Tolerance of *Trichoderma* sp. to Heavy Metals and its Antifungal Activity in Algerian Marine Environment

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Western Algerian waste line is a dump of any form of pollution. Therefore sampling sites are ports, also close to urban waste which shelter a wide variety of fungal population. After seawater samplings, isolation we screened 235 kind of fungi for their behaviors with (Pb, Zn, Cd and Cu). Fourteen *Trichoderma sp.* isolates were evaluated for their tolerance to heavy metals stress at different concentrations of (Pb, Zn, Cd and Cu). *Trichoderma sp.* was exposed to concentrations between (0 - 4000 mg/l). The stress of biomass caused by the presence of heavy metals remains minimal. Maximum optimal growth has been demonstrated in our study at high concentrations of metals. Fungi expand gradually in cultures of metallic solutions. Its fresh weight reached 1, 89 Gr on 200 mg/l and 0,556Gr on 2000 mg/l. In the Zinc ($Zn\ So_4$) solution the mycelium will grow mostly on 600 mg/l his weight achieved 0,97 Gr. Development in copper ($Cu\ So_4$) solution is minimal, the fungi has pushed on 2000 mg/l to reach 0,046Gr. The *Trichoderma sp.* show an important potential of tolerance, it growth and stays different comparing to others fungus; The results emphasized the heavy metals detoxification abilities of *Trichoderma sp.* Bioremediation strategy could enhance the treatment of contaminated areas.

Keywords: Marine, *Trichoderma sp.*, biomass, tolerance, heavy metals, western Algeria.

Pollution by heavy metals represents an important form of environnemental contamination; particularly pronounced in the summer season, without previous treatment, these waters sprinkled right represents, therefore, the major source of contamination of the marine ecosystem. It is the accumulation of pollutants through the food chain is the origin of the alteration of any natural environment.

The marine environment represents a final receptacle and dump any form of pollution, especially the urban wastewater discharges loaded for heavy metals, which is very bad to all life (Sun and *al.*, 2010; Leitao, 2009; Al Sohaibani and *al.,* 2009), paint pigments, electric accumulators and battery and other organic discharges, therefore there is accumulation through the links in the trophic chain (Kok and *al.,* 2001), these pollutants represent the largest source the spread of this scourge after the coastal zones of the Algerian coast and are home to a large fungal population, especially at the port sites. From where we started our study and our water samples.

Heavy metals contaminate soil and seawater, like: Lead, Chromium, Mercury, Uranium, Selenium, Zinc, Arsenic, Cadmium, Silver, Gold and Nikel, they are consistently present in the environment (Zhang et *al.,* 2016), important micronutrients, and necessary for balance from the environment and become toxic they exceed their threshold (Chibuike, 2014).

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Metallic pollution is the most answered these days given the industrial development, this form of contamination is non-biodegradable and the substances persist in the water. Existing techniques for metal removal are diverse; Reverse osmosis, electrolysis, chemical precipitation, and phytoremediation, ultra filtration. (Alluri and *al.,* 2007).

Several bioremediation techniques used for contaminated soil and water involved not just in physical and chemical treatments in the biological processes, like a lot of bacteria and fungi engaged for their tolerance and reducing power to heavy metals (Idriss et *al.,* 2004); the following are the reasons for the removal of pollutants or their recovery. In 2010, Yih–Min and *al*, demonstrated the usefulness of mushrooms *(Penicillium sp, Aspergillus niger* and *Trichoderma viride*) could degrade lead phosphate**.** Another work developed in Taiwan was talking about the immobilization of the Pb, Me and Cd by *Aspergillus terreus*. One year later Kumar and *al.,* conclude during their work that there is a decrease of Zinc and Cadmium by *Aspergillus niger* by the phenomenon of biosorption in 2011, the same result is found in 2015 by Tsekova**,** that has support the resistance of mycelium to Cd as well as Cu, still in the same year in Brazil the work of Cavalcanti luna also talks about the adaptation of *Aspergillus sp*. to Cu. The best last study was in 2017 Provedano-Priego et *al.,* working on a set of filamentous fungi (*Penicillium sp, Aspergillus niger* and *Trichoderma viride*) and the behavior with lead phosphate in the same year bioremediation by bacteria associated with fungi which was the subject of study in China; of Liu and *al.,* 2017; who have studied the behavior related to pollution by PAHs and heavy metals._

Bioremediation of the soil is also a major challenge to solve the pollution problem by indishable substances, *Trichoderma sp*. is the subject of resolution, and many works are chained in this context. In 2010, the study was designed to test the potential persistence of this plant-beneficial fungus in soils contaminated by mining waste, the persistence of the *Trichoderma sp*. in those extreme conditions was demonstrated by the study. Three year later; 2013, Abdi and *al.*, are studying the effects of microorganisms in the bioremediation of soil polluted by Zinc in Algeria.

2015, Song and *al,* still in China the soil study continues; work on trade between *Lolium perenne*, the Cd and soil and microbial biomass, and conclude that the involvement of fungal inoculate are characterized by the modification of soil acidity, rhizosphere and plants accompanied by Co2 production according to other works, inoculate provide an alternative for improving phytoextraction (Olsson and *al.,* 1996 and Lavelle, 1997).

Among the most concerned species are the *Trichoderma sp.* are the subject of many studies because they occur under difficult conditions and unusual and stimulates the growth of plants (Vinale and *al.,* 2008), the species of *Trichoderma sp*. improves the quality of the environment where it is, and sustainably protects against heavy metal pollution**,** (Téllez Vergas and *al.,* 2017), his job was to study the behavior of *Trichoderma sp* and Cu and to try to improve its phytotoxic effects in the onion (*Allium cepa* L.). It is also valid for plants, they have a great ecological role, it has been through several studies, and they contribute to biodegradation and phytoremediation through metabolic activities (kuiper and *al.,* 2004)

Trichoderma species are imperfect filamentous fungi with telomorphs and belong to the order hypocreales in the Ascomycete devision. *Trichoderma sp*. are among the most frequently isolated soil fungi and are well known for their biocontrol ability against a wide range of plant pathogenic fungi (Howell, 2003), they play in important role in ecology et biodegradation band bioaccumulation of high amounts of various metals from wastewater and soil (Ezzi and Lynch, 2005; Arnand and *al.,* 2006). Fungi has a very fast growth, and extensive on Sabouraud medium at 25°C. The colonies have woolly aspect white at the beginning then appear getting older green clumps, arranged in concentric rings on the culture medium the back remains colorless.

