ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230, **ISSN (online):** 2412-3986, **DOI:** **http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Influences of mycorrhizal inoculum on Cd and Pb uptake by wheat plant

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A B S T R A C T:

The investigation was carried out in the greenhouse, College of Science, Salahaddin University- Erbil, Iraq, from November 2018 to May 2019 for assessing the impact of arbuscular mycorrhizal fungi (AMF) in sterilized soil with addition of different Lead Pb levels (0, 50, 100, 150, 200 µg kg $^{-1}$), Cadmium Cd levels (0, 20, 40, 60, 80 mg kg $^{-1}$) and their interactions on vegetative growth characteristics, yield and Pb and Cd concentration in root and seeds of wheat, using factorial experiment according to Completely Randomized Design (Factorial C.R.D) with three replicates on vegetative characteristics, yield in addition to lead (Pb) and cadmium (Cd) concentration of roots and seeds of wheat cultivar (*Triticum aestivum* L.). The main results were summarized as follow:

The interaction effect between (Mycorrhizal inoculum levels of Pb), (Mycorrhizal inoculum levels of Cd) and (Mycorrhizal inoculum levels of Pb and Cd) caused an increased in grain yield, the highest values (1.28, 1.03 and 1.08) g pot $^{-1}$ were recorded from interaction treatments of (MPb_{150}, MCd_{80} and $MPb_{100}Cd_{40}$) respectively. The application of Pb, Cd and their interactions caused a decrease in mycorrhizal infection from (80%) to (23.33, 60.33 and 20.00%) respectively.

The mycorrhizal infection protected the plant from the toxic effect of Pb and Cd. The interaction treatments of (MPb and MCd) caused decreased in Pb concentration of wheat seeds from (10.07) µg kg^{-1} to (3.65 and3.33)µg kg^{-1} respectively. While the lowest Pb concentration (0.83) µg kg^{-1} was recorded from $MPb_{50}Cd_{20}$. The studied combination treatments also caused decrease in Cd concentration of wheat seeds.

KEY WORDS: Arbuscular mycorrhizal fungi (AMF), Cd, Pb, Wheat.
DOI: http://dx.doi.org/10.21271/ZJPAS.33.6.7
ZJPAS (2021) , 33(6);57-71 .

Aim of study

Thus the principal objectives of this investigation is to determine the effect of different levels of Pb (0, 50, 100, 150, 200 µg kg^{-1}) , Cd (0, 20, 40, 60, 80 µg kg^{-1}) and their combinations with arbuscular mycorrhizal fungi (AMF) inoculation on growth , yield and heavy concentration of wheat plant.

1.INTRODUCTION :

1.1- Heavy metals

Heavy metal stress is one of the most important stresses that negatively affect plant growth and the environment (Miransari, 2017). Heavy metal pollution in the soil causes serious environmental and health problems due to thier non-degradable nature, requiring biotechnological solutions (Zoomi et al., 2019).

Due to urbanization and industrialization, heavy metals are increasingly polluting the environment (Mythili and Kartikeyan, 2011), and they have become a major global concern due to their toxicity and threat to human life and the environment. Although some of these metals are essential for plant growth and development (Zn, Cu, Fe, Mn, Ni, Mo, Co), high levels and/or long-term presence of HMs in soils are generally regarded as a cause for concern by society because they can degrade soil and water quality and threaten sustainable food production (Kabata-Pendias and Mukherjee, 2007).

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Article History:
Received: 22/01/2021
Accepted: 08/05/2021
Published: 20/10 /2021

High levels of heavy metals, particularly lead (Pb), in the soil have a deleterious impact on the biochemical and physiological processes that control grain quality in cereals (Aslam et al., 2021). Phosphorous fertilizers and atmospheric deposition are two of the most significant sources of Cd in plants (Smolders and Mertens, 2013). Cadmium uptake in plants is influenced by soil pH, since as pH rises, Cd availability decreases (Eriksson, 1989). Various toxic metals, such as Cd, have been discovered to be extremely harmful to wheat (Rizwan et al., 2020). Because of Cd high mobility in the soil–plant system (Groppa et al., 2012), it is a hazardous element that can causes serious disruptions in plant physiological functions such as photosynthesis, water relations, and mineral uptake (Gill et al., 2012). Cd is a particularly hazardous contaminant due to its high toxicity and solubility (Jiang et al., 2001).

Cadmium and lead are poisonous to animals, plants and microorganisms, which are non-essential elements. There are significant variances in plant ability to collect heavy metals as well as plant responses to heavy metal toxicity (Vassilev et al., 2004). Heavy metals like cadmium and lead are known environmental pollutants that have a negative impact on a plant's morpho-physiological and biological characteristics. They have become an expanded element of the food chain and have an impact on human health as a result of their migratory mobility (Dhalaria et al., 2020).

1.2- Mycorrhizae

Arbuscular mycorrhizal fungi (AMF) form a symbiotic relationship with most terrestrial plants. In this symbiotic relationship, the fungus provides the host plant with water and nutrients in exchange for carbon, thereby improving growth and performance under stress and without stress (Miransari, 2017 and Begum et al., 2019). Inoculation of AMF allows host plants to tolerate various stress conditions such as drought, metal, and extreme temperatures, and increases plant performance (Mahmood and Rizvi, 2010 and Begum et al., 2019). AMF enhances metal toxicity by enhancing the ability of plants to withstand metal stress. Vesicles are similar to fungal vacuoles and accumulate large amounts of heavy metals with the help of the ubiquitous hyphal network (Dhalaria et al., 2020).

AMF have been reported to assuage heavy metal stress of plants by releasing organic acids, production of glomalin protein, metallothionine protein and secretion of plant growth promoting substances to the remediation process and helping plants to eliminate heavy metals from contaminated soil (Lokhandwala et al., 2017).

