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EFFECT OF HUMIC ACID AND ZEOLITE ON UPTAKE AND TRANSLOCATION OF MERCURY BY TURNIP PLANT IN A SALINE SOIL

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ABSTRACT

Contaminated soils can be remediated by various methods. For this purpose, 3 kg of saline soil in pot was treated with humic acid (HA) and zeolite (Zol) both at concentrations of 0, and 0.5 mg/kg soil, and were irrigated with three mercury levels of 0, 75 and 150 mg /L using mercuric chloride salt. Saline soil in pots were treated with three levels of Hg (0, 75 and 150 mg/L) by using mercuric chloride salt 10 days after planting. Plants were harvested 70 days after sowing, and root and shoot fresh and dry weights and mercury concentration in root and shoot of turnip were determined. The results showed that the maximum concentration of mercury occurred in roots followed by shoots. According to the results, exposure to the mercury concentrations of 75, and 150 mg/L significantly reduced the growth of turnip through diminishing the shoot fresh and dry weight, Root dry and fresh weights and Leaf No. and leaf area. As per BCF values humic acid and zeolite showed both has shown <1, 0.46 and 0.66, respectively. As per TF values humic acid and zeolite showed both has shown <1, 0.43 and 0.42 respectively.

KEY WORDS : Mercury toxicity, Organic, Inorganic, Treatments, Turnip plant.

INTRODUCTION

Mercury (Hg) pollution has become an ecological issue on a global (Schroeder and Munthe, 1998; Boening, 2002). Hg has been in use from the last 2500 years due to its unique physical and chemical properties (Crock *et al.,* 1996). Hg production since from the kickoff of the industrialization has been predestined at 0.64 million metric tons globally (Han *et al.,* 2004). Hg has no known physiological significance and, therefore, is not metabolized by most organisms. Even low concentrations introduced into biological systems may result in serious toxicity for sensitive species along the food chain (Suszeynsky and Shann, 1995). Soil, act as the most important resource of mercury, once it is released into the environment and can act as a mercury deposition record (Rufo *et al.,* 2007; Rodriguez martin, 2009). Mercury concentrations in soil ranged from 0.01 to 0.2 mg/kg (Adrino, 2001), but these concentrations are significantly higher in soils affected by Hg mining. Currently available technologies for the remediation of heavy metal contaminated soils relies heavily on 'dig-and-dump' or replacing the affected soil, immobilization or extraction by physicochemical techniques would be costly and is often suitable only for small areas (Martin and Bardos, 1996; Bio-Wise, 2000). It has been estimated that it would cost 40,000 to 70,000 US$ to remove each pound of mercury from the environment (Rugh, 1999). Recently, different studies have suggested that additions of organic matter (OM) to soils which would improve our understanding of the mechanisms on how decreases the environmental risks associated with Hg. Among the components of soil, organic matter (OM) is considered to be the most substantial agent for Hg biogeochemistry, Hg bioavailability, and Hg dangers due to OM interactions with Hg (Klapstein and O'Driscoll, 2018). Recently, different studies have suggested that additions of Zeolites are materials that can be applied for mercury eradication from soils (Haidouti, 1997) Zeolite and vermiculite have wide applications such as water adsorption, wastewater treatment, molecular sieves for plants, air filtration, etc. They are suitable materials for reducing heavy metals and effective in removing heavy metals as well as selective adsorption, dehydration rehydration, cation exchange capacity, and catalysis properties (Mumpton and Fishman, 1977). Leppert (1990) reported that zeolites, clinoptilolite in particular, have strong affinity for heavy metal ions. The mechanism of adsorption by zeolites was found to be ion-exchange. Zeolites contain various types of negatively charged sites and cationic sites (Abusafa and Yücel, 2002). The objective of the present study was effect of humic acid and zeolite on uptake and translocation of mercury by turnip plant in a saline soil.

