The effect of lead and cadmium on some physiological characteristics of the fungi that cause wheat damping-off and some biological fungi

Article · January 2021

DOI: 10.22034/iji.v8i0.765

CITATIONS
0

READS
145

3 authors:

Qusai Hattab Madhi
agriculture
19 PUBLICATIONS **33** CITATIONS
SEE PROFILE

Mohammed H Abass
University of Basrah
75 PUBLICATIONS **512** CITATIONS
SEE PROFILE

Abdulnabi Abdulameer Matrood
University of Basrah
89 PUBLICATIONS **169** CITATIONS
SEE PROFILE

Iran. J. Ichthyol. (Special issue 2021): 412-426

P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

Research Article

The effect of lead and cadmium on some physiological characteristics of the fungi that cause wheat damping-off and some biological fungi

Qusai Hattab MADHI*, Mohammed Hamza ABASS, Abdulnabi Abdul Ameer MATROOD

Department of Plant Protection, College of Agriculture, University of Basra, Basra, Iraq.
**Email: qusaihattab82@yahoo.com*

Abstract

Heavy metals are considered the most important environmental pollutants that negatively affect soils' microbial community, including fungal populations. The current study aimed to assess the effect of lead and cadmium on the physiological characteristics of pathogenic and biological fungi. The results revealed that Cd at a concentration of 10mg/kg and Pb at 600mg/kg had the highest inhibition effect on the radial growth of examined fungi, reaching 42.40 and 32.71%, respectively. The sensitivity results of fungi showed that *T. koningii* was less susceptible to Pb and Cd, followed by *R. solani*. It was also observed that the colour of *T. koningii* fungus changed from dark green to light green undergoing the effect of Pb and Cd and that the colour change may be due to the resistance characteristic of the fungus. The results also indicated that there was a significant effect of Pb and Cd on the spore germination of fungi, as the germination decreased to 8.49 and 8.19×10^4, respectively, by the effect of high concentrations of the two heavy metals, compared to the control treatment, which was 15.99×10^4, as well as the effect of Pb and Cd on the dry weight of fungi with an exception for *T. koningii*. All examined fungi showed a positive detection of protease, lipase and cellulase enzymes, and this enzymatic activity was affected by Pb and Cd concentrations. The antagonistic ability of the bioagent *T. koningii* against pathogenic fungi varied in the presence of Pb and Cd. Therefore, bioagent could control pathogenic fungi and biological treatment of heavy metals in polluted soils.

Keywords: Biotic stressors, Extracellular enzymes, Fungi, Heavy metals, Tolerance.

Citation: Madhi, Q.H.; Abass, M.H. & Matrood, A.A.A. 2021. The effect of lead and cadmium on some physiological characteristics of the fungi that cause wheat damping-off and some biological fungi. Iranian Journal of Ichthyology 8(ICAEAS special issue 2021): 412-426.

Introduction

Soil is one of the most important natural resources for humans, and in this regard, study of arable soil microbes function exposed to heavy metals pollution is extremely important in agricultural soil (Chu 2018). Heavy metals are defined based on three different criteria: density, atomic number, or chemical properties (Radojevic et al. 2007) and any elements with an atomic number greater than 20 (Yu 2001). Heavy metals play an essential role in the biological processes of microorganisms, e.g. cobalt, chromium, copper, nickel and zinc are essential nutrients, while some minerals such as aluminium, cadmium and lead have no biological role. The pollution of soil and water with heavy metals greatly impacted their microbial activity and physiological features (Jose et al. 2015). Heavy metals are essential for fungal growth and metabolism, but they can also be toxic when their concentrations are above a certain threshold; while others are toxic even in trace amounts as microbial growth is affected by the presence of heavy metals in the soil (Ezzouhri et al. 2009; Nongmaithem et al. 2014).

Fungi constitute a group of microorganisms widely distributed in the environment, especially in soil, and play a pivotal role as major decomposers in the soil ecosystem (Seth et al. 2016). Additionally, their presence in soils strongly influences ecosystem

structure and functioning and thus plays a major role in many environmental services (Orgiazzi et al., 2012). Heavy metals affect soil fungi by altering their fungal morphology and physiological activity, and the growth patterns, reproduction, enzyme production, and active uptake (Mala et al. 2006; Turnauk et al. 2006). El-Sersy & El- Sharouny (2007) indicated that Cd is highly toxic and has a deleterious effect on microbial communities and their functional activities in the soil and that microorganisms exposed to higher concentrations of this heavy metal could develop a resistance feature. The entry of heavy metals into the environment induces physiological and morphological adaptations in the microbial community specifically. Fungal species adopt one or more mineral carrying strategies, including extracellular mineral sequestration and deposition, suppressed flow, enhanced mineral flux, and intracellular / extracellular enzyme production, cell walls metal binding, isolation and intracellular morphogenesis (Vadkertioca & Slavikova 2006). Heavy metals in the environment can interact directly with the enzymes of the fungi outside the cell, however, to induce such physiological and morphological responses, the fungus must absorb the heavy metals. Some heavy metals are important for fungus metabolism. However, some metals are toxic in concentrations only a few times those required (Baldrian 2003). The interference of heavy metals with the physiological, morphological, enzymatic and reproductive processes of white mold fungi has environmental consequences as it affects growth or directly the enzymatic activities, which leads to changes in the community structure and the energy flow in the ecosystem (Turnauk et al. 2006).

Globally, several studies were conducted to determine the response of fungi to heavy metals. In Pakistan, Baldrin (2002) studied the response of *Piptoporus betulinus* to Cd in solid cultural media, which led to a significant decrease in colony growth, while in the liquid nutrient medium, colony growth decreased slightly. In a study conducted by Nasin (2008) to find out the effect of inorganic copper salts on the growth of some fungi, they observed a decrease in the growth rates due to their toxic effect. In Malaysia, Yazdani et al. (2010) studied the response of the fungi *Trichoderma atroviride* to copper. The results showed the ability of this fungus to adsorb by 85% and absorb it by 47%. The ionic forms of Cu and Zn inhibit the fungal growth of *Aspergilus niger, Candida albicans*, and *Cryptococcus neoformans* (Abu-Mejdad 2013), the growth of *Fusarium oxysporum* and *Pythium debaryanum* was significantly inhibited in the presence of Cu, Zn, Cd and Pb (Golubovic et al. 2010). Pilgrims (2004) showed that different concentrations of Zn and Mg ions had a greater effect on *Cryptococcus neoformans* than copper because these elements could charge the ends of the carboxylic chains with a positive charge is associated with the negative charges of the capsules surrounding the yeast. Then they entered into the fungal cell, which affects the cell growth and division process. Heavy metals have a role in inhibiting the multiplication of DNA and then inhibiting the division process, which leads to a decrease in the growth rate of the fungus. Iwona et al. (2018) indicated that *T. viride* could tolerate up to 400ppm Pb, Cd, Cr and Ni concentrations. The current research aims to determine the physiological responses of the both plant pathogenic fungi (*Rhizoctonia solani, F. solani* and *Macrophomina phaseolina)* as well as bioagents *T. koningii* and *Chaetomium globosum* to Cd and Pb *in vitro*.

Materials and Methods

The experiments were carried out in the laboratories of the Plant Protection Department, College of Agriculture, University of Basra.

Sources of fungal isolates: The isolates of the pathogenic fungi *R. solani, F. solani* and *M. phaseolina* were obtained from the roots and soil of the wheat plant according to Abass et al. (2021) and the two fungi *T. koningii* and *C. globosum* from the laboratories of the Plant Protection Department,

College of Agriculture, University of Basrah.