Several studies revealed that mycorrhiza could be used as a stress-reducing agent in soils contaminated by heavy metals helping plants to survive in such stressed conditions (Gong and Tian, 2019).

Although AMF collaborates with rhizosphere microbial communities, AMF may promote the revegetation of large amounts of metal-contaminated soil (Hao et al., 2021). Thus use of arbuscular mycorrhizal fungi (AMF) in agriculture has been reported to sustainably improve plant's tolerance to various heavy metal stresses (Wu et al., 2020).

Plants inoculated with AM grow faster than plants that are not, exhibit improved mineral nutrition, and have higher biomass. The functions of AM inoculated plants range from stress relief to bioremediation in MH-contaminated soils, with the aim of improving soil properties and plant performance in semi-arid and / or MH-contaminated sites (Elhindi et al., 2018).

1.3- Wheat plant

Among grains, wheat (*Triticum aestivum* L.) is the second most important crop and is mainly consumed as a source of carbohydrates and dietary protein and minerals. It ranks first in terms of world consumption (Food and Agriculture Organization of the United Nations (FAO), 2019).

As the world's population increases, wheat production must meet the world's needs and reduce the toxic components in its edible parts. It is reported that the use of AMF in agriculture can continuously improve the tolerance of plants to various heavy metal stresses (Wu et al., 2020).

Since there are little or no studies in Kurdistan region of Iraq about the role of mycorrhizal inoculation in reducing to toxicity of heavy metals for this reason this study was selected in order to focusing on the role of mycorrhizal inoculation in reducing the risk of Pb and Cd for plants, human and other organisms.

2. MATERIALS AND METHODS

2-1 Soil and pots preparation

Samples were collected from Khabat area in Erbil governorate, from soil surface (0-30) cm depth, which considered as the active root zone. Soil particle size distribution was determined by Hydrometer method (Salim and Ali, 2017) and textural class according to USDA system.

Plastic pots of (22) cm in diameter and (30) cm in height were used in this investigation. Each pot packed with (7 kg microwave sterile soil per pots according to (Razavi darbar and Lakzian, 2007), after sieving them through (4) mm sieves, seeds were surface sterilized with 0.1% $HgCl_2$ for 5 minutes, and then washed with distilled water as described by Clarck (1965), then left on sterilized surface at room temperature for 2 hours before planting. (5) sterilized wheat seeds were planted in each pot, and after germination thinned to three plants per pot (Hassan, 2011).

Plants irrigated with water to maintain soil moisture near the field capacity by weighting methods.

The soil was dried and passed through (2mm) sieve and some physico - chemical characteristics were analyzed according to the procedure described by Ryan et al., (2001) and results were summarized in Table (1).

The particle size distribution was determined using the pipette method as described by Gee and Bauder (1986).

Organic matter was measured by using loss on ignition method (Schulte and Hopkins, 1996). Total contents of Pb and Cd were digested by aqua regia (Sabiene et al., 2004), then their concentration were measured by using atomic absorption spectroscopy (AAS).

The concentration of main cations, anions, pH and EC were determined according to Ryan et al., (2001). The concentration of Pb and Cd were determined as describedin Allen et al., (1974).

Table (1): Some physical and chemical properties of the studied soil

Parameters	Value
PSD g kg^{-1} Clay	52.0
Silt	251
Sand	697
Soil texture	Sandy loam
Organic matter g kg^{-1}	6.00
Total Nitrogen %	0.075
Total phosphorus ppm(Olsen method)	116 ppm
pH	7.81
Electrical conductivity (dSm^{-1} at 25ºC)	0.67
Calcium carbonate % (titration with NaOH)	26.90
Soluble ions mmolec L^{-1}	
HCO^-_3	3.75
$SO_4^=$	1.19
Ca^{++}	2.29
Mg^{++}	0.81
Na^+	0.80
K^+	0.71
Cl^-	1.61
Fe^{++}	0.03
Total element (ppm)	
Pb	17.7
Cd	0.20

2-2 Infection Percentage

Mycorrhizal Inoculum, (powder preparation) consist of selected species specially for vegetables (Myco Grow for vegetables), contains concentrated spore mass of the following: *Glomus intraradices, G. mossaeae, G. aggregatum, G. etunicatum* obtained from FUNGI PERFECTI, LLC, USA, as prepared inoculum used in this experiment, adding with seeds during planting.

Detection the levels of mycorrhizal colonization after harvesting clearing and staining procedures requires root samples that should be washed free of soil under running tap water thoroughly. Washed roots were cut into 1.0 cm pieces, then treated and cleared with 10% KOH solution overnight, these root segments were washed again with distilled water, then acidified with 10% HCl for 10 minutes for removing excess KOH, washing again with distilled water, and stained with 0.05% trypan blue in lactophenol (Modified procedure of Phillips and Hayman, 1970).

Ten stained pieces of each taproot ends were mounted on slides, microscopically examined for VAMF colonization and presence of colonization was calculated by measuring the infected portion in relation to total length of root pieces then collected 10 values of each 1.0 cm to obtain total percent (Biermann and Lindermann, 1981).

The method described by (Giovannetti and Mosse, 1980) were used for root colonization assessment, root segments selected randomly from the stained samples were observed under an Olympus KH binocular microscope (×40 magnification). The colonization percentage was calculated as follows:

$$\text{Colonization percentage} = \frac{\text{Number of colonized root segment}}{\text{Total number of examined segments}} \times 100$$

2-3 Vegetative growth characteristics

Samples of wheat plant were taken after physiological maturity. Some vegetative growth characteristics were measured for each treatment included: The plant height (cm) **this parameter was measured from the contact point between the stem of the plant and the soil surface to the main stem,** spike length (cm), flag leaf area (cm^2) was estimated according to the following equation:

Leaf area = (length * width * 0.75).

weight of grain/ pot (g), weight of shoot/ pot (g) and weight of total dry matter of plant/ pot (g).

