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# Effects of PGPR and mycorrhiza on morphological traits and yield components of wheat under different lead levels

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**Abstract**

The problems associated with heavy metal Pollution are widespread and are particular ordinary in more developed countries. Phytoremediation is a non-destructive method of heavy metal-polluted soils. Although the numerous issues of this technology have not been widespread surveyed yet. To evaluate the effect of Plant Growth Promoting Rhizobacteria (PGPR) and mycorrhiza on seed weight, yield components and morphological traits of wheat, an experiment was done during 2012-2013 in Islamic Azad University, Karaj Branch, as factorial based on completely randomized design with 4 replications. The lead amounts in 4 levels (0 , 300, 600 and 900 mg/kg of soil), PGPR (Azosperillium, Azotobacter, Pseudomonas) in 2 levels (Application and non application) and mycorrhiza in 2 levels (Application and non application) were considered. The lead concentration decrease was noticed in all the growth parameters as the maximum anti stress efficiency was obtained from PGPR consumption for wheat height (45.93 cm), spike length (7.63 cm), spike weight (14.86 g), spikelet number in spike (12.82), seed number in spike (37.14) and seed weight in wheat (1.27 g). The maximum reduction (% of control) was noticed under the influence of higher dose of Pb (900 mg /kg) and non application of PGPR and mycorrhiza.

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## Introduction

Heavy metal toxicity and the risk of their residual in the soil and as a result in food chain is one of the main environmental and health difficulties of our modern population. Introductory sources of pollution is from the burning of fossil fuels, mining and melting of metallic ferrous ores, urban wastes, fertilizers, herbicides, and wastewater (Peng *et al.*, 2006; Xiong, 1998).

Industrial waste as the most important types of different kinds of metal pollution in water resources. Cd, Zn, Ni and Pb are the most main toxic heavy metals which find their way to the live organs through waste waters (Ajmal *et al.*, 1998). Because of their non- biodegradability and durability, heavy metals can store in food chains via plants and so may create a threat to hazard for human safety (Bakkaloglu *et al.*, 1998). Now, the most researches are purposed at finding means of separate these toxicity heavy metals from the soil, and so different ways have been proposed that contain both *ex-situ* and *in-situ* procedures. The *ex-situ* procedures need the removal of contaminated soil for treatment on or off site, and next return of the treated soil to the main site. So the common *ex-situ* procedures rely on excavating, detoxifying and destroying the Contaminants factor by physical or chemical methods in order to consistency, concentration, collection or completely destroy the pollutants (Ghosh & Singh, 2005). Lead toxicity is a recognized difficulty. Many researches indicate that its toxicity depends on chemical structure, the rout of its administration, and concentration, time and intensity of exposure. (Kulikowska *et al.*, 1994). Lead at 500 ppm in soil (or) solid waste considered the substance as "hazardous waste". Maximum permissible amount of lead in drinking water is 0.05 ppm. Sunflower maybe have high resistance and should be able of uptaking high amounts of lead (Usha *et al.*, 2011). Sharma and Dubey (2005) stated that, roots can uptake considerable amounts of lead whilst greatly restricting. Lead movement to shoot parts as observed in sunflower and sorghum. So, it can be proposed that Chinese cabbage was more efficiently at Pb extraction in the above ground parts than sunflower and sorghum. Therefore it appears that there is a good characteristic of Chinese cabbage, most investigators proposed that suitable plants for phytoextraction should accumulate heavy metals only in the roots (Prasad & Freitas, 2003). Tyksiński (2002) stated that because of biomass decrease of plants growing against heavy metals, polluted regions may also included in any disorders in the uptake, Transmission, and assimilation of macro and micronutrients. Root is one of the most important parts of plant and the ability of root system to uptake heavy metals has been intended as key point for promoting phytoremediation efficiency (Keller *et al.*, 2003). Better root growth would be privilege for phytoextraction because more roots can uptake metal (Liphadzi *et al.* 2006). due to evaluate the effects of PGPR and mycorrhiza on morphological traits and yield components of wheat under different lead levels this research was done.