Effect of heavy metals (HMs) on pathogenic and biological fungi: The concentrations of the two HMs of Pb and Cd were determined according to Madhi et al. (2020). Three concentrations of each element were determined as treatments. Cadmium chloride ($CdCl_2$) was used as a source of Cd and lead acetate $Pb(C_2H_3O)_2$ as a source of Pb, with concentrations of 200, 400 and 600mg/kg for Pb and 3, 6 and 10mg/kg of Cd. These concentrations were separately added to flasks containing the Potato Dextrose Agar (PDA) and the Malt Extract Agar (MEA) sterile. The flasks were shaken well to obtain a homogeneous spread, then poured into Petri dishes at a rate of 3 dishes for each concentration leaving a comparison treatment, and after hardening of the media, the inoculation of each plate center with a disc of 0.5cm in diameter taken from the edge of the fungal colonies was done. The plates were incubated at 25±2°C. After the arrival of the fungus growth in the comparison treatment to the border of the dishes, the following measurements were taken.

Radial toxicity: The fungal growth rate was measured by taking the average of two perpendicular diameters passing through the center of the petri dish after the fungal growth in the control treatment reached the edge of the dish. Then the percentage of radiation toxicity was calculated according to the following equation:

Radial toxicity= Diameter growth in the control treatment- diameter growth in the treatment/ Diameter growth in the control treatment *100

Sporulation: Disc with a diameter of 0.5cm were taken from the colonies of fungi developing in dishes treated with concentrations of Pb and Cd and put one disc and one of each concentration in a test tube containing 9ml of distilled water. The dilution was 10^{-1} and took 1ml of this dilution and put it in 9ml of distilled water to have a dilution of 10^{-2} until it reached 10^{-4}. The same method was used for the control treatment. Six replicates were used for each treatment. The test tubes were shaken vigorously to remove the fungal spores from the discs containing. A haemocytometer (0.025mm) was used to estimate the number of spores in 64 square. Eight readings were taken from each test tube, and the average number was calculated according to their numbers from the following equation:

$$\text{Number of spores in 1ml} = \text{Average number of spores} \times 2.5 \times \text{dilution factor}$$

Dry weight for growth: The liquid medium (PD) was prepared and distributed in 150ml flasks at a volume of 100ml per flask. The medium was sterilised, and after its temperature was reduced, it was inoculated with a disc of 0.5cm diameter for each fungus and each concentration of heavy metals. The flasks were incubated at 25±2°C for 10 days, and the flasks shacked every 2-3 days to ensure the distribution of the spores. The experiment was carried out with three

replicates for each fungus and concentration. The fungal mycelium was removed after the incubation with sterile forceps and washed several times with distilled water to remove traces of the medium, then dried on Whatman No.4 filter paper to remove the excess water. Dried mycelium was kept in an oven at 70°C for 24 hours (Mushin 1990).

Extracellular enzyme activity: Special media were used to qualitatively detect fungal isolates ability to produce enzymes. Discs with equal diameters were taken from the treatments, and the plates containing the culture media for the different enzyme tests were inoculated with three replicates for each treatment. Then the plates were incubated at 25±2°C. Before the fungal growth reached the edge of the plate and before filling the plate, enzymes were detected (Tables 1 and 2).

Cellulase activity: The method described by Reese and Mandels (1963) was followed to test the susceptibility of fungal isolates to exploit soluble cellulose. The medium was sterilized in an autoclave, except urea, prepared in a solution with sterile distilled water. Sterilizing was done by using a vacuum device, the solution through a 0.45μm fine membrane filter. Before the arrival of the fungal

Table 1. Components of cellulase enzyme medium.

Chemicals	Quantity (gm/L)
Urea	0.3
$MnSO_4.H_2O$	0.16
KH_2PO_4	2
$(NH_4)_2SO_4$	1.4
$MgSO_4.7H2O$	0.3
$CaCl_2$	0.3
$FeSO_4.7H_2O$	0.04
$ZnSO_4.7H_2O$	0.14
$COCl_2$	0.02
Peptone	0.8
CMC	10
Agar	20
Distilled water	1 Litter

Table 2. Components of the medium of the lipase.

Chemicals	Quantity
Tween 80	10 ML
Peptone	8 gm
$CaCl_2.2H_2O$	0.1 gm
Agar	20 gm
Dist. Water	1 L

growth to the edge of the plate, the cellulase reagent was added, which is a solution of iodine hydrochloric acid (HCl-Iodine Solution Cellulose) that prepared by mixing 100ml of 1N HCl and 500ml of 1% iodine (I) and potassium iodide (KI) 2%, as a function of weight: volume (Yeoh et al. 1985). The dye solution was added as 4ml/dish for 5-3min. Then the solution was poured from the plates. The ability of isolation to secrete the cellulase enzyme was inferred by forming a yellow halo around the colony.

Protease activity: Skim milk agar described in Cowan (1986) was used to test the ability of fungi to produce the protease enzyme by adding 20% skim milk to a sterile nutrient agar medium cooled to 45°C at pH=5. The dishes were inoculated with a 0.5cm diameter disc of the fungi colonies, with three replicates of each fungus, and the dishes were incubated at 25°C for three days. The activity of the fungus on the production of the protease enzyme was checked by the formation of a transparent halo around the colony.

Lipase activity: The medium described by Sierra (1957) was used to detect lipase activity; a Tween 80 was sterilized in a flask in the oven at 60°C for 15min. While the rest of the medium were sterilized by autoclaving. Then Tween 80 was added to the rest of the sterile medium, and its temperature decreased. The lipase enzyme was detected by forming white crystals under the mycelium or immersed in the culture medium surrounded by the fungal colony.

Statistical analysis: The experiment was designed using a Complete Randomization Design (CRD) and the Least Significant Difference (LSD) test to compare the averages at the 0.05 probability level. The Statistical Package for Social Science (SPSS. version 23) was used for data analysis. The results represented an average of three replicates per treatment.

Results and Discussion

Effect of lead and cadmium on radial growth of fungi *in vitro:* The results indicated that Cd of 10mg/kg treatment was the most effective in inhibiting the radial growth of fungi, as it recorded the highest inhibition rate of 42.40% with a significant difference than other treatments. Pb at