2-4 Plant analysis

After physiological maturity, the plant harvested, separated into different plant parts (grains and roots), then oven dried at 65 ºC for 24 hrs. Their dry weights were taken then grounded. Chemical analysis for grains and roots were determined after acid digestion for samples (0.5 g dried plant with H_2SO_4+ H_2O_2) according to Ryan et al., (2001), the concentration of Pb and Cd were determined by using atomic absorption spectrophotometer (Varian FAAS-240).

2-5 Experimental design

Pots were distributed in the greenhouse according to Factorial experiment in Completely Randomized Design (Factorial C.R.D), with three replications. The data were subjected to standard analysis of variance and means were compared at significant 5% level by Duncan test. Hence 13 experimental treatments were tested in each three replicates making the total of 33 pots.

3. RESULTS AND DISCUSION:

3.1- Vegetative growth characteristics including:

3.1.1- Plant height (cm)

As shown in table (2) that the maximum mean value for plant height was recorded from treatment MPb_{150} which was (46.10 cm), while the lowest mean value was recorded from a combination treatment of MPb_{50} that was (33.75 cm) and there is a significant differences between combination treatments of (MPb with different levels. On the other hand the combination treatments of (MCd and MPbCd) were not affected significantly on plant height (table 3 and 4). The results agree with those recorded by Nowak (2007).This may be due to the role of mycorrhizal infection in protecting plants from the toxic effect of heavy metals (Zoomi et al., 2019).

3.1.2- Spike Length (cm)

As shown from table (2, 3 and 4) the combination treatments of (MPb and MCd) were affected significantly on spike length the maximum mean values (11.13 and 11.10 cm) were recorded from combination treatments of MPb_{200} and MPb_0Cd_0 respectively. While the lowest values (9.3 and 8.43 cm) were obseved from combination treatments MPb_{200} and MCd_{20} respectively. The combination effect among the three studied factors was non-significant. This could be related to the role of mycorrhizal infection in increasing available nutrients then increase in biomass including spike length (Elhindi et al., 2018). Similar results were obtained by Ahmed (2012) in his study on the effect of Pb and Cd on the wheat plant.

3.1.3- Flag leaf area (cm^2)

Table (2, 3 and 4) explain the significant influence of combination treatments on flag leaf area the maximum values (2.01 and 2.85 cm^2) were recorded from combination treatment of MCd_{40} and $MPb_{150}Cd_{40}$), while the minimum value (1.22 cm2) was observed from MCd_{20} in spite of using one metals with low levels. This may be due to the reasons mentioned before, minimum mean value (1.22 cm^2) was recorded from combination treatment of MCd_{20} in spite of using one metals with low levels. This may be due to the reasons mentioned before.

3.1.4- Weight of grain /pot (g)

Table (2, 3 and 4) shows the significant influence of the studied combination treatments on grain yield. In general there is fluctuation in grain yield. The maximum mean value was recorded for a combination treatment of MPb150 which was(1.28 g) as shown in table (2), so the lowest value was recorded from combination treatment of MCd_{20} which was (0.18 g) as shown in table (3). This may be due to the higher toxic effect of Cd on plants in comparing with Pb (Ahmed, 2012). AMF binds Cd in the cell wall of mantle hyphae and inhibits its uptake, according to Garg and Chandel (2010), leading in better growth and production.

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Leaf area = (length * width * 0.75).

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Table (2): Effect of mycorrhizal inoculum on the vegetative characteristics and mycorrhizal percent of wheat treated with MPb.

Trait / Treatments	Plant height(cm)	Spike Length(cm)	Flag area (cm^2)	Grain W/pot (g)	Shoot W/pot (g)	T. D.W /pot (g)	Infection percentage %
MPb_0Cd_0 (control)	39.30 ^{ab}	11.10 ^{a}	1.55 ^{a}	0.84 ^{a}	1.12 ^{d}	1.96 ^{b}	٨٠.٠٠ ^{a}
MPb_{50}	٣٣.٧٥ ^{b}	10.53 ^{a}	1.45 ^{a}	0.95 ^{a}	1.38 ^{c}	2.33 ^{b}	٣٣.٣٠ ^{c}
MPb_{100}	٣٨.٨٠ ^{ab}	9.93 ^{a}	1.38 ^{a}	0.99 ^{a}	1.67 ^{b}	2.66 ^{ab}	٣٦.٦٦ ^{c}
MPb_{150}	٤٦.١٠ ^{a}	10.70 ^{a}	1.57 ^{a}	1.28 ^{a}	2.02 ^{a}	3.30 ^{a}	٤٦.٦٦ ^{b}
MPb_{200}	٣٨.٧٠ ^{ab}	11.13 ^{a}	1.56 ^{a}	0.65 ^{a}	1.24 ^{e}	1.89 ^{b}	23.33 ^{d}

Table (3): Effect of mycorrhizal inoculum on the vegetative characteristics and mycorrhizal percent of wheat treated with MCd.

Trait / Treatments	Plant height(cm)	Spike Length(cm)	Flag area (cm^2)	Grain W/pot (g)	Shoot W/pot (g)	T. D.W /pot (g)	Infection percentage %
MPb_0Cd_0 (control)	39.30 ^{a}	11.10 ^{a}	1.55 ^{bc}	0.84 ^{a}	1.12 ^{b}	1.96 ^{b}	80.00 ^{a}
MCd_{20}	٣٧.١٥ ^{a}	8.43 ^{b}	1.22 ^{c}	0.18 ^{b}	0.98 ^{c}	1.16 ^{d}	86.66 ^{a}
MCd_{40}	٣٦.٨٥ ^{a}	10.66 ^{a}	2.01 ^{a}	0.64 ^{a}	0.97 ^{d}	1.62 ^{bc}	33.33 ^{b}
MCd_{60}	٣٥.٤٥ ^{a}	9.33 ^{b}	1.30 ^{c}	0.21 ^{b}	1.12 ^{b}	1.34 ^{cd}	53.33 ^{c}
MCd_{80}	41.45 ^{a}	9.43 ^{b}	1.83 ^{ab}	1.03 ^{a}	1.43 ^{a}	2.47 ^{a}	63.33 ^{b}

Table (4): Effect of mycorrhizal inoculum on the vegetative characteristics and mycorrhizal percent of wheat treated with MPbCd.