Trichoderma sp. are most frequently isolated from the ground, they are known for their ability to reduce the risk of pollutants and toxic elements throughout the environment (Nonghmaithem, 2016), they tolerate more than one category of metal (Krediks et *al*., 2007) they play an ecological role in bioremediation, there are non-biodegradable pollutants that enter the food chain, however, metal ions influence the development, sporulation and enzymatic activity of *Trichoderma* (Papavizas and *al.,* 1985 and Jaworska and Dluzniewska, 2007), and can change enzymes and metabolites (Kredics et *al.,* 2001), *Trichoderma sp.* have acquired a very dramatic role in the ecological approach.

The isolates of *Trichoderma sp.* have tolerance to Ni, Cd and other heavy metals, as demonstrated by the study developed in 2016, in India by Nongmaithem and *al.,* who demonstrated the biosorbing potential of *Trichoderma sp*., in the same thematic still bioremediation by *Trichoderma sp.* another study was conducted by whole team in Mexico Telléz Vergas and *al.,* had goal in this study to Improve the phytotoxic effect caused by copper in the onion.

The fungus is unable to accumulate large concentrations of heavy metals essentially phytotoxic (Ruiz et *al.,* 2009). A high number of high numbers of heavy metals Cd, Cu, Cr, and Pb are remedied by fungi (Liu et *al.,* 2017)**.**

MATERIALS AND METHODS

Isolation and identification of fungus

The fungus studied is taken from wastewater discharges from three ports: Oran, Kharouba and Ghazaouet (Fig.1). The collected samples was transferred into a sterile plastic container, taken immediately to the laboratory and maintained at 4°C for further studies; the only medium used in our study is the Sabouraud; the fungus grows and develops in SDA (Sabouraud Dextrose Agar) solid medium.

Purified isolates were obtained by streaking repeatedly colonies in SDA medium and observation under light microscopy. Pure cultures of isolated microorganisms were identified using the keys of Pitt (1979) and Domsch & *al.,* 1980. The cultures were characterized to the genus level on the basis of macroscopic characteristics (colonial morphology, color and appearance of colony, shape) and microscopic characteristics (septation of mycelium, shape, diameter and texture of conidia).

We used the 1ml dilution method of 10^{-3}, 10^{-4}, 10^{-5} according to Nongmaithem, 2016 are incorporated in large glass kneaded boxes with Sabouraud medium. The suspensions were distributed uniformly over the medium surface by horizontal shaking the incubation is carried out in 7days at a temperature of 28°C±1°C, the colonies are identified with *Trichoderma sp* .

Colonies invade the whole surface; these green colonies are distinguished from other colonies of different strain.

Fungal strains were maintained and on Sabouraud dextrose agar during 14days at 28°C (Provedano-Priego & *al.,* 2017)

Tolerance to heavy metals

Metal tolerance assay in solid medium

The metal salts used were lead carbonate Pb^{2+}, copper Cu^{2+}, and zinc sulfate Zn^{2+}, Cadmium sulfate Cd^{2+}. Fungal strains were maintained and on Sabouraud dextrose agar during 14 days at 28°C (Provedano-Priego & *al.,* 2017)**.** For each metal, all Sabouraud plates supplemented with 0, 200, 400, 600, 800, 1000, 2000, 4000 mg/l with metal salts in triplicates and 0,6 cm diameter mycelial plug of *Trichoderma sp.* was inoculated centrally. Fungal strains were maintained and on Sabouraud dextrose agar during 14 days at 28°C (Provedano-Priego & *al.,* 2017) and the diameter growth (mm) was measured every alternate day. The effect of the heavy metal on the growth of the isolates tested was estimated by measuring the radius of the colony extension (mm) against the control (medium without metal) and the determination of the index of tolerance. The index is defined as the ratio of the extension radius of the treated colony to that of the untreated colony. Isolates showing resistance to Pb^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} were selected for the following experiments. (Ezzouhri et *al*., 2009), The plates were inoculated by placing 6 mm mycelial discs of 4 days old cultures of the *Trichoderma* isolates on the agar surface and incubated according (Nongmaithem et *al.*, 2016)

Dual fungal essay antifungal activities under metal stress

Sabouraud plates used were supplemented with metals as described in last paragraph with different concentrations. The control was established by inoculating agar plug instead of mycelial plugs of *Trichoderma.* Incubation was performed using similar conditions of incubation, and the radial growth of mycelium extension was measured at every alternate day. The antifungal activity was expressed as percentage inhibition of radial growth (PIRG%) as follows.

PIRG (%) = ((R1- R2)/R1) ×100%

Where; R1 indicates the radial growth of mycelium in control plates

R2: indicates the radial growth of mycelium co paired with fungus. The experiment was performed in triplicates (Nongmaithem et *al.,* 2016).

Metal tolerance assay in liquid medium

The resistance of the selected isolates to Pb^{2+}, $Cu^{2+,}$ Zn^{2+} and $Cd^{2+,}$ was determined by the dilution method. Metal ions were added separately to Sabouraud at concentrations of 0 to 4000 mg/l, the plates were inoculated with 6 mm agar plugs from young fungal colonies, pre-grown on SDA. Three replicates of each concentration and controls without metal were used. The inoculated plates were incubated at 25°C for at least 7 days.

For biomass preparation, a stock solution of heavy metals Pb $(NO_3)_2$, Cu So_4, $ZnSo_4$ and $Cd(No_3)_2$ was prepared by dissolving the appropriate quantity of metal salt, Sabouraud medium 100 ml was made in conical flasks or glass bottle, then appropriate quantities of metals salt were added to Sabouraud medium liquid to get required concentrations (0, 200, 400, 600, 800, 1000, 2000, 4000 mg/l). The non amended solution served as a control without heavy metals additional. The stock solution was sterilized and ready to receive mycelium fragment culture, in the strictest sterile conditions.