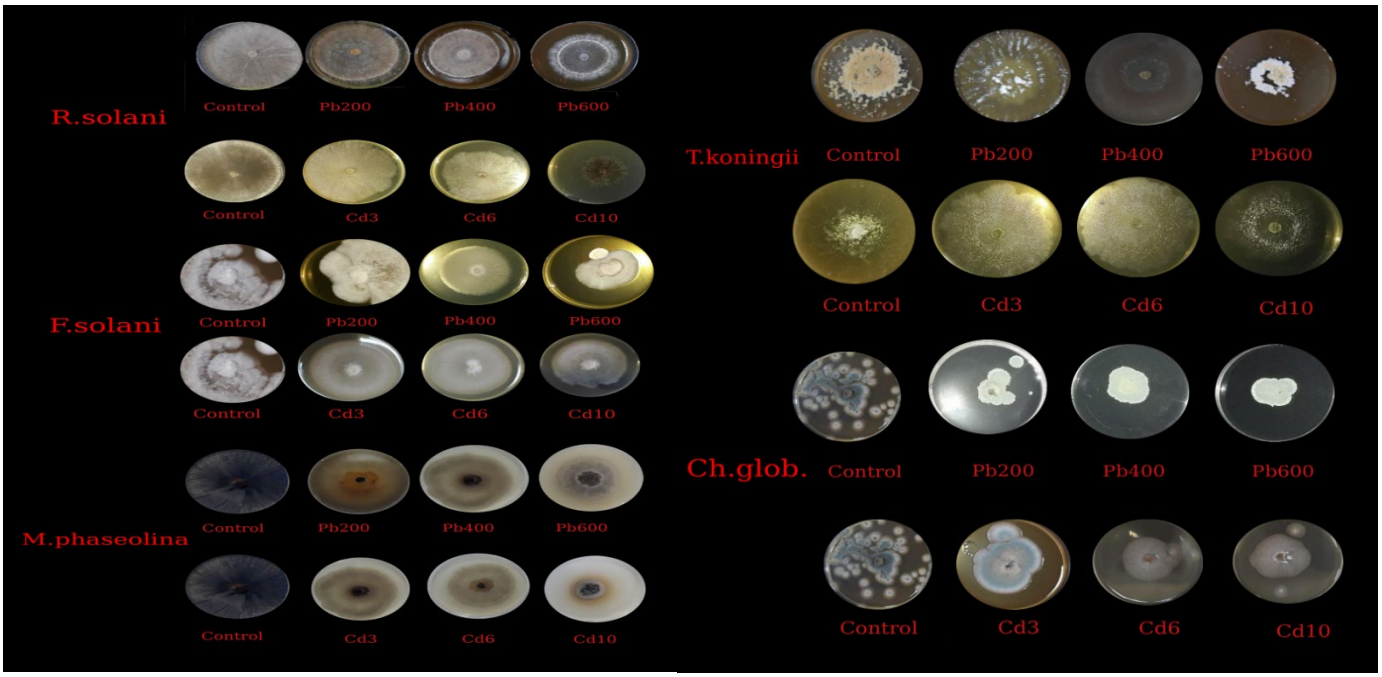

Fig.1. Effect of lead and cadmium on fungal colonies in PDA.

Table 3. Effect of lead and cadmium on radial growth of fungi in PDA and MEA media.

Fungi		HM						Average of medium	Average of fungi
	culture media	Pb200	Pb400	Pb600	Cd3	Cd6	Cd10		
R.solani	PDA	13.30	34.23	36.06	25.53	34.73	65.90	29.96	22.4
	MEA	5.55	10.96	28.26	7.96	16.25	34.93	14.84	
Average of HM and fungi		9.42	22.59	32.16	16.74	25.49	50.41		
F.solani	PDA	22.55	38.30	48.30	45.70	39.76	42.61	36.57	32.35
	MEA	20.40	34.01	34.70	30.62	34.63	52.02	28.13	
Average of HM and fungi		21.47	36.15	41.5	38.16	37.15	52.02		
M. phaseolina	PDA	0.00	45.50	55.53	36.96	48.30	82.93	38.89	29.76
	MEA	0.00	29.16	38.10	4.43	29.60	43.10	20.62	
Average of HM and fungi		0.00	37.33	46.71	20.69	38.95			
T. koningii	PDA	0.00	15.50	29.03	51.43	30.70	55.16	25.97	16.94
	MEA	0.00	0.00	0.00	0.00	1.46	53.86	7.90	
Average of HM and fungi		0.00	15.50	29.03	51.43	21.02	54.51		
C. globosum	PDA	28.26	41.63	55.86	27.90	29.76	69.96	36.20	30.61
	MEA	20.53	29.40	34.20	12.90	24.60	53.63	25.03	
Average of HM and fungi		24.39	35.51	44.88	20.4	27.18	61.79		
Average of HM	0.00	11.05	26.31	36.00	24.34	28.98	56.31	L.S.D= 7.06	
Average of HM and medium.	PDA	7.81	25.89	28.32	31.97	22.91	24.04	43.50	L.S.D= 7.07
	MEA	0.66	20.89	33.28	39.52	23.13	29.86	41.30	
Average of medium.	PDA	34.97	L.S.D=N.S.	L.S.D	Fungi	5.96	Fungi	5.96	
	MEA	19.31			Fungi	7.16	Triple	10.52	

600mg/Kg treatment was ranked second, which reported 32.71%, and it was not significantly different than Pb at 400mg/kg (Table 3, and Figs. 1, 2). *Trichoderma koningii* was the least affected by Pb and Cd concentrations, as it recorded the lowest inhibition rate of 16.94%, followed by *R. solani*, with an inhibition rate of 22.4%, while *C. globosum* and *M. phaseolina* were the most affected, as they recorded the highest inhibition rates of 30.61 and 29.76%, respectively. The results o indicated no

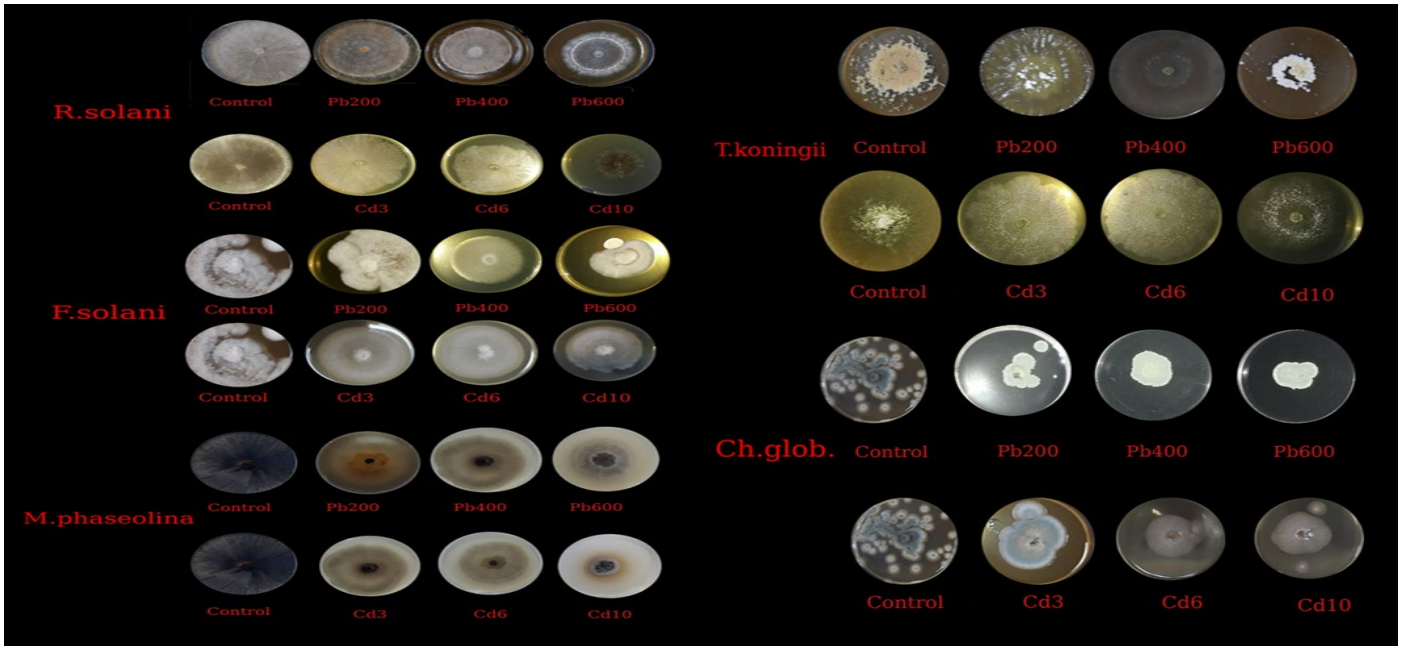

Fig.2. Effect of lead and cadmium on fungal colonies in MEA

Table 4. Effect of lead and cadmium on sporulation of fungi in PDA and MEA.

