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The Influence of Humic Acid and Nano-superabsorbent Application on the Growth of *Brassica Napus L.* in Lead-contaminated Soil

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Abstract. Toxic heavy metals are continuously released into the environment. Mining and industries can result in heavy metal contamination of urban and agricultural soil. Lead, because of its great variety of applications, is a common environmental contaminant widely distributed around the world. For the purpose of improving the lead-contaminated soil for agriculture, we have tested productive specie with high yield, the Rapeseed (*Brassica napus* L.). In particular, we have studied the effect of humic acid and nano-superabsorbent (hydrogel) on plant height, number of pods per plant, shoot dry weight and root dry weight. The treatments (with and without nano-superabsorbent, with and without humic acid) caused significant differences between treated and control plants. The effect of nano- superabsorbent on plant height, number of pods per plant, plant root and shoot dry weight was significant in levels of 1% and 5%, respectively. The usage of humic acid showed significantly negative effect on number of pods per plant, plant height, shoot dry weight ($p<0.01$) and root dry weight ($p<0.05$). The results of the pot experiment suggested that *B. napus L.* is suitable for being cultivated in moderately lead-contaminated soils (417 $mg.kg^{-1}$ Pb) and nano-superabsorbent could have positive effect on plant growth indexes.

Keywords: humic acid, nano-superabsorbent, Brassica napus, lead.

1. Introduction

Human activities release pollutants in the environment; heavy metals, in particular, originate from industrial emissions, mining activities, disposal of wastes and fertilizers and pesticides use [1]. The contamination of agricultural soils with metals belongs currently to the most important environmental issues [2]. Specially, Lead is one of the most widespread pollutants in soil. Lead is a non-essential element that occurs naturally in the environment. However, the highest concentrations found in nature are the result of human activities. Many of its physical and chemical properties such as softness, malleability, ductility, poor conductibility and resistance to corrosion, have favored that man uses lead and lead compounds since ancient times for a great variety of applications [3]. Lead is naturally present in soils. It is a trace constituent of common rock-forming and readily weatherable minerals such as K-feldspar, plagioclase and mica, and a major constituent of various sulphide, sulphate, oxide, carbonate and silicate minerals locally found in Pb ores and their weathering products. In uncontaminated soils, Pb^{2+} concentrations are generally below 50 $mg.kg^{-1}$ [4]. Lead is a ubiquitous environmental pollutant in industrial environment that poses serious threat to human health. It has been demonstrated that heavy metals may play a role in the pathogenesis of diseases of nervous system [5]. One strategy to minimize adverse effects of Lead (Pb^{2+}) toxicity on crop production is

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to develop plant species having higher genetical ability to tolerate lead toxicity. All plants have the potential to extract metals from soil, but some plants have shown the ability to extract, accumulate and tolerate high levels of heavy metals, which would be toxic to other organisms [6]. The use of some tolerant crops such as Rapeseed (*Brassica napus L.*) as economical plants would give contaminated soil a productive value and decrease remediation expenses. First of all Rapeseed is among the oldest cultivated oil-producing plants; in Europe it is cultivated since the 14th century. Today, Rapeseed varieties are mainly used in food applications, but to a growing extent also in the production of biofuel [7, 8]. Regardless of the plants used, availability of heavy metals to plant roots is considered. Chelators, such as humic substances, could be used for increasing the solubility of metal cations, and thus their bioavailability to plants. The term humic substances refer to a category of naturally occurring organic materials found in soils, sediments, and natural waters. They result from the decomposition of plant and animal residues [9]. Humic acids are those parts of humic substances which are not soluble in water under acidic conditions, but become soluble and extractable at higher pH values. Humic acids contain acidic groups such as carboxyl and phenolic OH functional groups, [10] and, therefore, provide organic macromolecules with an important role in the transport, bioavailability, and solubility of heavy metals [11]. Special hydrogels i.e., super absorbents absorb and store water hundreds times of their own weights [12]. Their use for agricultural applications has shown encouraging results; they have been observed to help reduce irrigation water consumption and the death rate of plants, improve fertilizer retention in the soil, and increase plant growth rate [13]. It is well recognized that hundreds of different gel-polymers exist [14] and that they influence the soil physical structure, plant growth and yield differently. According to Bres and Weston [15] differences in effectiveness of gel polymers might be due to gel- polymer type and amount applied. We are interested in the possibilities of *B. napus L.* (Rapeseed) as a candidate crop for several reasons. The objective of this research was to examine the effect of nano-superabsorbent and humic acid application on Rapeseed (*B. napus*), grown in a Lead-contaminated soil in the framework of a pot experiment. Stockosorb superabsorbent (hydrogel beads) were studied for the adsorption of Pb^{2+} from soil solution to investigate the plant behavior and growth indexes such as plant height, number of pods per plant, shoot and root dry weight. Humic acid was applied to lead-contaminated soil as chelator and its efficacy was determined in relation to the amounts of the mentioned indexes.