The plates inoculated with addition of mycelial disc that measures 6 mm of 4days old cultures of *Trichoderma* isolates into agar surface and incubated at 28 ± 1°C for 2 or 3 days. Fungus showing maximum radial growth on the media.

Three replicates are executed; the culture is done in (25°±2°C) for 11 days according to Su Yien Ting and after culture solutions are filtred with Millipore following studies established (Su Yien Ting and Jioe, 2016) and (Provedano –Priego et *al.*, 2017) . The biomass extracted is filtred with Whatman filters n° 1 or filter paper and then washed thoroughly with deionized water to remove the growth medium. The Harvested mycelia were oven –dried at 80°C for 48h and the dry weight was measured using a digital weight balance with occurancy of 0, 1 mg.

Statistical analyses

To evaluate the influence of heavy; metals the data was submitted to variance analysis using Statistica soft ware by Tukeys tests the means between treatments were compared by using test probabability for significant difference p< 0,05.

RESULTS

Screening for Heavy Metal growth profile

In this study, marine fungi were submitted to a preliminary experiment to screen their efficiency for ability to growth in cultures in the absence and presence of heavy metals on solid and liquid medium.

On solid medium; four kinds of heavy metals (Pb, Cu, Zn, and Cd) were tried with mycelium of *Trichoderma sp*. in different concentrations and whitout metals. Among this series for heavy metals only three metals were chosen for completing tests series of heavy metals liquid medium (Pb, Zn ,Cu), the Lead selection

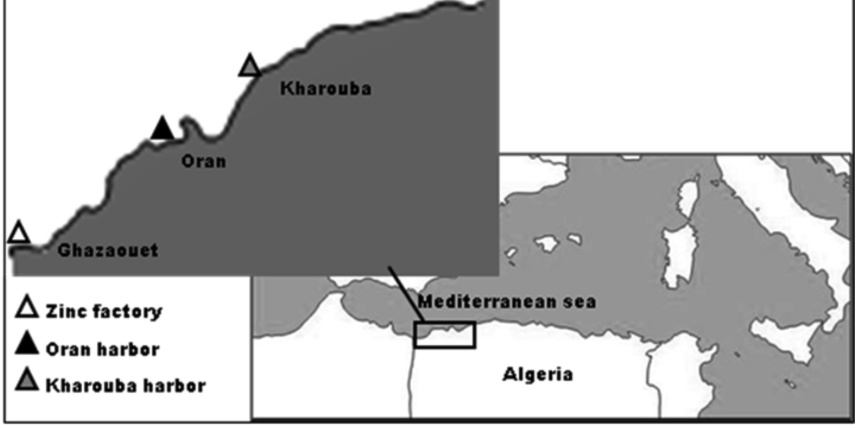

Fig.1. Map of the sampling sites of the western Algerian coast

for high toxicity for environnement, the Zinc and Copper for their importance for cellular and metabolic activities .

Tolerance of *Trichoderma sp.* to heavy metals in solid medium

The *Trichoderma sp.* were identified and selected for further study, based for variability

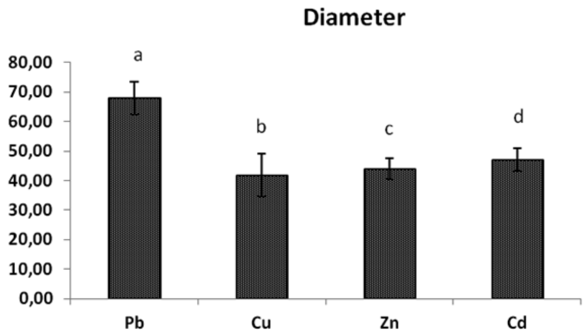

Fig. 2. Effect of heavy metals (Pb, Cu, Zn, Cd) concentrations on *Trichoderma sp*., Bars indicates standard deviation. Colums of differents treatements with different letters are significantly different at P<0,05

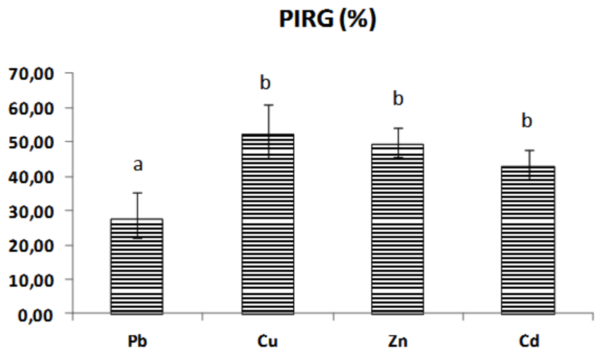

Fig. 3. Percentage of inhibition (PIRG) in solid medium of *Trichoderma sp.,* with heavy metals concentrations (Pb, Cu, Zn, Cd), Bar*s* indicate standard deviation. Colums of differents treatements with different letters are significantly different at P<0,05

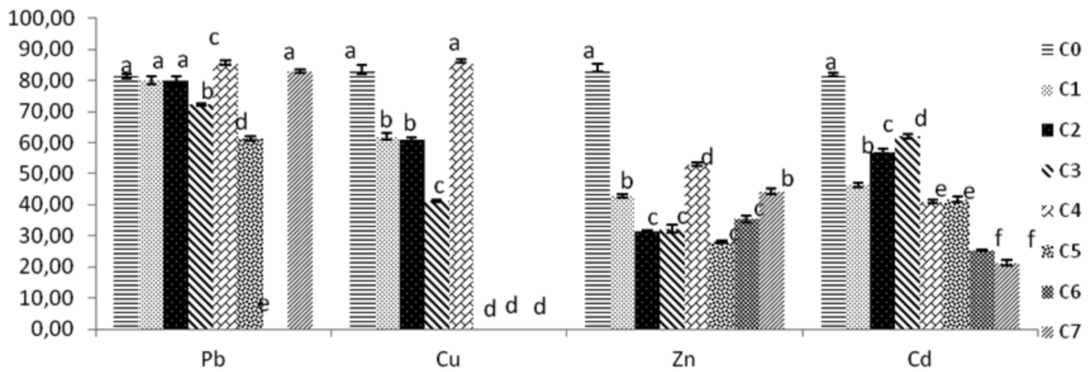

Fig. 4. Growth and tolerance of *Trichoderma sp* in solid culture SDA added with concentrations of (Pb, Cu, Zn, Cd). C0:0 mg/l, C1:200 mg/l, C2:400 mg/l, C3:600 mg/l, C4:800 mg/l, C5:1000 mg/l, C6:2000 mg/l, C7:4000mg/l. Bars indicate standard deviation. Colums of differents treatements with different letters are significantly different at P<0,05