Trait / Treatments	Plant height(cm)	Spike Length(cm)	Flag area (cm^2)	Grain W/pot (g)	Shoot W/pot (g)	T. D.W /pot (g)	Infection percentage %
MPb_0Cd_0 (control)	**39.30 [a]**	**11.10 [a]**	**1.55 [b]**	**0.84 [ab]**	**1.12 [d]**	**1.96[a]**	**80.00 [a]**
$MPb_{50}Cd_{20}$	**٣٧.٧٣[a]**	**10.23 [a]**	**1.60 [b]**	**0.52 [ab]**	**1.32 [b]**	**1.84 [ab]**	**66.66 [b]**
$MPb_{100}Cd_{40}$	**٤٠.٦٣ [a]**	**10.63 [a]**	**2.85[a]**	**1.08 [a]**	**1.39 [a]**	**2.47 [a]**	**33.33 [c]**
$MPb_{150}Cd_{60}$	**٤٠.٠٠ [a]**	**10.50 [a]**	**1.78 [b]**	**0.88 [ab]**	**1.17 [c]**	**2.05 [a]**	**20.0 [d]**
$MPb_{200}Cd_{80}$	**٣٨.١٣ [a]**	**10.23 [a]**	**1.73 [b]**	**0.24 [b]**	**0.98 [e]**	**1.22 [b]**	**20.0 [d]**

Note:
M: Mycorrhizal inoculum Pb: Lead Cd: Cadmium W: weight T. D.W: Total dry weight
•The same letters referred non-significant •The different letters referred –significant

Weissenhorn et al., (1995) noted that increasing mycorrhizal colonization in the soil causes increment in the amount of plant biomass and shoots as well as the decrease in concentrations of heavy metals such as Cd in roots and shoots. Accordingly, the root density of AMF is effective on heavy metal tolerance depending on mycorrhizal inoculum. The increase in infection percentage of plant roots with mycorrhizal fungi caused significant increase in wheat yield (Abdulkarim et al., 2020).

3.1.5- Shoot dry weight (g)

Table (2, 3 and 4) indicated the significant effect of the studied combination treatments on shoot dry weight of wheat plant. The highest values (2.02, 1.43 and 1.39) g were recorded from combination treatments of (MPb_{150}, MCd_{80} and $MPb_{100}Cd_{40}$) respectively. Results emphasized that Cd induced to decrease in the normal germination percentage and have an inhibitory effect on the initial growth of seedlings influenced the growth of the roots and aerial parts, and also reduced the production of green and dry mass of seedlings. Conclusively, the accumulation of Cd in the soil affected the viability and production of wheat largely due to the absorption of this metal by the plant roots (Kirmani et al., 2018).

3.1.6- Total dry weight/pot (g)

Table (2, 3 and 4) indicates that the combination treatment MPb_{150} gave the highest mean values for total shoot dry weight/pot which was (3.30 ,2.47 and 2.47) g were obtained from combination treatments of (MPb_{150}, MCd_{80} and $MPb_{150}Cd_{40}$) respectively, while the lowest values (1.89,1.16 and 1.22 g) were observed from MPb_{200}, MCd_{40} and $MPb_{200}Cd_{80}$) respectively. In general application of both Pb and Cd may have more negative effect than their individual application this may be due to antagonistic relation between them. On the other hand mycorrhizal infection prevent or at least decrease their toxic effect, the results of mycorrhizal infection explain above explanation since the mycorrhizal infection decreased from 80% to (23.33, 63.33 and 20.00% for application Pb , Cd and their combinations (table 2, 3 and 4).

In general, the highest values of most of the studied traits were recorded from interaction treatment of mycorrhizal inoculum and 150 ppm of Pb (MPb_{150}). This may due to the role of mycorrhizal infection in the protection of wheat plant from toxic effect of Pb and other heavy metals. Similar results were recorded by (Umar, 2017).

Figure (1) indicated to significant correlation between total dry weight and combination between mycorrhizal infection and levels of applied Pb (MPb) with the correlation coefficient of (r = 0.99**). It is an indicator that clarifies the role of mycorrhizal infection in protection of wheat plant from toxic effect of Pb. At high concentration of applied Pb a few amounts of HMs ions due to passing through the cell wall get deposited in fungal cell membrane or transported to the fungal cytoplasm then reduction the amount of metal ions as mentioned by (Dhalaria et al, 2020).

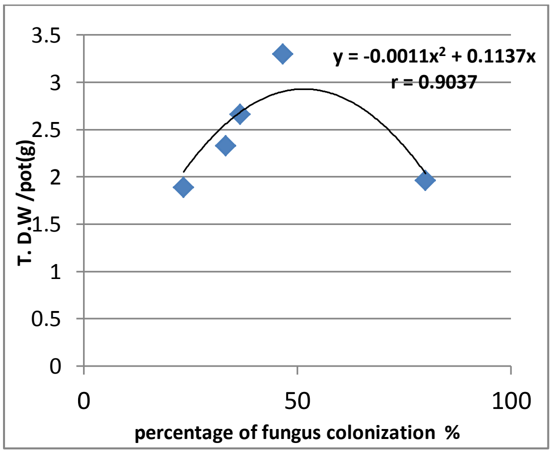
The result of statistical analysis showed significant correlation between % mycorrhizal infection and interaction treatments of (MCd) with correlation coefficient values of (r = 0.93** and

0.95**) for both T. D.W per pot and leaf area respectively (Figure 2 and 3). While the correlation coefficient was significant between MpbCd and T.D.W per pot only with correlation coefficient value of (r = 0.77**) as shown in figure (4).