MATERIALS AND METHODS

Soil sample was collected from the top layer (0–15 cm depth) soil, located near the city of Mashhad (Chenaran region), northeast of Iran. Once returned to the laboratory the soils were air-dried in the shade and sieved (≤ 2mm). The visible plant materials in sieved soils were then removed. In greenhouse experiment,3 kg of saline soil added in pots, (diameter 23 cm, height 21.5 cm), was treated with humic acid (HA) and zeolite (Zol) both at concentrations of 0, and 0.5 mg/kg soil. They were then irrigated with three mercury levels of 0.75, and 150 mg/L using mercuric chloride salt, and turnip seeds were sown and irrigated with tapwater. 10 days after germination, soil in pots were treated with 200 mL of mercury chloride solutions of Hg treatments (0, 75 and 150 Hg mg/L) along with tapwater in seven weeks. The experiment was performed as a completely randomized design (factorial) with three replications. Humic acid was provided from Merck Company USA (Sigma Aldrich, USA). Further, the zeolite (finely grounded) chemical properties used in this study are listed in Table 1.

Table 1. Properties of zeolite used in this study.

Parameter	Unit	Value
SiO_2	%	71.19
$Al2O_3$	%	8.40
$Fe2O_3$	%	0.88
SO_3	%	0.20
Na_2O	%	3.48
K_2O	%	2.74
CaO	%	0.18
MgO	%	0.28
TiO_2	%	0.14
Cl	%	0.67
L.O.I.*	%	11.76

Soil samples (0.5 g) were treated with 12 mL of Aqua Regia (HNO3: HCl, 1:3) solution. The mixtures were heated at a low temperature initially for 1 hour, and then 20 mL of 2% HNO_3 was added. Then the mixture was digested at high temperature for 30 min. The sample was diluted with 25 mL of 2 % HNO_3 and filtered with Whatman No.42 filter paper. The filtrate was analyzed by ICP-OES (SPECTRO ARCOS Model76004SSS Germany). (UNEP/IAEA.UNEP, 1985). Dried plant samples (0.5g) were digested with 5 mL of diacid (HNO_3:$HClO_4$ 1:3). The mixture was heated at a low temperature for 1 hr, the mixture was heated at a low temperature for 1 hour, and 3 mL of diacid was added. The sample was diluted with 50 mL of 2% HNO_3 and filtered with Whatman No.42 filter paper. The filtrate was analyzed by ICP-OES. (SPECTRO ARCOS Model 76004SSS Germany). (Gregerrt *et al.*, 2005). The ability of plant to accumulate metals from available soil source named as Bio- concentration factor (BCF) were defined as the ratio of metal concentration in roots to that in soil (Yoon *et al.,* 2006).

BCF= [metal] root/ [metal] soil (1)

Translocation Factor (TF): The ability of plant to accumulate metals from the roots to the aerial parts of the plants were obtained as the ratio of metal concentration in the shoots to the roots (Yoon *et al.,* 2006).

TF= [metal] Shoots/ [metal] roots (2)

The experiment was performed as a completely randomized design (factorial) with three replications, and included 27 pots for treated with three levels of Hg and with humic acid (HA) and zeolite (Zol) both at concentrations of 0, and 0.5 mg/kg soil. Data were subjected to statistical analysis using JMP8 software. The significant differences ($P<0.05$) and ($P<0.01$) between treatments and control were statistically evaluated by standard deviation and Student’s t-test methods.

RESULTS AND DISCUSSION

Shoot fresh and dry weights: Increasing levels of Hg treatments, significantly inhibited the shoot fresh and dry weights. The shoot fresh weight was decreased significantly by -43.75% and -75% under Hg levels of 75 and 150 mg/L compared to control (Fig. 1), respectively ($P<0.05$). Whereas, the shoot dry weight was decreased significantly by -16.66% and -58.3% under Hg levels of 75 and 150 mg/L (Fig. 2), respectively ($P<0.05$) compared to control.In general, the phytotoxicity of mercury exerted some effects on the biomass of the plant, while inhibiting the plant growth and showing long-terms impacts on the fertility of the soil (Sahi *et al.,* 2006). Root dry and fresh weights: Increasing levels of Hg treatments, significantly inhibited the root fresh and dry weights to a large extent. The root fresh weight was decreased significantly by -4.64% and -24.46% under Hg levels of 75 and 150 mg/L compared to control (Fig. 3), respectively ($P<0.05$). Whereas, the root dry weight was decreased significantly by -18.58 % and -41.98% under Hg levels of 75 and 150 mg/L (Fig. 4), respectively ($P<0.05$) compared to control. Leaf No. and leaf area*:* With increasing levels of Hg treatments, significant reduction of the leaf area was noted. The leaf area was decreased significantly by -14.42% and -40.06% under Hg levels of 75 and 150 mg/L (Fig. 5), respectively ($P<0.05$) compared to control. Whereas, the leaf no was decreased significantly by -25% and % -41.66% under Hg levels of 75 and 150 mg/L (Fig. 6), respectively ($P<0.05$) compared to control.The responses of plants to various stimuli (biotic and abiotic) is arisen in the leaf, which is considered to