growth and mycelial cultural diameter. The expansion of *Trichoderma sp.* revelate an abilility to growth in heavy metals at high and various concentrations, the maximum growth rate was observed in Pb rates followed by Cadmium cultural growth rate, then mycelial rates of Zinc, lastly Copper growth rates.

All the diameters of mycelium heavy metals rates are different significantly between them, the diameter of Pb rates growth stay the best diameter and most important remains the highest significantly (p< 0,05) to metals Cu, Zn, Cd, its general overage is 67,93 mm of mycelial growth with 27,58% of inhibition PIRG (Table.1).

The mycelial growth in Copper Cu rates is the most reduced significantly with mycelial diameter rate of 41, 72 mm and 52, 24 % of inhibition PIRG (Fig.2, 3).

The percentage for inhibition of Pb is significantly different to others metals , the inhibition of fungi is minimal and reduced , and important with copper rates the Copper inhibits the mycelial growth significantly p< 0,05 , there is no significant difference with PIRG inhibition of fungi by Zinc and Cadmium rates p >0,05. (Fig. 2, 3), (Table.1).

For the Pb, the concentrations C_1 and C_2, show no significant diffence with the control rate p>0, 05.

From C_3 there is a significant diameter decrease p<0,05 , the growth mycelium resume his growth at C_4, the diameter mycelial growth decrease with increasing concentration until complately inhibition, from C_5 to C_7; which corresponds at 4000 mg/l of Pb.

The mycelial rates growth of Cu, at concentration C_1 and C_2 the growth of *Trichoderma sp*. show a significant development of fungi to compare with control m at C_4 the culture of mycelium is inferior significantly to C_0 Control p<0,05 .

Fungi in concentrations C_5, C_6, C_7 of Cu, the growth of fungi is inhibited significantly, no growth is detected (fig.4).

The mycelia growth is affected by Zinc concentrations. All diameters fungi growths are lower and reduced significantly to compare with control fungi growth who reaches 84 mm, a good growth is observed with C4 Zinc concentration with 53 mm mycelair diameter growth. Except the others rates of remaining concentrations of fungi in C_2, C_3, C_5, C_6 which are not significant difference (p >0, 05) (Fig.4).

Inhibition increases with high concentrations from C_1 to C_3; then it decreases, growth is inhibited at C_4 rate Zinc then resume at higher concentrations of zinc but these fluctuations are not significant with C_0 control and C_4.the inhibition of *Trichoderma sp*. growth with Zinc concentrations decreases until C_7 (Fig. 5).

The Cd rates growth shown all diameters in various concentrations significantly different compared with control. The best fungi growth is reported at C_3, from this concentration there is mention of a decrease of diameter growth. The inhibition of mycelium by Cd increases with high concentrations rates. The maximal PIRG inhibition rate reported at 4000 mg/l of C_7, and significantly high to others concentrations Cd rates p<0, 05 (Fig.5).

Tolerance of *Trichoderma sp.* to heavy metals in liquid medium

After culture on liquid Sabouraud medium the mycelium is weighed and dried 24h at 80°C, the mass of fungi got before and after culture is weighed is named dry weight, among the metal salts only three were required chosen for their tolerance with *Trichoderma sp*. to push while working on the most marked concentrations where we have a maximum of positive reponse from 0 to 2000 mg/l (Table. 2).

After 24h at 80°C, biomass dried and weighed, the dry weight of Zn solution medium is most increase significantly compared to dry weight of fungi in Pb, and Cu solutions after time dry, the weight of biomass change but there is no significant dimininution. The weight of general biomass of fungi in Pb and Cu medium solution is almost the same because there is no significant difference p > 0, 05 between the two (Fig. 6).

The results of dry weights found are statistically similar to results of biomass of *Triochoderma sp.* before dry.

The biomass growth in Pb solutions medium decreases significantly until C_4 which corresponds at 800 mg/l , the biomass believes more at C_5; 1000 mg/l and exceeds the weight registered at different lower concentrations all different compared to control C_0.

After drying biomass 48h at 80°C; the

dry weight down significantly, the lowest dry weight raised at C_2 (Fig.8). The medium Cu solution inhibits the growth of biomass apart for solution with high concentration 1000 mg/l; at this concentration proliferation reaches its maximum and exceeded the control biomass. The dry weight of this series of Cu medium culture is maximal at 1000 mg/l almost the same as the witness .on the