Fungi	culture media	HM							Average of med. and fungi	Average of fungi
		Cont.	Pb200	Pb400	Pb600	Cd3	Cd6	Cd10		
F. solani	PDA	13.86	12.80	10.90	8.90	9.96	6.66	3.50	9.51	11.96
	MEA	34.50	16.33	9.13	12.73	12.06	8.90	7.25	14.41	
Average of HM and fungi		24.18	14.56	10.01	10.81	11.01	7.78	5.37		
M. phaseolina	PDA	19.40	13.86	12.00	7.86	8.56	5.50	3.90	10.23	11.05
	MEA	25.66	16.80	12.66	8.40	8.40	6.20	5.53	11.87	
Average of HM and fungi		22.53	15.33	12.33	8.13	8.48	5.85	4.71		
T. koningii	PDA	17.56	14.30	12.06	9.13	9.23	8.50	5.86	10.11	11.55
	MEA	19.63	17.23	16.56	9.90	12.73	8.66	6.23	12.99	
Average of HM and fungi		18.59	15.76	14.31	9.51	10.98	8.58	6.04		
C.globosum	PDA	11.46	7.83	6.90	6.66	6.16	5.66	2.66	6.76	8.70
	MEA	17.63	12.73	9.13	8.83	10.33	8.13	6.46	6.46	
Average of HM and fungi		14.54	10.28	8.01	7.74	8.24	6.89	3.56		
Average of HM		19.96	13.98	11.16	9.05	9.67	8.30	5.67	L.S.D=4.45	
Average of HM and med.	PDA	16.08	12.19	8.47	7.47	10.40	8.52	5.01	L.S.D= N.S	
	MEA	23.06	15.16	10.20	7.41	12.82	8.73	5.33		
Mean of med.	PDA	9.68	L.S.D=N.S.	L.S.D	Fungi	3.15	Fungi and med	N.S		
	MEA	12.07			Fungi and HM	1.63	Triple	N.S		

significant differences between the PDA and MEA on the radial growth of fungi. A significant interaction was observed between Pb and Cd and PDA and MEA on the radial growth of fungi.

The results of the interactions indicated a significant difference between the PDA and MEA culture media and *T. koningii* recorded the lowest inhibition rate of 25.97 and 7.90%, respectively, by

the effect of the two media. It did not differ significantly from *R. solani* and *F. solani*, while *M. phaseolina* recorded the highest inhibition rate reaching 38.89 and 20.62%, respectively, and it did not differ significantly from *C. globosum*.

The examined fungal isolates were resistant to low concentrations of heavy metals and showed strong growth, but the higher concentrations caused decreased growth. The decrease in growth rate is a typical response of fungi to toxic substances (Turnauk et al. 2006). *Fusarium solani* was not affected by the low concentration (100mg/ml) Ni and Co, but the radial growth of the fungus was inhibited at their high concentration of 300mg/ml (Meenal & Sarita 2000). Sanyal et al. (2005) also mentioned that *F. oxysporum* was affected by Pb. It was also found that *Penicillium chrysogenum* was resistant to Cr, but it was sensitive to Pb. This means that the level of resistance differed between the different isolates. Babich & Stotzky (1979) indicated that the growth rates of *R. solani, F. solani*, *T. viride*, and *Cunninghamella echinulata* decreased initially at 10g/ml of Pb and gradually decreased with increasing the concentration to 500gml. In a study by Nongmaithem et al. (2014), the concentrations of 2 and 3ppm of Ni and Cd were highly toxic to *R. solani*. They had significantly reduced radial growth, biomass production and sclerotia development. The effect of Zn and Pb was studied in different concentrations on the growth rate of *T. viride,* as the fungus was resistant to heavy metals, and the lower the concentration, the higher the rate of growth of fungi was caused (Ekundayo et al. 2018). Cd, Co and Ni at concentrations of 500mg/ml affected the growth and survival of *M. phaseolina* and inhibited the growth to 78.8, 73.6 and 11.8%, respectively (Dubey 1988).

The colony's morphology changed with higher concentrations of heavy metals, and the production of pigments indicates the precipitation of metal ions on the fungal cell wall (Gruhn et al. 1991). The change in the colour of the fungal cell wall may be due to the mineral effect (Martino 2000). Morphological changes were observed in all the isolated fungi upon exposure to heavy metals on the agar media, as the colour changed from pink to white in *Paecilomyces* and *Scopulariopsis* genera. In *Trichoderma* and *Aspergillus*, the green colour changed to white, and the red colour appeared in *Aspergillus* and the sides of its colonies in the culture medium and may be due to cadmium binding to cell-wall protein in fungi (Yazdani 2010; Velmurugan et al. 2010). Jarosz-Wilkołazka et al. (2006) indicated that CD induced the formation of an orange-brown pigment in growth biennis, and the aerial growths disappeared and changed colour from green to white in the culture medium. A possible explanation for these different morphological changes between isolates might be the extensive detoxification / tolerance mechanisms that each isolate applies (Zafer & Ahmed 2007). *Aspergillus aculeatus* showed small and separated colonies on medium containing cadmium. In colonies were dark brown and white in Pb, and the presence of Cr led to the growth of a smaller white colony of *A. sclerotiorum*, but under the influence of Cd and Pb, smaller and more separated colonies could be observed. In the control treatment, the colonies were larger than all other treatments in the presence of Cr and Pb (Fiza et al. 2020).

The results indicated that Pb and Cd had a significant effect on spore germination, as the control treatment was recorded a rate of 15.99×10^4 (Table 4). The results of sporulation showed a decreased pattern with an increase of the HMs concentrations, as Pb of 600 and Cd 10mg/kg reported the lowest sporulation rate of 8.49 and 8.19×10^4, respectively. The media PDA and MEA did not have any significant effect on fungal sporulation, and no interaction was observed between the fungi and the culture media, while the results showed an interaction between fungi and heavy metals. The heavy metals reduced the sporulation of *Glomus etunicatum* and *G. intraradices*. Zn concentration of 0.1mM led to inhibition of sporulation and had a moderate effect on the density of mycelium, as for the concentration of 0.1mM of Pb and Cd and 10m molar of Zn, the

Table 5. Effect of lead and cadmium on the dry weight of fungi in PD and ME.

Fungi	culture media	HM							Average of med. and	Average of fungi
		Cont.	Pb200	Pb400	Pb600	Cd3	Cd6	Cd10		
R.solani	PDA	0.688	0.674	0.324	0.266	0.582	0.303	0.164	0.428	0.394
	MEA	0.667	0.625	0.185	0.171	0.535	0.188	0.136	0.358	
Average of HM and fungi		0.677	0.649	0.254	0.218	0.558	0.245	0.15		
F.solani	PDA	0.602	0.224	0.174	0.124	0.204	0.157	0.119	0.230	0.195
	MEA	0.118	0.185	0.162	0.135	0.177	0.148	0.124	0.160	
Average of HM and fungi		0.395	0.204	0.168	0.129	0.190	0.152	0.121		
M.phaseolina	PDA	0.404	0.233	0.167	0.124	0.168	0.224	0.114	0.204	0.190
	MEA	0.352	0.187	0.156	0.120	0.148	0.136	0.113	0.177	
Average of HM and fungi		0.378	0.21	0.161	0.122	0.156	0.18			
T.koningii	PDA	0.894	0.605	0.364	0.304	0.435	0.354	0.275	0.461	0.413
	MEA	0.736	0.504	0.225	0.285	0.314	0.258	0.223	0.363	
Average of HM and fungi		0.815	0.554	0.294	0.294	0.374	0.306	0.249		
C.globosum	PDA	0.366	0.256	0.181	0.145	0.213	0.125	0.107	0.199	0.179
	MEA	0.188	0.180	0.173	0.122	0.178	0.161	0.103	0.157	
Average of HM and fungi		0.277	0.218	0.177	0.133	0.195	0.143	0.105		
Average of HM		0.508	0.367	0.211	0.179	0.295	0.205	0.147	L.S.D= 0.12	
Average of HM and med.	PDA	7.81	25.89	28.32	31.97	22.91	24.04	43.50	L.S.D= 0.15	
	MEA	0.66	20.89	33.28	39.52	23.13	29.86	41.30		
Average of med.	PDA	0.296	L.S.D=0.05.	L.S.D	Fungi	0.21	Fungi and med.	0.03		
	MEA	0.264			Fungi and HM	0.17	Triple	0.16		

sporulation was accompanied by inhibited fungal growth (Pawlowska & Charvat 2004). The increased level of heavy metals decreased the germination of *G. mosseae* (Vivas et al. 2005). Levinskaite (2001) showed that Cd and Ni at 0.2mM had no effect on sporulation of *P. funiculossum* and *P. atramentosum*, but when increase to 0.5 mM had a negative impact on sporulation. *Trichoderma* spp. can sporulate well on exposure to arsenic (Cao et al. 2008). Zn and Pb at 5 and 10 ppm did not affect the growth of fungi or sporulation of *T. viride*, and the contents of cysteine and glutathione were high always observed when the fungi grew in the presence of heavy metals (Babu et al. 2014).