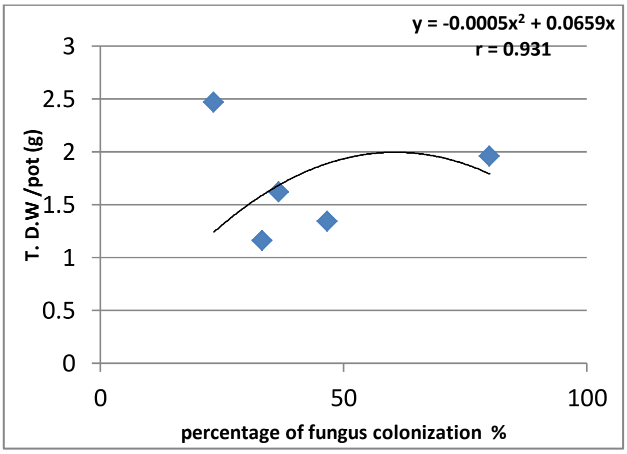
Abdulkarim, (2011) reported that in the case of mycorrhizal infection, leaf area and dry weight of leaves, steam, and roots increased compared to the control treatment. In mycorrhizal plants, the HMs tolerance potential is correlated with the yield of mycelial biomass and fungal growth, as metals remain accommodated in the fungal mycelium. Moreover, the vesicles in the fungi also increase with the increase in the concentration of HMs (Yang et al., 2015).

3.2- Infection percent %

Table (3) referred to interaction between mycorrhizal inoculum and levels Cd (MCd). In general the role of mycorrhiza is very clear which caused increase in some traits value and the negative role of Cd was minimized or changed to positive effect, in spite of the high toxicity Cd for plants, the mycorrhizal infection protected the wheat plant from this toxic element and the plant completed its life cycle successfully.



Fig(1): Relation between total shoot dry weight of wheat treated with MPb and percentage of fungus colonization



Fig(2): Relation between total shoot dry weight of wheat treated with MCd and percentage of fungus colonization

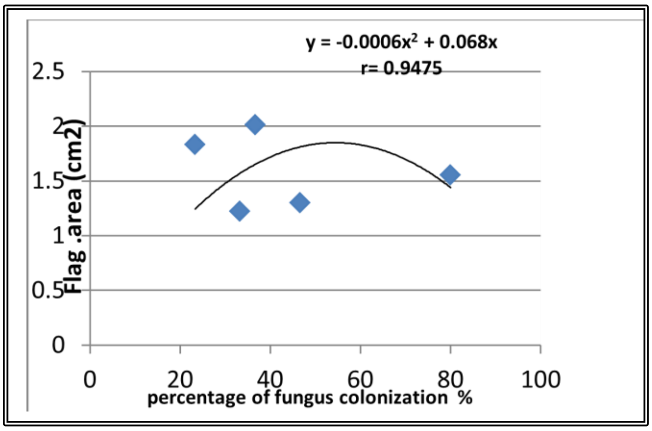


Fig (3): Relation between flag area of wheat leaves treated with MCd and percentage of fungus colonization

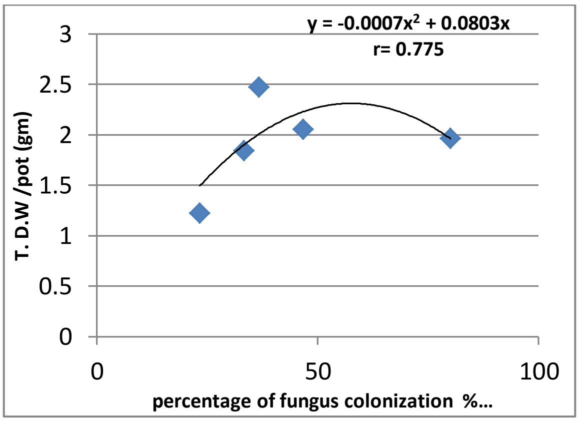


Fig (4): Relation between flag area of wheat leaves treated with MPbCd and percentage of fungus colonization

It has been shown from table (2, 3 and 4) that there are a significant differences between all treatments of both metals according to infection percent, the maximum mean value was recorded from (control) MPb_0Cd_0 which was (80%) while the lowest value was recorded from both combination treatments of $MPb_{100}Cd_{40}$, $MPb_{150}Cd_{60}$ which was 20%. This decrease may be due to unsuitable condition for wheat growth under high concentration of toxic heavy metal that may causes decrease in carbohydrate production then decrease in infection to a certain level. This observation is consistent with the findings of Koul et al. (2001), who found that high concentrations of heavy metals inhibited or even eliminated mycorrhizal colonization. With the addition of high Cd concentrations, root colonization rates in mycorrhizal plants were much reduced (Kanwal, 2015). AMF root colonization was reduced when Cd and Pb were added to the growing substrate (Nowak, 2007).

3.3- Concentration of Pb and Cd in wheat seeds and roots:

As shown from table (5) the mycorrhizal inoculum affected significantly on Pb concentration of wheat seed. The highest value (29.44 µg kg^{-1}) was recorded from interaction treatment of $MPb_{200}Cd_{80,}$ while the lowest value was recorded from combined application of $MPb_{50}Cd_{20}$ which was (0.83 µg kg^{-1}). Non-significant differences in Cd value were recorded among interaction treatments when the highest value of Cd was (1.99 µg kg^{-1}) recorded in treatment MPb_{100} while the lowest value was (0.24 µg kg^{-1}) recorded in treatment $MPb_{200.}$ This may be due to antagonistic relation between Pb and Cd as mentioned before.