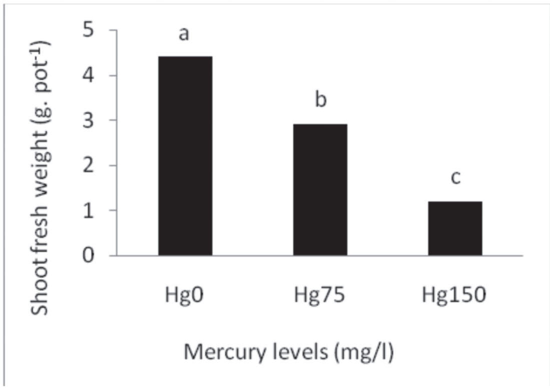

Fig. 1. Effect of Hg treatments on shoot fresh weight

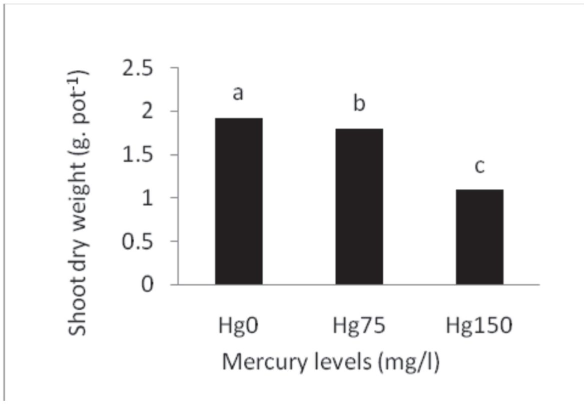

Fig. 2. Effect of Hg treatments on shoot dry weight

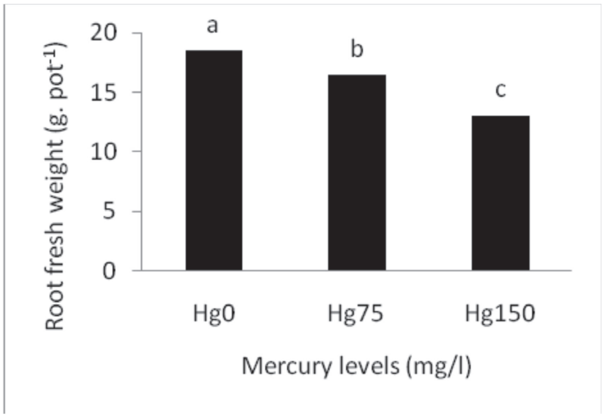

Fig. 3. Effect of Hg treatments on root fresh weight

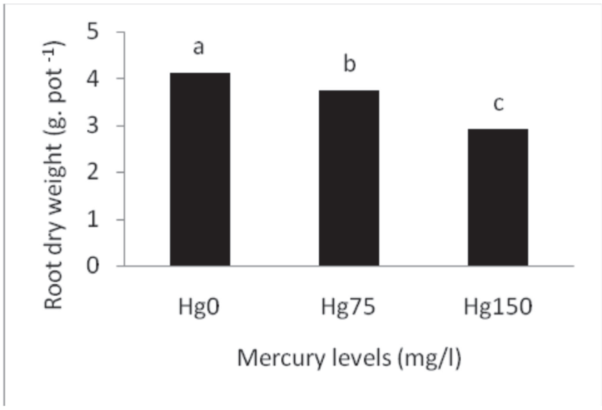

Fig. 4. Effect of Hg treatments on root dry weight

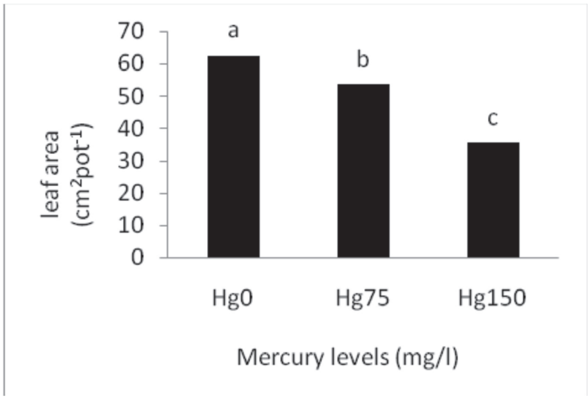

Fig. 5. Effect of Hg treatments on Leaf area

be a physiological parameter for the same reason. For the most part, this has been attributed to a combination of factors, including light, accumulated dry materials, photosynthetic capacity, growth, metabolism, and performance (Severino *et al.*, 2004). In general, as such, the decreased leaf area could be the mechanism through which *J. curcas* species minimize their loss of water through transpiration as a result of the inhibited formation of aquaporins, which enable water transfer. The physiological processes in plants may alter markedly due to exposure to heavy metals, along with the adverse effects of these compounds on the growth and development of plants (Cárdenas-Hernández *et al.*, 2009).

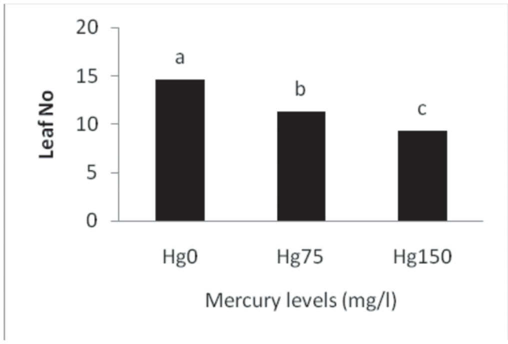

Fig. 6. Effect of Hg treatments on leaf No

Bio-Concentration Factor (BCF)