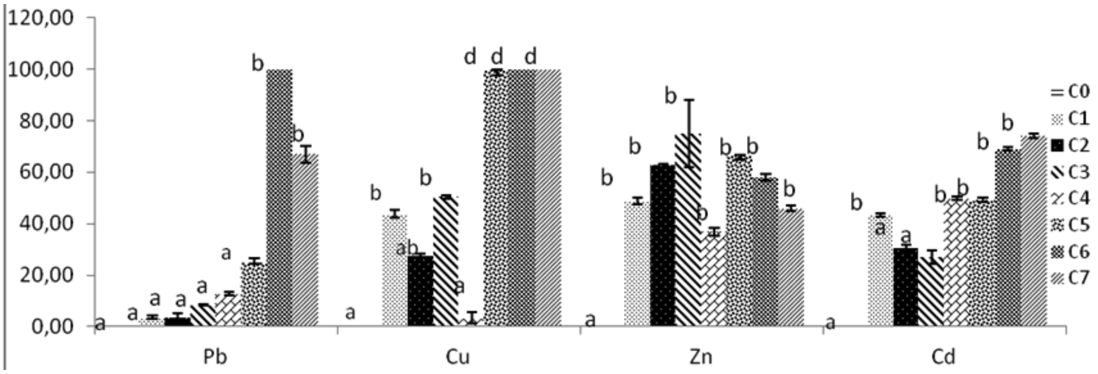

Fig. 5. Percentage of inhibition radial growth (PIRG) of *Trichoderma sp* by heavy metals in solid culture SDA. C0:0mg/l, C1:200mg/l, C2:400mg/l, C3:600mg/l, C4:800mg/l, C5:1000mg/l, C6:2000mg/l, C7:4000mg/l. Bars indicate standard deviation. Colums of differents treatements with different letters are significantly different at P<0,05

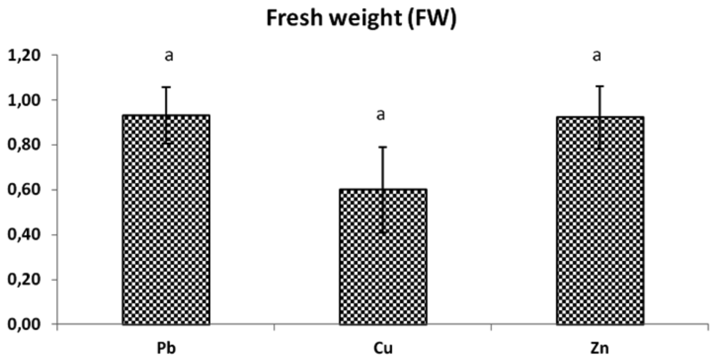

Fig. 6. Total biomass fresh weight (Fw) of *Trichoderma sp.* in liquid medium at concentrations of heavy metals (Pb, Cu, Zn), Bars indicate standard deviation . Colums of differents treatements with different letters are significantly different at P<0,05

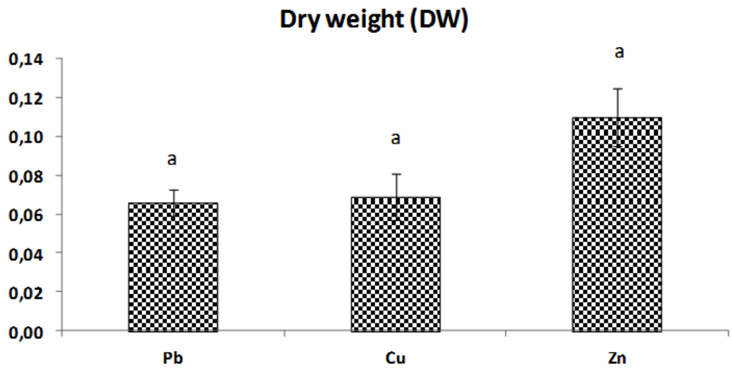

Fig. 7. Total biomass dry weight (Dw) of *Trichoderma sp.* at concentrations of heavy metals (Pb, Cu, Zn), Bars indicate standard deviation. Colums of differents treatements with different letters are significantly different at P<0,05

other hand the dry weight is reduced significantly all over concentrations after dry. In Zn medium solution, the weight of biomass unregistered for C_5 is similar to control weight without difference, the *Trichoderma sp*. biomass is different and very reduced compared to control biomass for C_2, C_3, C_4 at these levels, weights is similar between them without comparison, P|>0,05 (Fig.8) .

After exposure to heat, the fresh weight decreases in volume and weight, it is reduced after

Table 1. General mycelia growth and inhibition of *Trichoderma sp*. by different heavy metals (Pb, Cu, Zn, and Cd) at different concentrations (0 to 4000) mg/l in Sabouraud dextrose agar rates

	Diameter (mm)	PIRG (%)
Pb	67,93± 5,56[a]	27,58 ± 7,9[a]
Cu	41,72± 7,27[b]	52,24 ± 8,67[b]
Zn	43,93 ± 3,54[c]	49,21 ± 4,75[b]
Cd	47,08 ± 3,86[d]	42,91 ± 4,66[b]

Table 2. General weight of biomass before and after dry of *Trichoderma sp* .with different heavy metals (Pb, Cu, Zn) at different concentrations (0 to 2000) mg/l in medium SDA

	F.W (g)	D.W (g)
Pb	0,93 ± 0,13 [a]	0,07 ± 0,01 [a]
Cu	0,60 ± 0,19 [a]	0,07± 0,01 [a]
Zn	0,92 ± 0,4 [b]	0,11 ± 0,02 [b]

F.W: fresh weight.
D.W: dry weight.

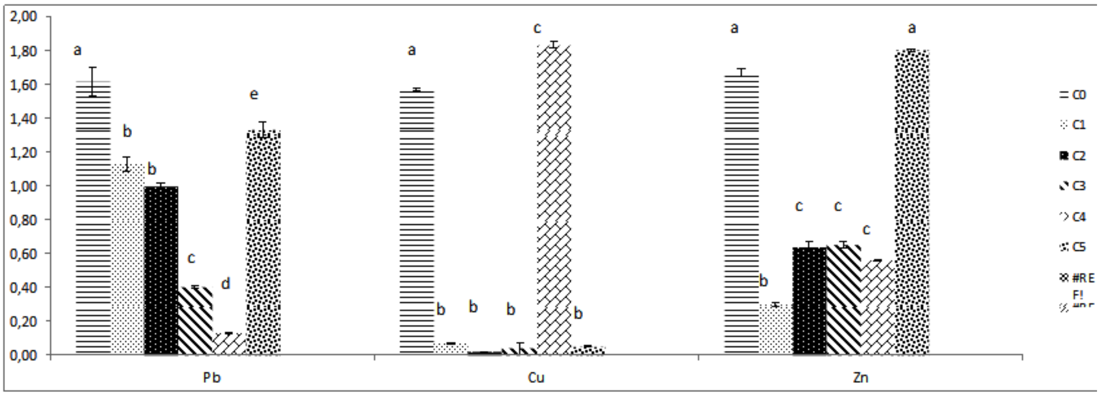

Fig. 8. The different results of biomass fresh weight of *Trichoderma sp*. under various concentrations of heavy metals (Pb, Cu, Zn) .Colums of differents treatements with different letters are significantly different at P<0,05