2. Materials and methods

The experiment was conducted in the framework of a pot experiment in clay loam agricultural soil with three replications in greenhouse of Islamic Azad University (Khorasgan branch), Isfahan, Iran. The soil was air-dried, sieved through a 2-mm sieve and then some physical and chemical properties of the soil were measured which are summarized in Table 1. The initial total lead concentration of the soil, as determined by the Ryan method [16], was 17.5 $mg.kg^{-1}$ and the initial bioavailable lead content, determined by diethylenetriaminepentaacetic acid (DTPA) extraction method [17] amounted to 1.62 $mg.kg^{-1}$. The soil was artificially contaminated to approximately 417 $mgkg^{-1}$ Pb^{2+} with spraying (Pb $(CH3OO)_2$. 3H2O) and then treated to three cycles of saturation with de-ionized water and air-dried before being used for pot experiments. Lead concentration was measured again after contamination. Pots were filled with 7 kg of contaminated soil (Pb^{2+} = 417 ppm) and then divided into two parts as with nano-superabsorbent (5 gr per pot) and without nano-superabsorbent. Initially, 700 cc distilled water was added to 5 gr nano-superabsorbent (hydrogel beads) and after swelling was mixed with soil. The other part was divided again into two parts as with humic acid (125 gr per pot) and without humic acid. Rape seed (B. napus) seeds were cultivated and watered on the basis of 75% F.C. No fertilizer was needed due to adequate amount of N, P and K in soil as is shown in table 1. Two weeks after germination the seedlings were thinned to 6 plants per pot and kept constant till end of experiment. After 8 weeks, plants height and the number of pods per plant were measured in each pot and then plants were harvested. Shoot and root were separated and individually dried and weighted. Results showed in fig.1.

Table1. Selected physicochemical properties of the tested soil before experiment

Pb DTPA	Pb total	P available	K available	N total	O.M	C.E.C	EC	pH CaCl2	Sand	Clay	Silt

	$mg.kg^{-1}$			%		$cmol.kg^{-1}$	$dS.m^{-1}$			%	
1.62	17	46.05	449.7	0.13	0.25	10.7	2.4	7.9	16.17	20.83	63

3. Results

Dry matter weight of the shoots and roots, plant height and the number of pods per plant are shown in Fig. 1.

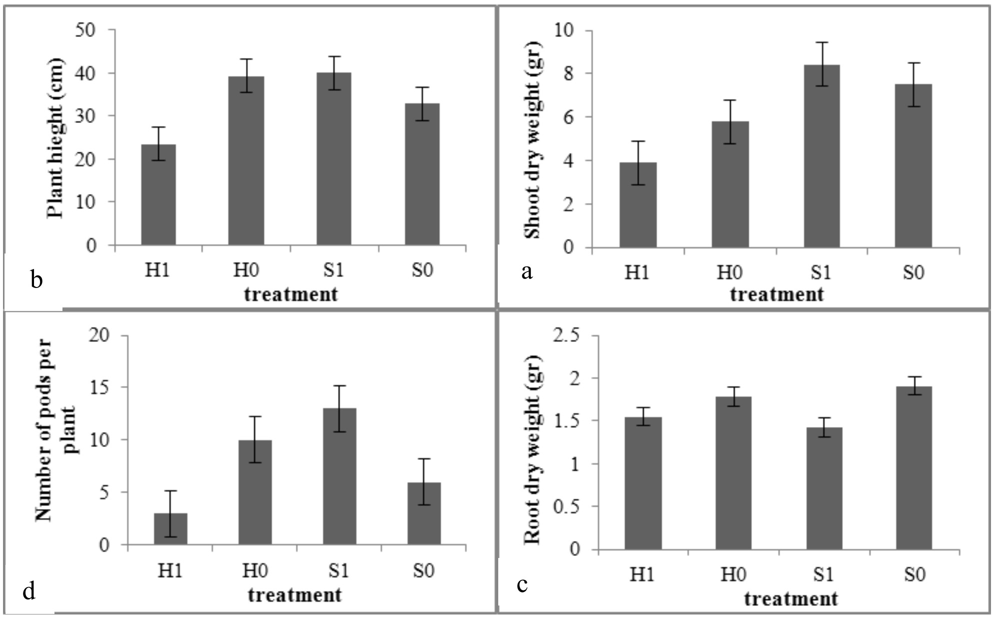

Fig.1. The effect of nano-superabsorbent and humic acids application on shoot dry weight (a), plant height (b), root dry weight (c) and number of pods per plant (d) of Rapeseed. Error bars represent ±SE of triplicates (n = 3). Treatments are as: H_1: humic acid, H_0: without humic acid, S_1: nano-superabsorbent, S_0: without nano-superabsorbent.