Figure 7 reveals the data for mercury levels on the values of BCF in saline soil with 75 and 150 mg/L Hg in the saline soil, the BCF was 2.27 and 2.39, respectively. Results of in this case showed that the values of BCF were ≥1,which indicates height translocation of Hg from soil to root in plant. The root system serves an enormous surface area that absorbs and accumulates the water and nutrients that are essential for growth, but also absorbs other non-essential contaminants (Arthur *et al.*, 2005), because there is a tendency to form a heavy metal complex with inorganic compounds found in the body of organisms (Selin, 1993). Figure 8 demonstrates the data for HA and zeolite Zol treatments on the values of BCF in saline soil. with 0.5 mg/kg HA and 0/5 Zol mg/kg treatments in the saline soil, the values of BCF was 0.46 and 0.66, respectively, compared to the control (3.54). Results of in this case showed that the values of BCF were ≤ 1,which indicates poor translocation of Hg from soil to root in plant. Humic acid (HA) has been confirmed to have a strong ability for forming complex with metals in soil including mercury (Hg), thus reducing the ratio Hg in the plant (Qing and Mou, 1993). According to (Stumm, 1992), the reduction of mercury uptake is mainly attributed to its immobilization via ion-exchange operation on the micro-porous minerals such as zeolites, phyllosilicates, as well as to the adsorption operation on the exterior of the micro- and non-microporous minerals. Figure 9 illustrates the data