## Materials and methods

Green house pot culture experiments were conducted during 2012-2013 in Islamic Azad University, Karaj Branch to study the effect of heavy metal Lead, PGPR and mycorrhiza on morphological traits and yield components of wheat (Bahar variety) under different lead levels. The experiment was done as factorial based on completely randomized design with 4 replications. The lead amounts in 4 levels (0, 300, 600 and 900 mg/kg of soil), PGPR (Azosperillium, Azotobacter, Pseudomonas) in 2 levels (Application and non application) and mycorrhiza (Mix variety) in 2 levels (Application and non application) were considered. The ratio 3:1:1 of sand, clay and manure fertilizer were used as media. Wheat seeds were obtained from Seed and Plant Improvement Institute, Karaj, Iran. Seeds were poured in 3% (v/v) of formaldehyde for 3 minutes and washed with distilled water for 4 times to avoid fungal infection. The seeds were sown directly in 7 kg soil capacity pots. To avoid loss of nutrients and trace elements out of the pots, plastic plate were placed under each pot and the collected leaches were put back to experimental pots. The plant samples were dried in an oven at 75$^{oC}$ for 2 days, and the dry mass was determined. After the experiment were completed, the plant height, spike length, spike weight, spiklet number in spike, seed number and seed weight in plant determined using 10 samples. All data were analyzed using the SAS software for Windows Standard Version, and differences between individual means were tested using the Duncan Multiple range test ($p < 0.05$).

## Results

The effect of different lead concentration, PGPR and mycorrhiza on morphological traits and yield components of seed weight in wheat is presented in Table 1. Doses of Pb were found to have inhibitory effects on mentioned traits but exposure in lead concentration to 900 mg Pb/kg caused a significant decrease in plant height (35.05 cm), spike length (6.45 cm), spike weight (10.19 g), spiklet number in spike (10.49), seed number in spike (26.68) and seed weight in plant (0.97 g). The simple effect of different lead concentration indicated that they were located in different statistical groups.

**Table 1.** Analysis of variance for experimental characters.

| S.O.V. | d. f. | Mean of Square (MS) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Plant height | Spike length | Spike weight | Spiklet number in spike | Seed number in spike | Seed weight in plant |
| Lead | 1 | 826.42** | 10.08** | 3.61** | 42.52** | 673.41** | 0.53 ** |
| PGPR | 1 | 632.96** | 4.12** | 0.90** | 10.80** | 360.05** | 0.44** |
| Mycorrhiza | 1 | 256.04** | 1.30* | 0.29 n.s | 3.85* | 144.0** | 0.11* |
| PGPR × Lead | 3 | 8.18 n.s | 0.05 n.s | 0.03 n.s | 0.005 n.s | 2.81 n.s | 0.005 n.s |
| Mycorrhiza × | 3 | 0.93 n.s | 0.01 n.s | 0.01 n.s | 0.02 n.s | 0.72 n.s | 0.008 n.s |
| Mycorrhiza × | 1 | 22.66 n.s | 0.04 n.s | 0.03 n.s | 0.78 n.s | 27.3 n.s | 0.007 n.s |
| Mycorrhiza × PGPR × Lead | 3 | 0.47 n.s | 0.004 n.s | 0.002 n.s | 0.009 n.s | 0.58 n.s | 0.004 n.s |
| Error | 48 | 22.54 | 0.29 | 0.08 | 0.67 | 12.04 | 0.02 |
| CV (%) | 30 | 11.09 | 7.32 | 8.11 | 6.59 | 9.91 | 12.52 |