Effect of lead and cadmium on the dry weight of fungi in PD and ME media: The results indicated that high concentrations of Pb and Cd reduced the dry weight of the fungi, as Pb 600mg/g recorded a decrease of 0.215g, and Cd 10mg/g recorded 0.204g where control treatment was 0.341g (Table 5). The results also indicated a significant decrease in the dry weight of the fungi, and that the lowest decrease was recorded by *C. globosum* which was 0.179g. The dry weight of *T. koningii* was not affected by the Pb and Cd, as it recorded a dry weight of 0.413g compared to the control treatment recorded as 0.394g. The results of the statistical analysis indicated that there is an interaction between heavy metals and the cultural media, and that the high concentrations of lead and cadmium reduced the dry weight significantly, as it was recorded as 0.187 and 0.150g, respectively, for the PD and 0.172 and 0.145g, respectively, for the ME compared to the control treatment for the PD and ME, which were 0.586 and 0.430 g, respectively. The results also indicated an interaction between the culture media and the fungi, and *T. koningii* recorded the highest dry weight rate compared to the rest of the fungi.

The dry weight of the fungi of *P. globosum* and *Alternaria chlamydosporia* were decreased by increasing the concentration of Pb and Cu in the

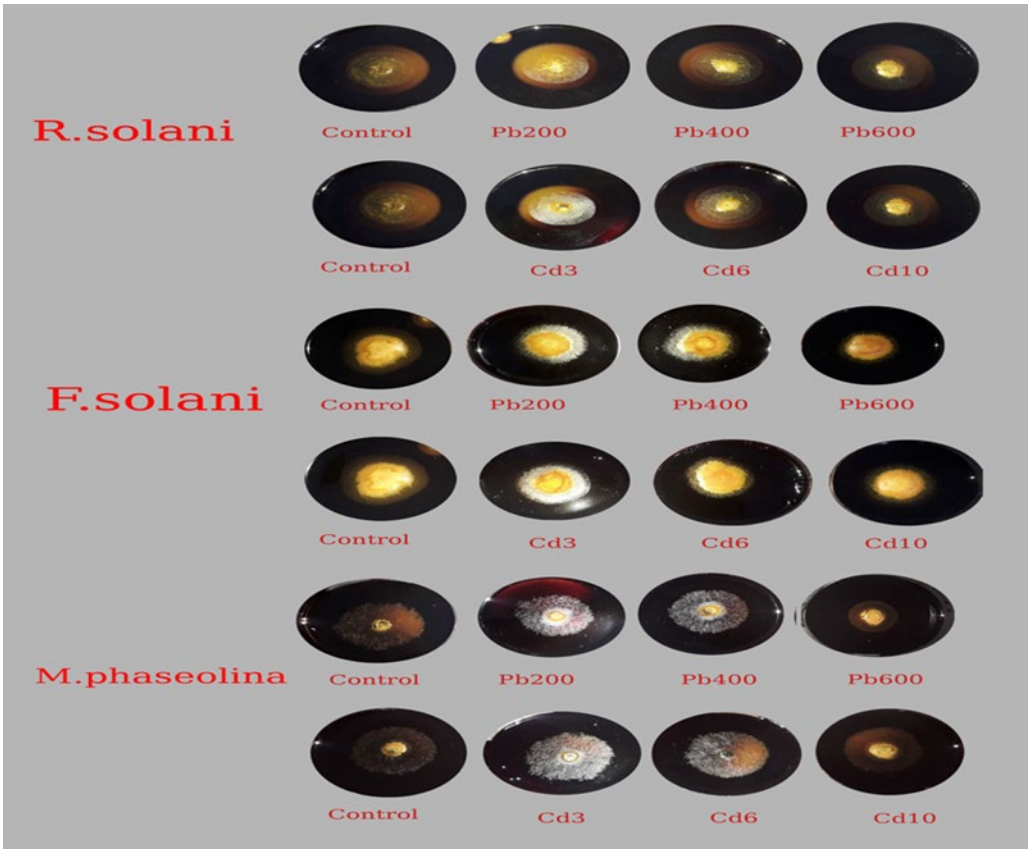

Fig.3. The effect of lead and cadmium on the cellulase enzyme activity.

Table 6. Effect of lead and cadmium on cellulase enzyme activity.

Concen.	Fungi			Average of concen.
	R.solani	*F.solani*	*M.phaseolina*	
Control	1.700	1.633	1.566	1.633
Pb200	1.533	1.466	1.433	1.477
Pb400	1.433	1.400	1.333	1.388
Pb600	1.000	0.866	0.833	0.900
Cd3	1.466	1.466	1.300	1.411
Cd6	1.266	1.200	1.100	1.188
Cd10	0.966	0.866	0.600	0.811
Average of fungi	1.337	1.271	1.166	L.SD Fungi = 0.048
L.S.D Concen.= 0. 711		L.SD fung + Concen. =0.127		

growth medium when the fungi stimulated at the concentration of 2mM of Pb by about 23 and 9.6%, respectively while the dry weight decreased to 17 and 29%, respectively at concentrations 6 and 12mM (Al-Kadeeb 2007). The dry weight of *Phanerochaete chrysosporium*, *A. awamori*, *A. flavus* and *T. viride* in the presence of Cd at 50ppm ranged from 0.02 to 0.5g. Joshi et al. (2011) indicated that the maximum dry weight 0.5g of *P. chrysosporium, A. flavus* and *A. awamori* in a PD medium contained 50ppm of Cd, *T. viride* scored 0.08 at 50ppm with Cd, and the dry weight in the presence of Pb ranged from 50ppm. Kacprzak & Malina (2005) reported that the dry weight of the fungi decreased with increasing Zn and Fe, and *T. atroviride* recorded the highest dry weight. The dry weight of *Cylindrocarpon destructans* decreased by increasing Pb, Zn, Cu and Cd (Rozycki 1993).