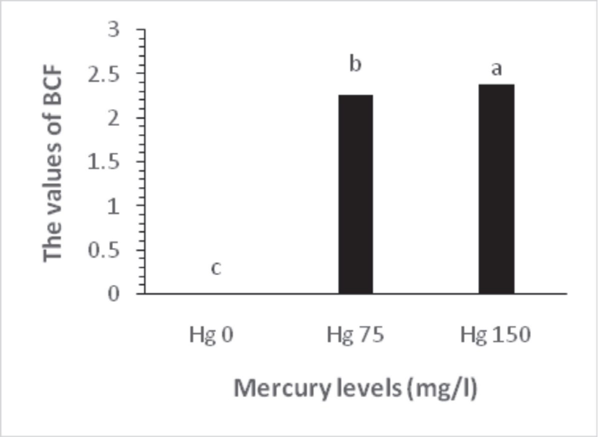

Fig. 7. Effect Hg levels on the values of BCF under salinity stress.

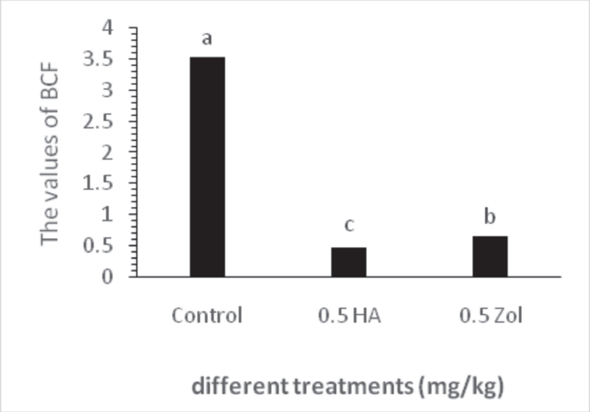

Fig. 8. Effect of different treatments on the values of BCF under salinity stress.

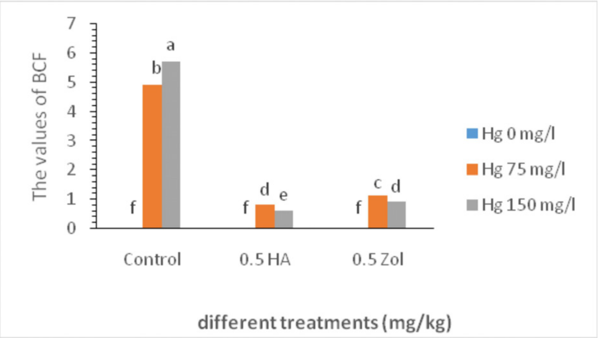

Fig. 9. Interaction effects of Hg levels and different treatments on BCF in saline soil

for the interaction effect of HA, Zol, and Hg levels on the values of BCF in saline soil. When 0.5 mg/kg HA and Zol with 75 mg/L Hg treatments were applied to the saline soil. The BCF was 0.8 and 1.1 respectively, compared to the control (4.91). Likewise, with the 0.5 mg/kg HA and Zol with 150 mg/L Hg treatments. The values of BCF was 0.58 and 0.89 respectively, compared to the control (5.72). Results of in this case showed that the values of BCF were 1,which indicates poor translocation of Hg from soil to root in plant. Note that the positive impact of HA was greater than that of Zol (Wang *et al.,* 1997), observed that the increase of HA in soils affects the reduction of Hg soil content which could articulate strong binding of mercury by organic matter (OM) in soil, thus reduce its absorption by the plant. Studies with zeolites revealed that these are the materials that can be used for mercury elimination from soils, thereby reducing the proportion of mercury in the plant (Haidouti, 1997).

Translocation Factor (TF)