**Table 2.** Simple effect of Lead, PGPR and Mycorrhiza on some morphological traits and yield components of wheat.

| Factors | Lead (mg.kg-1) | Plant height (cm) | Spike length (cm) | Spike weight (g) | Spiklet number in spike | Seed number in spike | Seed weight in plant (g) |
|---|---|---|---|---|---|---|---|
| Lead | 0 | 52.08a | 8.33a | 18.52a | 14.52a | 42.14a | 1.40a |
| | 300 | 43. 95b | 7.61b | 14.93b | 13.13b | 36.88b | 1.26b |
| | 600 | 40.06c | 7.11c | 12.08c | 11.78c | 33.36c | 1.11c |
| | 900 | 35.05d | 6.45d | 10.19d | 10.49d | 26.68d | 0.97d |
| PGPR | Non application | 39.64b | 7.12b | 13.00b | 12.00b | 32.39b | 1.10b |
| | application | 45.93a | 7.63a | 14.86a | 12.82a | 37.14a | 1.27a |
| Mycorrhiza | Non application | 40.78b | 7.23b | 13.40a | 12.17b | 33.26b | 1.14b |
| | application | 44.78a | 7.52a | 14.46a | 12.66a | 36.26a | 1.23a |

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test at 5%.

The simple effects of PGPR on morphological traits were noticed that after its consumption during growth stage, all studied characters recovered, so the plant height (45.93 cm), spike length (7.63 cm), spike weight (14.86 g), spiklet number in spike (12.82), seed number in spike (37.14) and seed weight in plant (1.27 g). Among yield components, PGPR application was more effective on spike weight than mycorrhiza (Table 2). Simple effect of mycorrhiza on experimental traits showed that instead of spike weight in other cases was observed significant changes. In this case the highest plant height (44.78 cm), spike length (7.52 cm), spike weight (14.46 g), spiklet number in spike (12.66), seed number in spike (36.26) and seed weight in plant (1.23 g) were gained. Double interaction of lead and PGPR had significant effect on experimental traits. All double and triple interaction effects were not significant on morphological traits and yield components of wheat, although in this condition, the maximum reduction of seed weight for wheat was noticed in Pb 900 mg Pb/kg and non application of PGPR (0.91 g) and lack of mycorrhiza (0.94 g) (Table 3 and 4). The fertilization character which involves via spiklet number in spike and seed number in spike was noticed that in sever lead stress (900 mg Pb/kg), PGPR using can prevent 19% and 7.5% significantly reduction in seed number in spike and spiklet number in spike alternatively (Table 3).

**Table 3.** Mean comparison of Lead and PGPR on some morphological traits and yield components of wheat.

| PGPR | Lead (mg.kg$^{-1}$) | Plant height (cm) | Spike lenght (cm) | Spike weight (g) | Spiklet number in spike | Seed number in spike | Seed weight in plant (g) |
|---|---|---|---|---|---|---|---|
| Non application | 0 | 48.00a | 8.10a | 17.02a | 13.81a | 39.45a | 1.29a |
| | 300 | 41.42a | 7.36a | 13.71a | 12.73a | 34.81a | 1.19a |
| | 600 | 37.41a | 6.85a | 11.52a | 11.38a | 31.41a | 1.03a |
| | 900 | 31.72a | 6.17a | 9.74a | 10.08a | 23.91a | 0.91a |
| application | 0 | 56.15a | 8.56a | 20.03a | 14.68a | 44.83a | 1.51a |
| | 300 | 46.47a | 7.85a | 16.15a | 13.53a | 38.96a | 1.33a |
| | 600 | 42.71a | 7.37a | 12.65a | 12.18a | 35.31a | 1.20a |
| | 900 | 38.38a | 6.73a | 10.64a | 10.90a | 29.45a | 1.04a |

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test at 5%.