The usage of humic acid showed significantly negative effect on plant height, shoots dry weight ($p<0.01$) and root dry weight ($p<0.05$). It is because of enhancement in bioavailability of toxic Pb^{2+} by humic acid chelates. However, the maximum shoot dry weight (8.4 gr) was observed in pots without addition of humic acid and nano-superabsorbent. The results also showed that the effect of nano-superabsorbent on plant height, number of pods per plant, plant root and shoot dry weight was significant in levels of 1% and 5%, respectively. The highest amount of plant height (40 cm), number of pods per plant (13 pods) and root dry weight (1.91 gr) was achieved in pots with addition of 5 gr nano-superabsorbent. Although the plant height, number of pods per plant and root dry weight increased, dry shoot weight decreased significantly with addition of nano-superabsorbent.

4. Discussion

The statistical analysis revealed that effect of humic acid and hydrogel usage was significant for harvest index. All measured parameters of *B. napus* changed by addition of humic acid and hydrogel to contaminated soil. Humic acid usage decreased above ground dry weight, root dry weight and plant height (30, 14 and 40% respectively) and had extreme decrease (about 80%) in number of pods per plant which greatly affected the productivity of Rapeseed. This result is because of enhancement in bioavailability of lead from soil and decreasing in pH by humic acid chelates. The bioavailability of heavy metals in soil is influenced by many factors, such as the organic matter content [18], the cation exchange capacity [19], and especially, the pH which is partially influenced by organic acids exudate by plants. Cieslinski et al. [20] and Nigam et al. [21] showed that organic acids had a positive effect on the metal extraction by plants. Also Evangelou and Marsi [22] and Halim et al. [23] came to similar results on the enhancement of the bioavailability and mobility of heavy metals in soil by humic acids. Thus, more bioavailability of lead resulted to higher concentration of Pb^{2+} in plant and its growth indexes decreased. The plants growing in the non-humic acid treated soil received a much smaller lead amount due to its lower bioavailability in the soil and revealed higher biomass and growth. Previous studies have also been reported that the addition of high concentrations of humic substances to soils would have an inhibitory effect on plant growth which is the same as our result in this study [24]. The mean plant root dry weight ($p<0.01$) and shoot dry weight ($p<0.05$) showed significant differences between pots with and without nano-superabsorbent. The highest increase in measured parameter was achieved at the number of pods per plant compared to control treatment (fig.1). The result indicated that lead may be absorbed to hydrogel beads surface and was less available to plant and its negative effect on growth decreases. Adsorption of Pb^{2+} on hydrogel beads is due to the attractive electrostatic interaction between the hydrogel beads and the lead or lead hydroxide species. Significant increases in total leaf area dry weight and relative growth rate [25] and high tomato yields [26] were also reported. Allahdadi et al. [27] studied the impact of Superabsorbent A200 on drought stress of soybean (*Glycine max L.*). The results showed that the highest yield and yield components obtained from 225 kg/ha Superabsorbent polymer. The number of pod per main stem and branches, percentage of seed oil and nodule dry weight were increased with application of 150 kg/ha Superabsorbent polymer (SAP). In addition, because for inducing high yield adequate water is necessary, the application of hydrogel, especially in heavy textured soils, resulted in better and more effective use of water and nutrition with increasing the available water for plant and at last, increased the yield. By contrast, plant root dry weight decreased in nano-superabsorbent treatment in comparison with control. Superabsorbent polymer could reserve different amounts of water in it and increases the soil ability of water storing and at last in water deficiency. Hence, the plant needed to develop and produced lower amount of root net and biomass in the soil to uptake its sufficient water.

5. Conclusion

In conclusion this study has shown that Rape seed (*B. napus*) could possibly be used prosperously in marginally lead-polluted soil where its growth would be acceptable. Humic acid do have a positive effect on toxic heavy metal bioavailability in soil and in response to toxicity of Pb, the plant growth indexes decreased. Nano-superabsorbent could have considerable influence on growth of Rape seed in contaminated soil due to adsorbtion of Pb on its surface and make it unavailable for plant. According to these results it can be suggested that usage of nano-superabsorbent (hydrogel) can reduce the harmful effects of Pb and improves plant resistance.

6. References

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