Figure 10 reveals the data for mercury levels (mg/L) on the values of TF in saline soil. with 75 and 150 mg/L Hg in the saline soil, the TF was 0.68 and 0.64, respectively. Results of in this case showed that the values of TF were ≤1, which indicates poor translocation of Hg from root to shoot in plant. In general, several other studies have confirmed that mercury mostly accumulates in the roots of the plants growing in the soils that are contaminated with mercury (Kalac and Svoboda, 2000). Figure 11 demonstrates the data for HA and zeolite Zol treatments on the values of TF in saline soil with 0.5 mg/kg HA and 0.5 Zol mg/kg treatments in the saline soil, the values of TF was 0.43 and 0.42, respectively, compared to the control (0.46). Results of in this case showed that the values of TF were ≤ 1, which indicates poor translocation of Hg from root to shoot in plant. Note that the positive impact of HA was greater than that of Zol. Figure 12 illustrates the data for the interaction effect of HA, Zol, and Hg levels on the values of TF in saline soil. When 0.5 mg/kg HA and Zol with 75 mg/L Hg treatments were applied to the saline soil. The TF was 0.63 and 0.67 respectively, compared to the control (0.73). Likewise, with the 0.5 mg/kg HA and Zol with 150 mg/L Hg treatments. the values of TF was 0.66 and 0.6 respectively, compared to the control (0.65). Results of in this case showed that the values of TF were ≤ 1, which indicates poor translocation of Hg from soil to root in plant. Note that the positive impact of HA was greater than that of Zol.

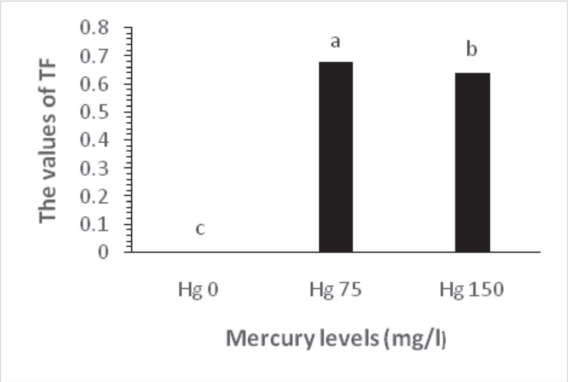

Fig. 10. Effect Hg levels on the values of TF under salinity stress.

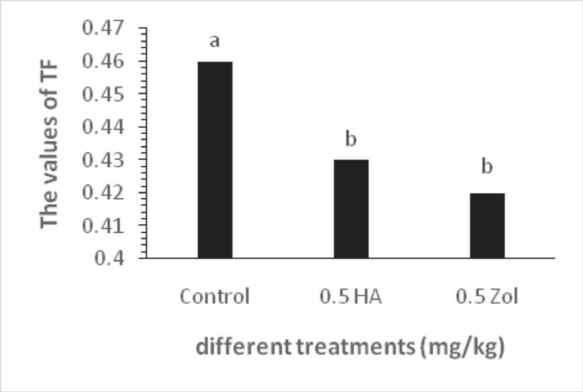

Fig. 11. Effect of different treatments on the values of TF under salinity stress.

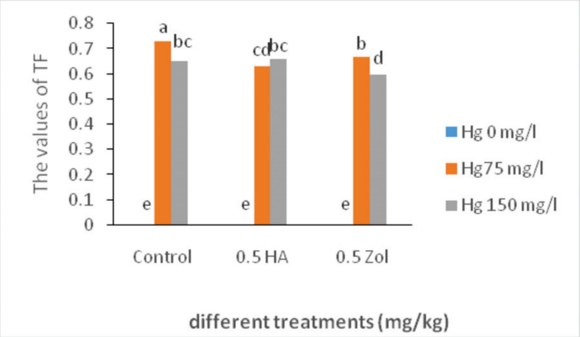

Fig. 12. Interaction effects of Hg levels and different treatments on the values of TF in saline soil

CONCLUSION

According to the results, exposure to the mercury concentrations of 75, and 150 mg/L significantly reduced the growth of turnip through diminishing the shoot fresh and dry weight, Root dry and fresh weights and Leaf No. and leaf area, while, the values of BCF and TF were < 1, which indicates poor translocation of Hg from soil to root and from root to shoot in all treatments, although Hg was available to the plants. showed BCF values also reduced for HA and Zol, BCF value reached to 0.46 and 0.66, respectively, compared to the control (3.54). The TF values also reduced for HA and Zol, TF value reached to 0.43 and 0.42, respectively, compared to the control (0.46). The result showed that both HA and Zol treatments play an important role in controlling Hg in saline soil. So that both HA and Zol increased the retention of Hg in saline soil. Therefore, both HA and Zol reduced the BCF values in soil and prevented transportation in the saline soil. However, the positive impact of HA was more than Zol.

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