with control. H_2O_2 which is poison product of SOD's yield is used or transformed by CAT (Garnczarska and Ratajczak, 2000).

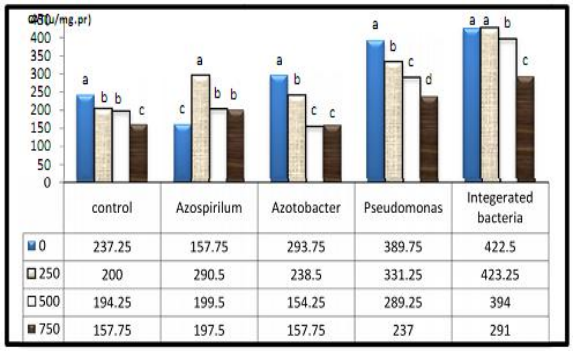

Fig. 3. Interaction effect of Pb and bacteria on CAT's activity in *Hordeum vulgare*.

GPX preserves lipid membrane against oxide damages (Mashhadi Akbar Boojar and Goodarzi, 2007). Enhancement of enzymes activity under bacteria treatment indicates that probably activities of CAT and GPX prevent accumulation of H_2O_2, since it transformed O_2^- to H_2O_2 by SOD yields and transformed O_2^- poisoning effects to H_2O and O_2.

Therefore, by making a balance between ROS production and eliminating them, systems survival is guaranteed, since ROS is poisoning and should separated by enzymes activity, enhancement of GPX activity had an important role in reduction of H_2O_2 through GSH activity (Khatun *et al*., 2008). It seems that synthesis of Glutathione was similar in both plants' types.

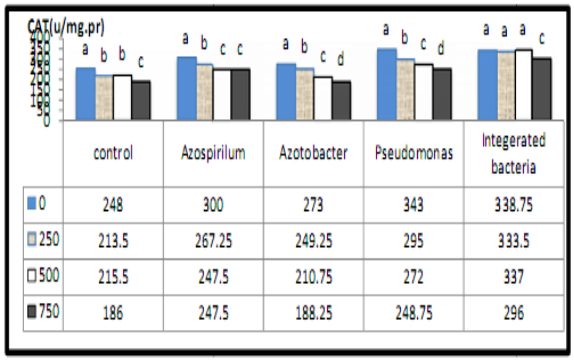

Fig. 4. Interaction effect of Pb and bacteria on CAT's activity in *Brassica napus GPX's activity in Hordeum vulgare*

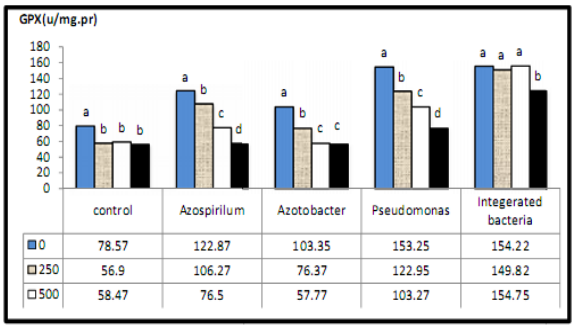

Fig. 5. Interaction effect of Pb and bacteria on GPX activity in *Brassica napus*

Comparison between control plants and those which are grown in Cu environment shows that enhancement of GPX enzymes is the results of increasing concentration of Ca. As it was mentioned, antioxidant enzymes always haven't enhancement under oxidant stress of heavy metals, and they show different reaction due to different plant's types, since antioxidant components that are existed in plants' oils have different yield and their mechanism mostly is related to mixture and condition of experimental system (Wong *et al*., 2005).

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