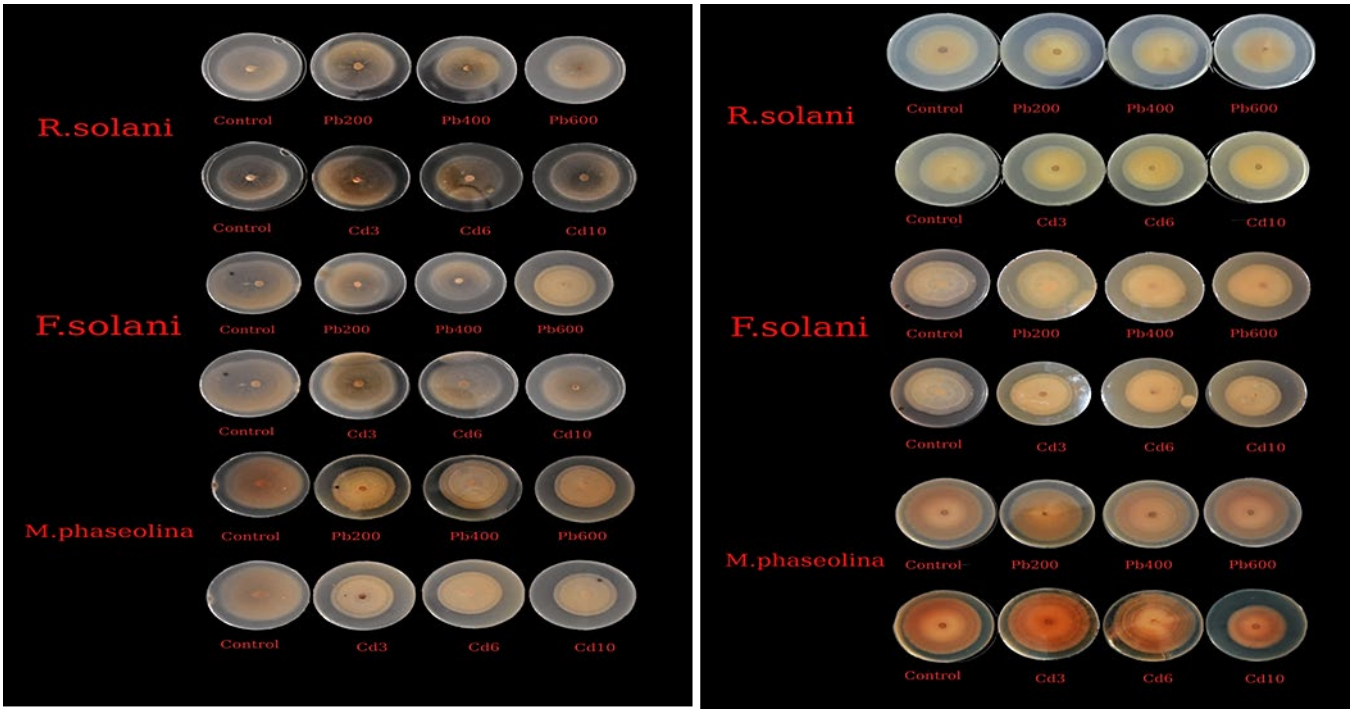

Fig.4. The effect of lead and cadmium on the lipase enzyme activity.

Table 7. Effect of lead and cadmium on the activity of the protease enzyme.

Concen.	Fungi			Average of concen.
	R.solani	*F.soalni*	*M.phaseolina*	
Control	1.966	1.666	0.966	1.533
Pb200	1.866	1.533	0.800	1.400
Pb400	1.766	1.466	0.733	1.322
Pb600	1.633	1.366	0.633	1.211
Cd3	1.833	1.433	0.733	1.333
Cd6	1.700	1.266	0.600	1.189
Cd10	1.575	1.233	0.566	1.111
Average of Fungi	1.757	1.423	0.719	L.S.D Fungi= 0.12
L.S.D Concen.= 0.18	L.S.D. Fungi + concen. =N.S.			

Effect of Pb and Cd on extracellular enzymes activity: The results indicated a significant difference between the fungi in their ability to secrete the cellulase enzyme, as *R. solani* recorded a halo diameter of 1.337cm, followed by *F. solani* and *M. phaseolina*, which recorded a halo diameter of 1.271 and 1.166cm; respectively (Table 6; Fig. 3). The results also indicated that the high concentrations of Pb and Cd had a significant effect in reduction of the halo diameter, as the halo diameter decreased to 0.900cm with the effect of Pb at 600mg/kg and to 0.811cm with the effect of cadmium at 10mg/kg compared to the control which reached to 1.633cm. The results showed an interaction between the fungi and the concentrations of Pb and Cd.

The results showed that all examined fungi had a positive detection of the protease enzyme on the medium but with different activities. *Rhizoctonia solani* recorded the highest enzyme activity of 1.757cm as a transparent region surrounding the fungus colony, followed by *F. solani* and *M. phaseolina*, which reached 1.423 and 0.719cm,

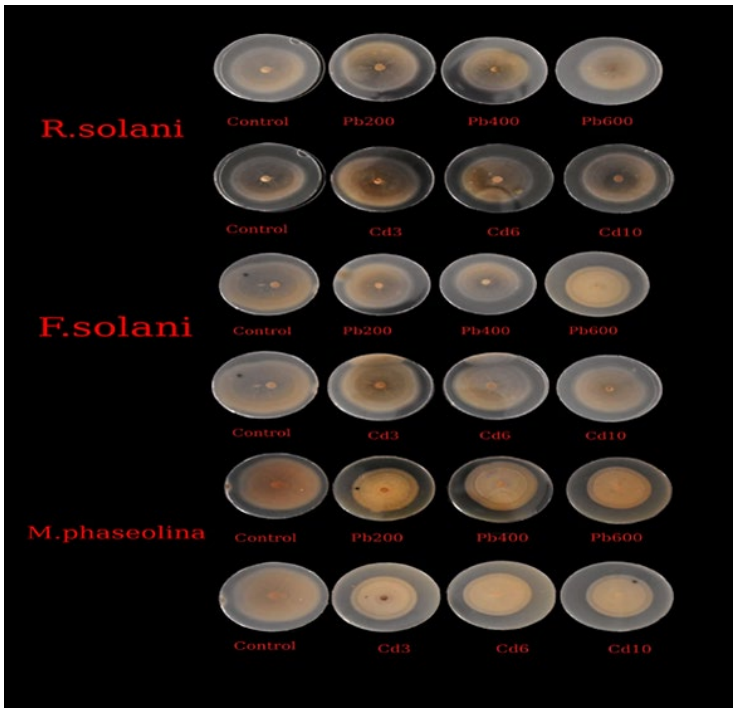

Fig.5. The effect of lead and cadmium on lipase enzyme activity.

respectively (Table 7, Fig. 4). The results also indicated that the concentration of Pb 600mg/kg and Cd 10mg/kg were the most influential on the protease enzyme activity, with halo diameter decreased to 1.211 and 1.111cm, respectively, whereas that of the control treatment was 1.533cm. The results indicated no interference between the fungi and the concentrations of Pb and Cd.

The fungi showed variation in their ability to produce the lipase enzyme (Table 8, Fig. 5), as *R. solani* recorded the highest enzyme activity of 1.55cm and did not differ significantly from *F. solani,* which recorded a halo diameter of 1.067cm, but it differed significantly from *M. phaseolina* that reported 0.695cm. The results indicated that the concentrations of Pb and Cd had a clear effect on the effectiveness of the lipase enzyme, as it was observed to decrease the diameter of the halo with an increase in the concentrations, as it fell to 1.022 and 1.044cm by the effect of lead 600mg/kg and cadmium 10mg/kg, respectively, compared to the control treatment amounted 1.233cm. The results indicated an interaction between the fungi and the concentrations of Pb and Cd.

Fungi produce enzymes inside and outside the cells that help them overcome the concentration of heavy metals and possibly acquire active absorption, complexity, crystallization, and bio-absorption of their cell walls (Zafar et al. 2007). Baldrian (2003) pointed out the possibility of a heavy metal reaction in the environment is directly associated with the extracellular enzymes produced by fungi, but for the physiological response to occur the fungi must take in the heavy metals. The inhibitory effect of heavy metals may be attributed to being potent inhibitors of enzymatic reactions. An example of that is mercury, whose toxic effect is mainly attributed to it by binding to SH groups present in the active or regulatory sites of the enzyme and causing them to be permanently inactivated, while Cu and Cd in addition to binding to aromatic amino acid residues in enzyme molecules can also cause oxidative damage to proteins by inducing oxidative stress associated with the production of reactive oxygen species such as hydroxyls or superoxide radicals (Stohs & Bagchi 1995). The enzymes produced outside the cell often

Table 8. Effect of lead and cadmium on the activity of the lipase enzyme.