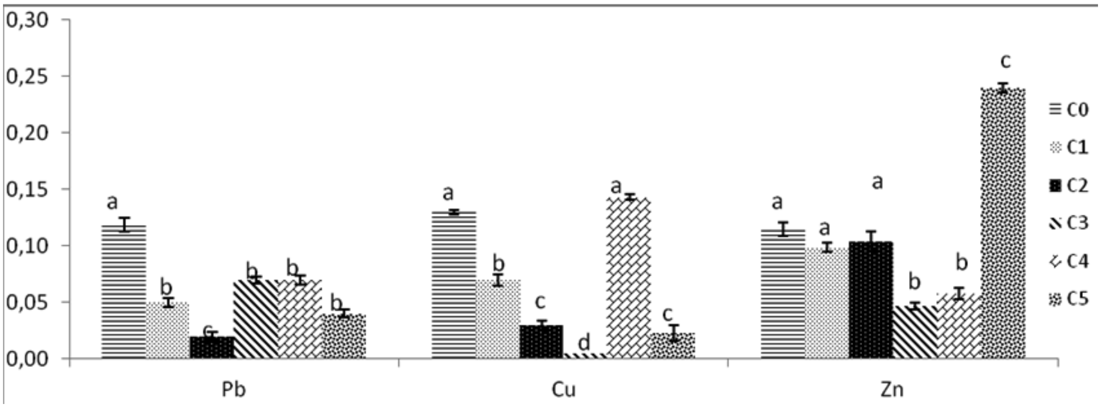

Fig .9. Weight dry of biomass of *Trichoderma sp*. after drying fresh weight 48h at 80°C under various concentrations of heavy metals (Pb, Cu, Zn). Colums of differents treatements with different letters are significantly different at P<0,05

Table 3. Results of the variance analysis (Test F, ANOVA) of; D, PIRG, F.w and D.w of *Trichoderma sp*.

F (Metal x Concentration)	F (Concentration)	F (Metal)	
1,62$^{N.S}$	7,12**	7,69**	D
13,68**	4,68**	7,5**	PIRG
4,99**	10,18**	5, 84*	F.W
1,10$^{N.S}$	20,34**	3,24*	D.W

D : Diameter ; PIRG:Percentage of inhibition of radial growth in solid medium ; F.W :Fresh weight ; D.W : Dry weight , (* : significant (p< 0,05) ; ** : Very significant (p<0,001) ; ^{ns} : Not significant
df (Metal) = 3.92; df (Concentration) = 7.88; df (Metal x Concentration) = 31.64

dry but remains high significantly compared to control. The weight of C_1 and C_2 remain similar to control no difference between them.

DISCUSSION

Bioremediation is an important solution for elimination and reduction of various polluants from contamined sites , soil land industrial wastewater , with the help of biological organisms such as fungus , bacteria, and plants by degradation ; certain fungal, algal and bacterial are capable of accumulation (Kumar and *al.,* 2011), especially the filamentous fungi, *Trichoderma sp.* isolated from wastewater studied . Through our final results in this study the tolerance profile was evaluated. The level resistance capacity was studied in medium solid and liquid; the mycelial growth was presented as follows Pb>Cd>Zn>Cu (Fig.2).

Lead is considered one of the most toxic and dangerous heavy metals, that caused problems pollution in marine environnement, and many modifications in mycelium and induce production of melanin chlamydospore development, a fugal reponse based in biological adaptation for fungi, cells to synthesize some enzymes for lead uptake (Ezzouhri, 2009 and Provedano and *al*., 2017).

Bioremediation strategy converting the contaminant metals into theirbless toxic forms , fungal stains was able to tolerate Pb lead and transform metallic lead into lead phosphate ;mineral phases , this stable minerals could reduce the bioavailability of lead, (Provedano and *al*., 2017)

Biomineralisation of lead phosphates (e.g pyromorphyte) by fungi is a well known process (Leitao, 2009).

Trichoderma sp. were identified with selected culturals variability existed among the isolates different characteristics with changement of color caused aspect sporulation in different heavy metals cultures raised , the *Trichoderma sp.* pushed well and fits with different concentrations of $Pb(No_3)_2$, the maximum radial growth was shown at 800 mg/l (C4) with 85,67mm and 4000 mg/l (C7) with 81,33, the optimum growth enregistred similar to diameter control equivalent to 83mm , it tolerated high concentration of lead. The effect of lead tolerance was tested on Sabouraud medium liquid added with metal salts. The inhibition is from 1000 mg/l to 2000 mg/l (Fig.4), the same tolerance results are observed with another fungus the endurance of Pb of each fungal strain was recorded by inoculating them separately in lead amended plates. *A. niger* RH 17 and *A. niger* RH 18 showed the highest resistance, being 6000 and 7000 mg/l , respectively, warranting them to be successful candidates for metal detoxification. The colony diameter of *A. niger* RH 17 after 7 days was 48.4 mm, while in another study (Price *et al.,* 2001) then study of Povedano and *al.,* in 2017 *Penicellium Chrysogenum, Aspergillus niger* and *Trichoderma viride* could tolerate high concentration Pb , able to resist concentrations up to 8mM of lead , *Trichoderma viride* tolerate this concentration.