**Table 4.** Mean comparison of Lead and Mycorhiza on some morphological traits and yield components of wheat.

| Mycorhiza | Lead (mg.kg$^{-1}$) | Plant height (cm) | Spike length (cm) | Spike weight (g) | Spiklet number in spike | Seed number in spike | Seed weight in plant (g) |
|---|---|---|---|---|---|---|---|
| Non application | 0 | 49.84a | 8.23a | 17.54a | 14.00a | 40.75a | 1.32a |
| | 300 | 42.29a | 7.44a | 14.38a | 12.86a | 35.15a | 1.22a |
| | 600 | 38.01a | 6.96a | 11.73a | 11.52a | 32.10a | 1.09a |
| | 900 | 33.00a | 6.30a | 9.94a | 10.30a | 25.07a | 0.94a |
| application | 0 | 54.31a | 8.43a | 19.51a | 14.50a | 43.53a | 1.47a |
| | 300 | 45.61a | 7.77a | 15.47a | 13.41a | 38.62a | 1.29a |
| | 600 | 42.11 | 7.26a | 12.44a | 12.05a | 34.62a | 1.14a |
| | 900 | 37.10a | 6.60a | 10.44a | 10.68a | 28.28a | 1.00a |

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test 5%.

## Discussion

To achieve a sufficiently high amount of the shoot parts of wheat used in the process is the basis of induced phytoremediation efficiency. This research findings indicated a significant reduction in seed in spiklet (18.81%) against 900 mg Pb/g without PGPR consumption, as compared to the application (Table 2), which is consistent with several references reports. Based on Gruca- Krolikowska & Wacławski (2006), the main reason for the decrease in productivity of plants growing on heavy metal polluted areas is a considerable decline in the photosynthesis efficiency due to disorder of chlorophyll biosynthesis. In researches can be some verification of this idea, because there was a considerable enhancement in the wheat height ranging from 31.72 cm to 38.38 cm in PGPR application and 54.31 cm to 37.10 cm in mycorrhiza consumption in different lead levels. Sharma and Dubey (2005) showed that surplus lead amounts gained a number of toxicity signs in plants such as increase in growth, chlorosis and blackening of the root sections, this comply with the observations of this study. Consequently, Pb in the soil has been shown to be able to complex other plant elements such as phosphorus, so explanation them both access for absorption (Xie *et al.,* 2006). Wheat length, spike length and weight and seed weight in plant are important traits which are influencing nutrients uptake. In this study, acceptable increases of morphological traits and seed weight per plant were observed after PGPR and mycorrhiza consumption**.** It seems that this leads anti stresses factors stimulate IAA biosynthesis and this phytohormon has an effective duty in growing root section, which may be the key point to enhancement root dry matter and promotion lead absorption as well as transportation from roots to shoots, and as a result, increasing the phytoextraction Performance of lead. Huang and Cunningham (1996) indicated that 20 $\mu$M Pb significantly inhibited Ca, Mg, and Fe accumulation in shoot parts of maize and ragweed.

**Table 5.** Mean comparison of PGPR and Mycorhiza on some morphological traits and yield components of wheat.

| Mycorhiza | PGPR | Plant height (cm) | Spike lenght (cm) | Spike weight (g) | Spiklet number in spike | Seed number in spike | Seed weight in plant (g) |
|---|---|---|---|---|---|---|---|
| Non application | Non application | 37.04a | 6.95a | 12.30a | 11.65a | 30.24a | 1.05a |
| | application | 45.22a | 7.51a | 14.49a | 12.69a | 36.29a | 1.24a |
| application | Non application | 42.23a | 7.29a | 13.69a | 12.36a | 34.55a | 1.15a |
| | application | 47.33a | 7.74a | 15.24a | 12.96a | 37.98a | 1.30a |

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test at 5%.

## Conclusion

Our finding indicated that PGPR and mycorrhiza consumption by stimulating IAA could be considered as a practical method for phytoextraction of lead polluted soils. It have been found that application of this heavy metals anti stress microorganisms at non, moderate and high concentrations can effectively increase wheat tolerance to this heavy metal and prevent increasing yield and yield components of wheat.

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