Table (5) explains that the mycorrhizal inoculum in case of interaction between the studied two and three factors were affected significantly on Pb and Cd concentration of wheat roots. The highest values of Pb concentration (225.25 and 37.50 µg kg^{-1}) were recorded from interaction treatments of $MPb_{50}Cd_{20}$ and MCd40 respectively. While the lowest values (30.50 and 175 µg kg^{-1}) were recorded from interaction treatment of MPb_{50} and $MPb_{150}Cd_{50}$ respectively.

Mycorrhizal inoculum in case of two factors and three factors interactions were affected significantly on Cd concentration of wheat roots also, the highest and lowest Cd concentration in case of two factors interactions (5.50 and 1.75 µg kg^{-1}) were observed from interaction treatments of MPb200 and MCd60 respectively. While in case of interaction among the three studied factors the highest and lowest root Cd concentration (5.75 and 3.25 7.50 µg kg^{-1}) were obtained from interaction treatment of ($MPb_{150}Cd_{60}$ and $MPb_{100}Cd_{40}$) respectively.

Table (5) explains that the interactions between (M and Pb) and (M and Cd) caused significant decrease in concentration of Pb and Cd in root of wheat plant. This may be due to VAF's ability to fix heavy metals in the cell wall and store them in the vacuole, or chelate with other substances in the cytoplasm and so minimize metal toxicity in plants (Punamiya et al., 2010). On the other hand, the interaction between the three studied factors (MPbCd) caused significant decrease in Cd concentration only. In general, the above combination treatments caused decrease in Pb and Cd concentration in the root wheat. This pointed out the role of mycorrhizal infection in decrees the absorption of heavy metals by plants.

Table (5): Effect of mycorrhizal inoculum on Pb plus Cd concentration in seed and root of wheat plants at different levels of Pb and Cd.

Combination Treatments	Pb root µg kg^{-}	Pb seed µg kg^{-}	Cd root µg kg^{-}	Cd seed µg kg^{-}
MPb_0Cd_0 (control)	174.00 ^{a}	10.07 ^{a}	7.50 ^{a}	1.24 ^{a}
MPb_{50}	30.50 ^{b}	9.86 ^{a}	3.25 ^{bc}	0.99 ^{a}
MPb_{100}	31.25 ^{b}	12.58 ^{a}	4.50 ^{b}	1.99 ^{a}
MPb_{150}	45.75 ^{b}	4.96 ^{b}	2.25 ^{c}	1.49 ^{a}

MPb_{200}	**42.25 [b]**	**3.65 [b]**	**1.75 [c]**	**0.24 [a]**
MCd_{20}	**68.75 [bc]**	**3.65 [bc]**	**4.50 [b]**	**1.50 [a]**
MCd_{40}	**121.75 [ab]**	**9.65 [a]**	**4.75 [b]**	**0.74 [a]**
MCd_{60}	**37.50 [c]**	**5.00 [b]**	**5.50 [ab]**	**0.91 [a]**
MCd_{80}	**142.25 [a]**	**3.33 c**	**4.50 [b]**	**0.83 [a]**
$MPb_{50}Cd_{20}$	**225.25[a]**	**0.83 [c]**	**5.50 [ab]**	**0.65 [a]**
$MPb_{100}Cd_{40}$	**196.75[a]**	**12.65 [b]**	**3.25 [b]**	**1.41 [a]**
$MPb_{150}Cd_{60}$	**175.00 [a]**	**7.99 [b]**	**5.75 [ab]**	**0.48 [a]**
$Mpb_{200}Cd_{80}$	**193.00 [a]**	**29.44 [a]**	**4.00 [ab]**	**0.83 [a]**

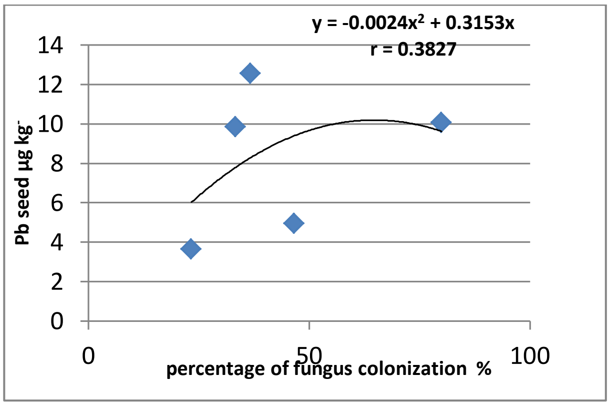
The most important and interested point in this study is that the infection of wheat plant with mycorrhiza at using different levels of Pb, Cd and (Pb + Cd) caused decreased in concentration of Pb and Cd In the wheat seeds. Metal dilution in plant tissues is thought to be caused by increased growth or chelation in the rhizospheric soil (Audet, 2014).

The high concentration of Pb and Cd in control treatment may be due to the role of sterilization in increasing the availability of the precipitate parts of Pb and Cd.

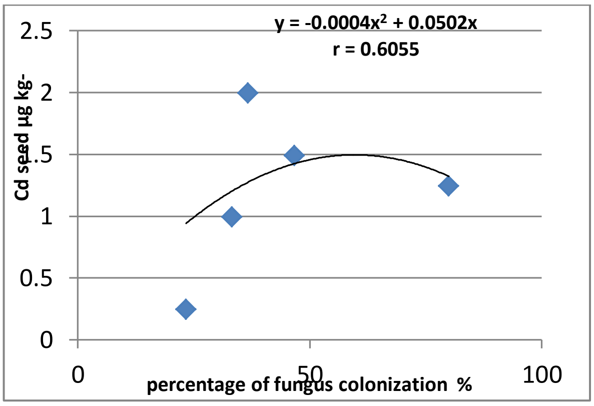
The concentration of Pb and Cd in the wheat seeds is very low in comparing with their concentration in the root (Tale, 5). It means the accumulation of in Pb and Cd were occurred in root not in edible part of plant (seed). The higher heavy metal concentration in AM plants could be explained by the fact that AM infection increased plant uptake of metals by mechanisms such as enlargement of the absorbing area, volume of accessible soil, and efficient hyphal translocation (Yu et al., 2004). The accumulation of the heavy metals Pb and Cd was more intensive in the root than in the shoot (Kastori et al., 1992).