Concen.	Fungi			Average of concen.
	R.solani	*F.soalni*	*M.phaseolina*	
Control	1.700	1.200	0.800	1.233
Pb200	1.633	1.133	0.766	1.178
Pb400	1.566	1.066	0.700	1.111
Pb600	1.466	0.966	0.633	1.022
Cd3	1.600	1.033	0.733	1.122
Cd6	1.500	0.933	0.666	1.033
Cd10	1.433	1.133	0.566	1.044
Average of Fungi	1.55	1.067	0.695	L.S.D Fungi= 0.338
L.S.D Concen.= 0.127	L.S.D. Fungi + concen. =0.222			

encounter high concentrations of heavy metals and cannot be protected by cell-related metal detoxification mechanisms as well. After the heavy metals enter the cell, they can also influence the production of extracellular enzymes on levels of transcription and translation regulation (Fogarty & Tobin 1996). Vig et al. (2003) stated that fungi under heavy metal stress could exploit growth energy to maintain their damaged cells, and this may explain why fungi can maintain their ability to dissolve and develop a halo in a solid medium., Muhammad et al. (2005) also stated that the appearance of the halo in a solid medium is related to the interaction of organic acids released by the fungus, whose production is stimulated by the presence of heavy metals. Organic acids help fungi adapt to heavy metals through their ability to chelate heavy metal compounds. Hartikainem et al. (2012) indicated that copper and zinc concentrations affect the production of extracellular enzymes for ascomycetes, basidiomycetes, and zygomycetes fungi. There is a variation in the responses of copper and zinc concentrations concerning the production of endoglucanases among the tested fungi, Cu was more toxic than zinc for all fungi as well as copper reduced the activity cellulase enzyme.

Kredics et al. (2001) indicated that 1mM mercury concentration inhibited enzymes such as B-1,4-N-acetylglucosamindase, trypsin, chymotrypsin-like, and protease, and to a lesser extent concerning aluminum, as a concentration of 1mM of Pb inhibited the protease enzyme. Heavy metals inhibited cellulase, pectinase, and protease enzymes of the fungus *Hebeloma crustuliniforme* (Dahm & Strzelczyk 1996). Kacprzak and Malina (2005) reported that *T. viride* and *Mortierella exigua* had the ability to form chemical complexes between metal ions and extracellular enzymes. Sipos et al. (2010) also indicated that the degradation of cellulose forming the plant cell wall upon infection is due to the activity of the cellulase enzyme produced by the pathogen (Sipos et al. 2010). Syamsia et al. (2019) indicated that the secretion of cellulase enzyme around the fungal colony contains a medium containing cellulose and is characterized by the formation of a clear region indicating the activity of the cellulase enzyme, the difference in the clear region formed around colonies of fungi isolates showed differences in the ability to produce the enzyme cellulose.

Conclusions

Lead and cadmium negatively affect most of the physiological properties of fungi such as radial growth, sporulation and dry weight. This effect increases with increasing concentrations, and a decrease in extracellular enzymes was observed as a result of the effect of lead and cadmium.

Acknowledgments

The authors are grateful to A. Abboud Al-Khalifa and D. Sabeeh Altai for performing the statistical analyzes.

References

Al-Kadeeb, A.S. 2007. Effect of lead and copper on the growth of heavy metal resistance fungi isolated from second industrial city in Riyadh, Saudi Arabia. Journal of Applied Sciences 7(7): 1019-1024.

Abu-Mejdad, N.M.J.A. 2013. Response of some fungal species to the effect of copper, magnesium and zinc under the laboratory condition. European Journal of Experimental Biology 3: 535-540.

Abass, M.H.; Madhi, Q.H.; Matrood, A.A. 2021. Identity and prevalence of wheat damping-off fungal pathogens in different fields of Basrah and Maysan provinces. Bulletin of the National Research Centre 45*:* 51

Babich, H. & Stotzky, G. 1979. Abiotic Factors Affecting the Toxicity of Lead to Fungi. Applied and Environmental Microbiology 38(3): 506-513.

Baldrian, P. 2002. Mycological Society of America Journal 94(3): 428-436.

Baldrian, P. 2003. Interactions of heavy metals with white-rot fungi. Enzyme Micro-biology and Technology 32: 78-91.

1.1Babu, A.G.; Shim, J.; Bang K.S.; Shea, P.J. & Oh, B.T. 2014. *Trichoderma virens* PDR- 28: a heavy metal-tolerant and plant growth-promoting fungus for remediation and bioenergy crop production on mine tailing soil. Journal of Environmental Management 132: 129-134.

Cowan, J.T. 1986. *Manual for the identification of medical bacteria* .2nd Cambridge University. Press London. pp: 146-156.

Cao, L.; Jiang, M.; Zeng, Z.; Du, A. & Tan, H. 2008. *Trichoderma atroviride* F6 improves phytoextraction efficiency of mustard (*Brassica juncea* (L.) Coss. var. foliosa Bailey) in Cd, Ni contaminated soils. Chemosphere 71: 1769-1773.

Chu, D. 2018. Effects of heavy metals on soil microbial community. IOP Conf. Series: Earth and Environmental Science 113: 012009.

Dubey, R.C. & Dwivedi, R.S. 1988. Effect of heavy metals frowth and survival of *Macrophomina phaseolina* (Tassi) Goid. Biology and Fertility Soils 6: 311-314.

Dahm, H. & Strzelczyk, E. 1996. Effect of heavy metals on enzymes production by *Hebeloma crustuliniforme*. Acta Mycolgica 31(2): 181-189.

El-Sersy, N.A. & El-sharouny, E.E. 2007. Nickel biosorption by free and immobilized cells of marine *Bacillus subttilis* N10. Journal of Biotechnogy 6: 316-321.

Ezzouhri, L.; Castro, E.; Moya, M.; Espinola, F. & Lairin, K. 2009. Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier. African Journal of Microbiology Research 3: 35-48.

Ekundayo, E.A.; Akharaiyi1, F.C.; Ekundayo, F.O.; Prebor, E.; Ogunmefun, O.T. & Oluwafemi, Y. 2018. In vitro Interactions of Fungal Isolates obtained from Selected Soil Samples in Ado-Ekiti Metropolis and their Tolerance to Selected Fungicide and Heavy Metals. International Journal of Current Microbiology and Applied Sciences 7(3): 3573-3585.

Fogarty, R.V. & Tobin, J.M. 1996. Fungal melanins and their interactions with metals. Enzyme and Microbial Technology 19: 311-317.

Fiza, L.; Muhammad, F.; Hussain M.; Urooj, H.; Samiah, A.; Saddam, S.; Wajid, Z.; Ali R.; Jianxin S.; Shengquan, C. & Qunlu, L. 2020. Evaluation of metal tolerance of fungal strains isolated from contaminated mining soil of Nanjing, China. Biology 9(12): 469.

Gruhn, C.M. & Miller Jr, O.K. 1991. Effect of copper on tyrosinase activity and polyamine content of some ectomycorrhizal fungi. Mycological Research 95(3): 268-272.

Golubovic Curguz, V.; Raicevic, V.; Tabakovic Tosic, M.; Veselinovic, M. & Jovanovic, L.J. 2010. Same physiological characteristics of three ectomycorrhizal fungi from *Suillus* genus. Mineral Biotecnology 22: 1-7.

Iwona, M.; Agnieszka, W.; Van, C.; M., Renata, R.; Nska, S. & Philippe, J. 2018. The role of heavy metals in plant response to biotic stress. Molecules 23(9): 2320.