Sun and Shao in 2007, talk about resistance of *Penicellium sp* to Lead , the hyphae grown at 4milliMol this fungi accumulate Pb in cellule structure , had intracellular dispositifs , Both fungal strains showed maximum Pb removal at 1000 mg/l metal concentration. And supports for resistance phenomene exhibited by fungis which translates by extra cellular bioaccumulation which provide strength and tolerance according to Shao study in 2007. In our study lead diameter of *Trichoderma sp.* decrease significantly at 1000 mg/l and bounces 4000 mg/l, was highly efficient in lead absorption since it absorbed 90% of the contaminating solution

$Pb(NO_3)_2$, after 48 hours. Heavy metals 2007, Shao and Sun suggested that intracellular accumulation is an important mechanism for fungi from deep sea sediment to resist heavy metals. These fungi are a potential treatment for heavy metal pollution, otherwise the fresh weight of the mycelium decreases before and after drying with the increase of concentration, the highest weight of Pb enregistred is of 1, 33g , the fungi tolerate solution concentration of last concentration in this series test heavy metals treatment fixed at 1000 mg/l , the fungi grown all over , even after drying the biomass is clearly absorbent to Lead salt accumulated , into fungi cells, generally the microorganisms evaluated exhibited higher reduction of biomass when cultivated in the presence of metal ions , inhibition of growth and active metal process accumulation (Leyval and *al.,* 1997., Pepper, 2000., Malik, 2004., Lopez, Vazquez., 2003).

The Cadmium tolerance represented in figure indicated the strong capacity of *Trichoderma sp.* at C3 corresponding to 600 mg/l , a diameter growth enregistreted is 62 mm after this concentration the Cadmium; become toxic and slow down the growth of culture mycelium of *Trichoderma sp*., at 4000 mg/l the inhibition is maximal the fungi can't growth at this high concentration , according to our results *Trichoderma sp.* resist to Cd until 600 mg/l (Fig. 4) , in 2016 by Nongmaithem and *al.,* study the fungi achieved 73, 99% , biomass production decline with increasing metal concentration at 150 ppm. This tests show the ability to remove the cadmium for culture rate medium depends of cellular growth and metabolism action; reduction of production in biomass indicate the effects of various concentrations salts metals on fungi reaction and total Cadmium retained by fungi . Many species of fungi showed a potential for removal, among them *Fusarium sp., Penicellium sp*. et *Aspergillus flavus* and *Trichoderma konjinji* , *Trichoderma harzianum* isolated from polluted industrial area have a capacity to remove metal ions in aqueous solution , the removal metal ions cell do not depend of metabolism (Kapoor and Viraraghavan., 1997., Gharieb and Gadd., 2004., Gogsungur, and *al.,* , 2005., Acosta and *al.,* 2007., Massaccesi and *al.*, 2002) , an other study in 2011 supports the *Trichoderma harzianum*; capacity for removal cadmium with a high level tolerance to growth in the presence of high Cd concentrations. This fungi used in this study is able to grow at high concentrations, can be used in bioremediation. Li and *al.,* 2007 talk about ability to growth in Cadmium concentration above 5miliMol in strain *Pseudomonas* , this organism was able to remove 99% of Cadmium, Lopez and Vazquez demonstrated that the presence of Cadmium; inhibited the growth cells of *Trichoderma atroviride* in 50% to concentrations lower than 1miliMol, the *Trichoderma harzianum* isolate showed an other way a high resistance to Cadmium in concentrations 1mililMol, 2miliMol, 3miliMol, the literature shows that microorganisms like fungi have a capacity to accumulate polyphosphate used in bioremediation technology (Keasling and Hupf., 1996 and Keasling and *al.,* 1999., Torres and *al.,* 1998., Moreno, and *al.,* 2001., Van Loosdrecht and *al.,*1997).

The genus of *Trichoderma* is able to modify the rhizosphere of plants, and product an important substance organic acid such gluconic acid furamic and citric acid approved in study of Kapazak and *al.*, 2014. An other study appropriate talking about *Aspergillus niger* to remove and accumulate Cadmium and Zinc the removal was less significant by accumulated biomass in 2011; the similar results were reported by Ahmed and *al.,* in 2006, where *Aspergillus niger* and *Penicellium sp*. were tested for tolerance and high remove action to Cr and Ni .

Copper is an important co-factor enzymatic processes, represents the third most abundant transition metal found in living organisms (Brandolini and *al.,* 2002). The growth of all fungi tested was decreased after addition of Copper in comparison with Zinc. The blue color of the isolates' mycelia on agar media amended with copper salts is of Cu ions to in medium rate.

The *Trichoderma viridie* turned blue on agar media at all concentration according to Ezzouhri study in 2011, similar morphologic and physic characteristics were deferred in our study the mycelia agar rate color also turn in blue in presence of $CuSo_4$ ions . *Trichoderma* have resistance and high capacity to biosorb Cadmium and other heavy metals (Volesky, 1990; Zafar et *al.,* 2007; Lopez-Errasquin and Vasquez, 2003).

In study of Levinstakete in 2002 growth of *T.viride* and *P. Chrysogenum* were slowed down at 1,5 and 2 miliMol Cr in the medium. Metals such as Zn, Cu, are essential to biological actions, their toxicity may be presented differently *Trichoderma sp*. tolerate Copper at 800 mg/l maximum, up to this concentration the fungi is inhibited and don't growth, the growth diameter decrease with increase metal concentration; the same remarks have been mentioned on the whole series on liquid medium heavy metals solution the fungi don't have capacity to have a good growing activity but , the mycelium biomass achieved the maximum in 800 mg/l solution concentration , the biomass before and after drying always stays at the pick of its growth at 800 mg/l corresponding to C4 in representation (Fig. 6, 7) , the structure of *T. viride*, so it was excluded and the study was carried out to optimize the Cr (VI) removal efficiency by a marine isolate named *T. viride.* Micro-organisms isolated from industrial processes and polluted environments with high metal concentrations exhibit considerable tolerance to these elements. This tolerance may be due to abiotic factors (pH, temperature, nutriments in the environment or growth media). Growth parameters, sporulation, diameter biomass and physical details represent a set used to determine level of toxicity of Copper an other type of heavy metals In study of Téllez-Vergaz and *al.,* 2017; who is interested to evaluate metal toxicity in plants talk about parameters growth of plants; stem elongation number of roots and (Ait Ali and *al.,* 2002; Shi et *al.,* 2010), survival oignon biomass depends for $CuSo_4$ concentration.