The role of mycorrhizal infection is very clear in decrease heavy metals Pb and Cd in seed of wheat plant. This may be due to the capability of AMF in heavy metal translocation and distribution in inner root parenchyma cells (Kaldorf et al,. 1999). Or due to the following mechanisms: 1- Active efflux pumping of metals out of the cell by transporter system. 2- Heavy metal sequestration in vacuoles. 3- Exclusion of chelates to extracellular space (Umar, 2017). Or may be due to increase of root biomass and adsorption of metals on hypha wall and glomalin exudation as mentioned by (Janeeshma and Puthur, 2020).

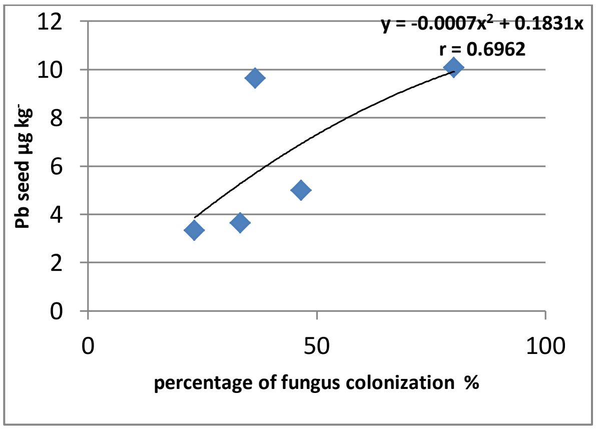
The most important and interesting point in this study is that the infection of wheat plant with mycorrhiza at using different levels of Pb, Cd and (Pb + Cd) caused decrease in concentration of Pb and Cd in the seeds. Carvalho et al., (2006) investigated that AMF colonization enabled the fungi to retain Cd in the mycelium and promoted plant tolerance to Cd, which led to an increase in the absorption area of the roots in the soil and resulted in better plant growth (Jankong and Visoottiviseth, 2008).



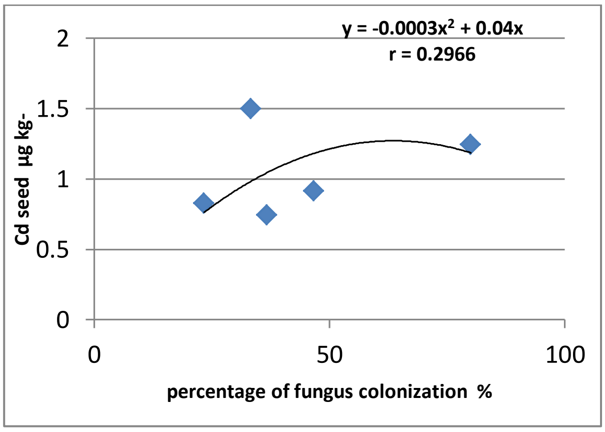
Fig(5): Relation between Pb content of wheat seed MPb and percentage of fungus colonization



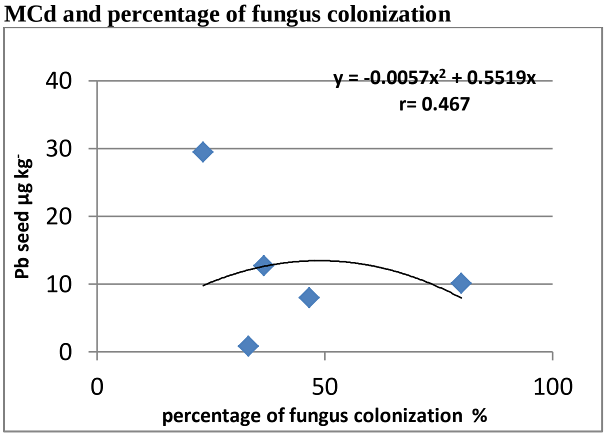
Fig(6): Relation between Cd content of wheat seed treated treated with with MPb and percentage of fungus colonization



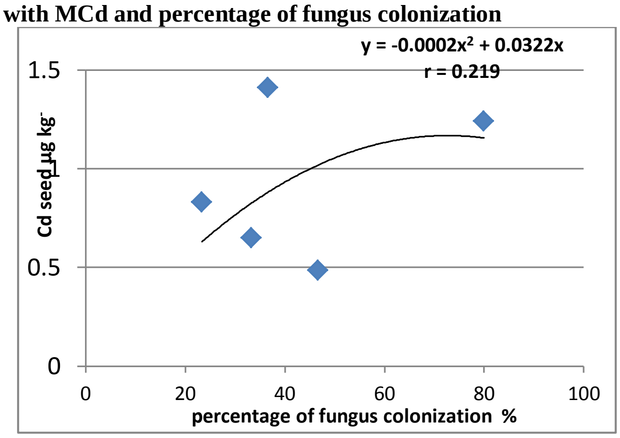
Fig(7): Relation between Pb content of wheat seed MCd and percentage of fungus colonization



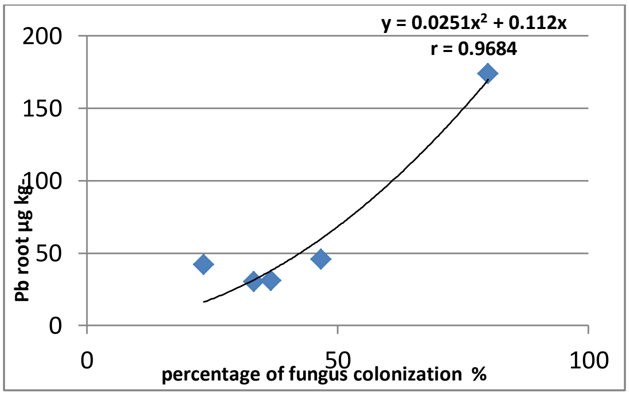
Fig(8): Relation between Cd content of wheat seed treated treated with with MCd and percentage of fungus colonization