Joshi, P.K.; Anand, S.; Sonu, M.; Raman, K. & Namita, S. 2011. Bioremediation of Heavy Metals in Liquid Media Through Fungi Isolated from Contaminated Sources. Indian Journal Microbiology 51(4): 482-487.

José, R.; Zúñiga-Silvaa, W.; Chan-Cupulb, O.; Loerac, N.; Nogawa, G.F. & Nordberg, K.M. 2015. *Cadmium-Handbook on the Toxicology of Metals (Fourth Edition)*. Chapter 32. Handbook on the Toxicology of

Metals 667.

Kredics, L.I.; Doczi, Z.A. & Manczinger, L. 2001. Effect of heavy metals on growth an extracellular enzyme activities of mycoparasitic *Trichoderma strains*. Bulletin of Environmental Contamination and Toxicology 66: 249.254.

Kacprzak, M. & Malina, G. 2005. The tolerance and $Zn2^{+}$, $Ba2^{+}$ and $Fe3^{+}$ accumulation by *Trichoderma atroviride* and *Mortierella exigua* isolated from contaminated soil. Canadian Journal of Soil Science 85(2): 283-290.

Levinskaite, L. 2001. Effect of heavy metals on the individual development of two fungi from the genus *Penicillium*. Biologija 1: 25-30.

Mushin, T.M. 1990. Effect of salt on the growth of fungi associated with halophytes in vitro. Basra Journal of Agricultural Science 3(1-2): 151-159.

Meenal, K. & Sarita, N. 2000. Metal Tolerance of *Fusarium solani*. Ecological Environmental and Conseve 6 (4): 391-395.

Martino, E.; Turnau, K.; Girlanda, M.; Bonfante, P. & Perotto, S. 2000. Ericoid Mycorrhizal fungi from heavy metal polluted soils: Their identification and growth in the presence of zinc ions. Myclogical Research 104: 338-344.

Muhammad, A.; Xu, J.; Li, Z.; Wang, H. & Yao, H. 2005. Effects of lead and cadmium nitrate on biomass and substrate utilization pattern of soil microbial communities. Chemosphere 60(4): 508-514.

Mala, J.G.S.; Nair, B.U. & Puvanakrishnan, R. 2006. Accumulation and biosorption of chromium by *Aspergillus niger* MTCC 2594. The Journal of General and Applied Microbiology 52: 179.

Madhi, Q.H.; Abass, M.H. & Matrood, A.A. 2020. Heavy metals pollution of wheat fields (soil and leaves) sampled from Basrah and Maysan provinces. *Periódico Tchê Química*. ISSN 2179-0302. vol.17.

Nasin, G.; Ilyas, N.; Ali, A. & Munawar, A. 2008. Effect of salt primingon in vitro growth on some soil fungi. Pakistan Journal of Botany 40(1): 427-431.

Nongmaithem, N.; Roy, A. & Bhattacharya, P.M. 2014. In vitro evaluation of tolerance to cadmium and nickel by *Rhizoctonia solani*. International Journal of Current Research 6(3): 5349-350.

Orgiazzi, A.; Lumini, E.; Nilsson, R.H.; Girlanda, M. & Vizzini, A. 2012. Unravelling soil fungal communities from different Mediterranean land use backgrounds. PLoS ONE 7(4): e34847.

Pawlowska, T.E. & Iris, C. 2004. Heavy-Metal Stress and Developmental Patterns of Arbuscular Mycorrhizal Fungi. Applied and Environmental Microbiology 70(11): 6643-6649.

Reese, N. & Mandels, M. 1963. Enzymic hydrolysis of cellulose and its derivatives. In: *Method in Carbohydrate Chemistry*. Vol. 3. R.L. whisler (ed.). Acadmic Press, New York, 139 - 143p.

Rozycki, H. 1993. Effect of Heavy Metals (Pb, Zn, Cu and Cd) on Mycelial Growth of *Cylindrocarpon destructans* (Zinssm.) Scholten. Zentralbl Mikrobiology 148: 265- 275.

Radojevic, M.; Abdullah, M.H. & Aris, A.Z. 2007. *Analisis air*. Scholar Press, Selangor.

Sierra, G. A. 1957. Simple method for the detectioin of lipolytic activity of microorganism and some observation on the influence of the contact between cells a fatty substrate. Antonie van Leeuwenhoek 23: 15-22.

Stohs, S.J. & Bagchi, D. 1995. Oxidative mechanisms in the toxicity of heavy metals. Free Radical Biology and Medicine 199518: 321-336.

Sanyal, A.; Rautaray, D.; Bansal, V.; Ahmad, A. & Sastry, M. 2005.Heavy metal remediation by a fungus as a mean of lead and cadmium carbonate crystals. Langmuir 21: 7220.

Sipos, B.; Benkő, Z.; Dienes, D.; Réczey, K.; Viikari, L. & Siika-aho, M. 2010. Characterisation of specific activities and hydrolytic properties of cell-wall-degrading enzymes produced by *Trichoderma reesei* C30 on different carbon sources. Applied Biochemistry and Biotechnology 161(1-8): 347-364.

Seth, R.K.; Alam, S. & Shukla, D.N. 2016. Isolation and identification of Soil Fungi from Wheat Cultivated Area of Uttar Pradesh. Journal of Plant Pathology and Microbiology 7(11): 1-3.

Syamsia, S.; Abubakar, I.; Irma, H.; Amanda, P. & Noerfitryani N. 2019. Screening endophytic fungi from local rice for lignocellulolytic enzyme production. 1st International Conference on Science and Technology, ICOST 2019, 2-3 May, Makassar, Indonesia.

Turnauk, O.E.; Ryszka P.; Zubek S.; Anielska, T.; Gawronski, S. & Jurkiewicz, A. 2006. Role of

mycorrhizal fungi in phytoremediation and toxicity monitoring of heavy metal rich industrial wastes in southern Poland. Soil and Water Pollution Monitoring, Protection and Remediation 3(23): 533.

Vig, K.; Megharaj, M.; Sethunathan, N. & Naidu, R. 2003. Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. *Advances* in *Environmental* Research 8(1): 121-135.

Vivas, A.; Barea, J.M. & Azcon, R. 2005. *Brevibacillus brevis* isolated from cadmium- or zinc-contaminated soils improves in vitro spore germination and growth of *Glomus mosseae* under High Cd or Zn Concentrations. Microbiology Ecology 49: 416-424.

Vadkertiová, R. & Sláviková, E. 2006. Metal tolerance of yeasts isolated from water, soil and plant environments. Journal of Basic Microbiology 46(2): 145-152.

Velmurugan, N.; Hwang, G.; Sathishkumar, M.; Choi, T.K.; Lee, K.J. & Oh, B.T. 2010. Isolation, identification, Pb (II) biosorption isotherms and kinetics of a lead adsorbing *penicillium sp*. MRF-1 from South Korean mine soil. Journal of Environmental Sciences 22(7): 1049-1056.

Yu, M.H. 2001. *Environment Toxicology: Impacts of Environmental Toxicants on Living Systems*. (3rd Edn) CRS Press LLC, Florida.

Yazdani, M.; Yap, C.K. & Abdullah, F. 2010. Adsorption and absorption of Cu in *Trichoderma atroviride*. Pertanika Journal of Tropical Agriculture 33(1): 71-77.

Yazdani, M.; Yap, C.K.; Abdullah, F. & Tan, S.G. 2010. An in vitro study on the adsorption, absorption and uptake capacity of Zn by the bioremediator *Trichoderma atroviride*. Environmental Asia 3(1):53-59.

Zafar, S.; Aqil, F. & Ahmad, I. 2007. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresource Technology 98(13): 2557-2561.