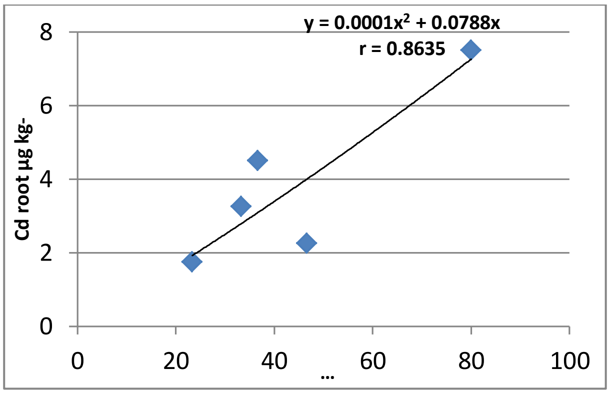
Fig(9): Relation between Pb content of wheat seed with MPbCd and percentage of fungus colonization



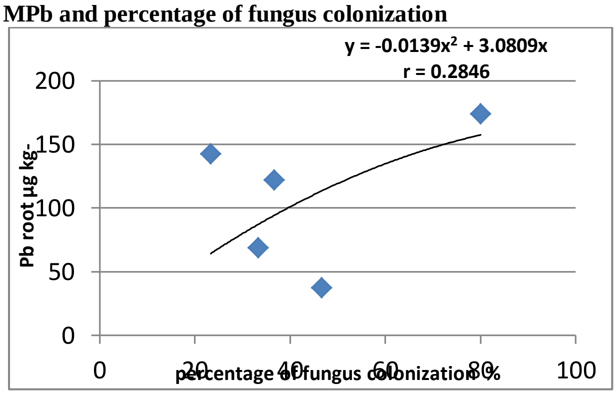
Fig(10): Relation between Cd content of wheat seed treated treated with MPbCd and percentage of fungus colonization



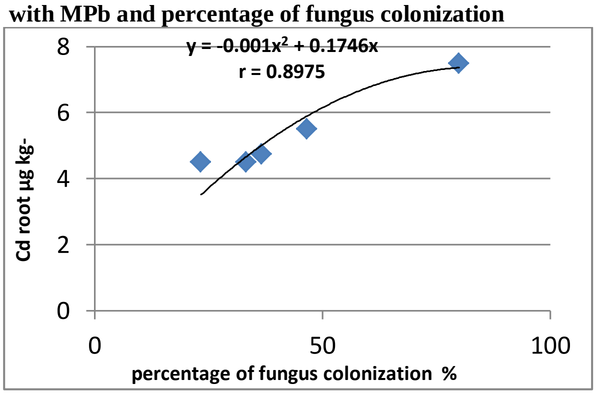
Fig(11): Relation between Pb content of wheat root MPb and percentage of fungus colonization



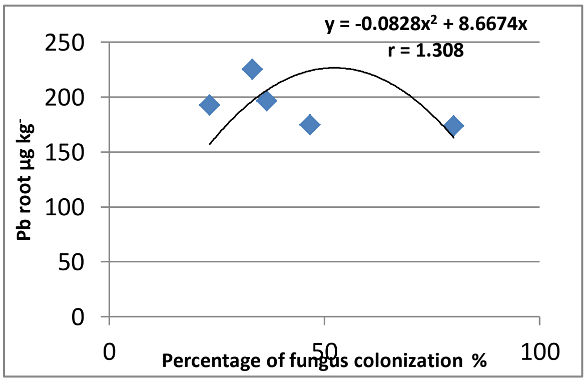
Fig(12): Relation between Cd content of wheat root treated treated with with MPb and percentage of fungus colonization



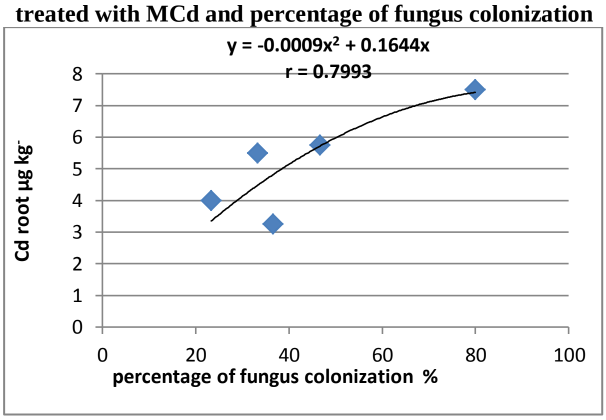
Fig(13): Relation between Pb content of wheat root MCd and percentage of fungus colonization



Fig(14): Relation between Cd content of wheat root treated with treated with MCd and percentage of fungus colonization



Fig(15): Relation between Pb content of wheat root MPbCd and percentage of fungus colonization



Fig(16): Relation between Cd content of wheat root treated with treated with MPbCd and percentage of fungus colonization

The Pb concentration decreased from 10.057 µg kg^{-1} to (3.65 and 3.33) µg kg^{-1} for combination treatments of (MPb_{200} and MCd_{80}) respectively, while the combination treatment of (MPb_{50} and MCd_{20}) caused decrease in Pb concentration, to 0.83 µg kg^{-1}. Which means metals accumulation by mycorrhizal in wheat root tissues, suggesting that an exclusion strategy for metal tolerance widely exists in them. The results agree with Ramtahal et al., (2012) and Kanwal et al., (2015).

4. CONCLUSION:

The mycorrhizal inoculation had significant effect in decreasing the risk of Pb and Cd on plant and human, due to the decrease in their concentration in edible part of whet plant. The studied combination treatment caused (2.78 to 12.13) and (1.91 -5.17) times decrease in concentration of Pb and Cd in wheat seed respectively.

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