Trichoderma sp. isolated from polluted sea water sites exhibit a potential tolerance and a good capacity to accumulate Cu from metal contaminated sediment, the growth was not affected at 100 ppm, according to Iskandar and *al.,* in 2011, 120 ppm was able to slow down the growth of *Trichoderma asperellum* isolate. *Trichoderma sp.* was affected in our study to 200 mg/l equivalent to 200 ppm and inhibited mycelial growth at 43, 85% and fungi is inhibited to high concentration at 1000 to 4000 mg/l , absence of growth , the biomass of *Aspergillus niger* had reduced at 2miliMol in presence of copper , this fungus was able to grow at concentrations up to 300 ppm in the culture medium (Tsekova and Tedorova, 2002) exactly at the result of our study which determines the resistance potential and capacity growing to 800 ppm or 800 mg/l for *Trichoderma sp*.

The microorganisms reduce the concentration of Cu and sequester a substance poly phosphate, a biodegradation of Cu and heavy metals is dependent to metabolism of polyphosphate, this polymer provides resistance to many heavy metals, in various microorganisms; yeasts, bacteria and filamentous fungi.(Keasling, 1997, 2000., Lima and *al.,* 2003, Alvarez, Jerez., 2004., Remonsellez and *al.,* 2006).

The $CuSo_4$ accumulated in fungi cell , which does interest to evaluate potential effect to ameliorate the toxic effects of Cu onion plants (Hajierghari, 2010, Cacciola and *al*., 2015, Nonghmaithem and *al.,* 2016) , growth inhibition is the principal effect of Cu reported and the first symptom of toxicity of Cu to inhibit photosynthesis by accumulation on the leaves (Geremias et *al*., 2011), following Luo and *al*., 2016; this study interested many species selected for capability tolerance to heavy metals and bio accumulation Cu above 1000 ppm .

In *Trichoderma harzianum* showed that presence of Cadmium reduced the polyphosphate activity. The hydrolisis of organic phosphorus acid phosphatase protect structure cell of fungi and help to resist at high concentration (Gadd, 2010).

The *Aspergillus niger* was considered to be also a greatest potential biotechnological and industrial uses , the microbial cells are used in biotransformation process, and the biomass was an excellent biosorbent to heavy metals ions (Remonsellez and *al.,* 2006, Gadd, 2010) in the literature the microorganisms and filamentous fungi such as *Aspergillus , Mucor, Trichoderma, Rhizopus* , product various metabolites able to reduce pollution heavy metals, and a potential to remove Cu ions (Adrio and Demain., 2003, Bennet, 2010, Djiksterhuis and Wosten., 2013).

The Zinc is an essential element for organisms, become toxic at high concentration. The major part of fungus group were able to grow at 12, 5 miliMol and more higher concentration. The aspect morphological were affected at high concentration ; many descriptifs details change ; after effect of high Zn concentration, with secretion of substance colored in yellow , who which shows stress response in Zn solid medium rates . In the present study the concentration decreasing comparing to mycelial diameter growth control. The *Trichoderma sp*. growth at differentes concentrations ; the fungi diameter increase more and more until 800 mg/l who matches to 53 mm of diameter growth, followed by diameter growth enregistred at 4000 mg/l, the fungi tolerate and resist the most high concentration , the *Trichoderma sp.* was inhibited at 1000 mg/l; le Zn is toxic and slows down the mycelium expansion, a resumption of development is noticed; the fungi bounces and become thik but with zone of inhibition detected which marks the circumference yellow; Fomina et *al*., 2005 reported that heavy metals accumulated accompanied with excretion of different substances organics acids such as lactic acid, oxalic acid, acetic acide. The damages were related to cells membrane and cytoplasm caused by metals ions. the properties of growing fluctuations caused by fungi accumulate Zn in cell wall selected for resistance to heavy metals; the zinc trained fungus according to study for (Kumar and *al.,* 2011) observed *A, niger* solubilize Zn in malt grown solution. Kok and *al.,* in 2001 talk gets good results proves that *Aspergillus niger* have metal tolerance against Zn and Cd, and support that various filamentous fungi like *Trichoderma sp.* slected for our study were an excellent product in bioremediation activity.

Levinskaite (2001) demonstrated that the growth rate of *Penicillium atratmentosum* , 25SL decreased slowly as the Zn ions concentration increased up to 40 miliMol. In Zn solution liquid medium the biomass increasing with high concentration solution for all fungus such as *Trichoderma sp*. growth at 1000 mg/l and uptake Zn from metallic solution after drying the weight dry stay the most important at 1000 mg/l. the removal of Zn is egual than the Cu; Price et *al.,* talk in 2001 about *A.niger* growing to concentration up to 0,1miliMol Zn the same profile with copper is mentioned.

The *Trichoderma sp*. increases with increasing metal solution concentration, this fungi proliferates and absorbed des Zn ions from solution ; Price in 2001 talk about Cu and Zn removal by *Aspergillus niger* at 0,1miliMole solution concentration. Similar results shown that *Aspergillus niger* growth in solutions of Zn So4 and could remove Zinc from solutions the A.niger removed 0,533 mg in 0, 01 solution ZnSo4. Das in 2008 precise that specific Zn uptake decreased with the increase in biomass living; the process followed in second mate kinetics biosorption. The biomass uptake cadmium was higher than zinc, and in dual system combination solution the uptake of zinc is the higher according to Kok 2011, an other similar ascertainment was mentioned approving the higher capacity removing zinc from wastewater that better than fungus grown in culture media amended with zinc (Price, 2001), *Aspergillus niger* was grown in solution in which Zn increased from 10 too 500 ¼m.

In Lead medium solutions, the biomass of *Trichoderma sp*. decreased with increase metal concentration, the fungi weakness does not absorb until solution at 1000 mg/l equivalent to 1,33 g , from here this large significant decrease is related to absorption process, the ions. Accumulated depend of cell density present in medium culture; the adsorption capacity were expressed as Cd>Pb>Cu (Fig.8) for *Aspergillus flavus* with study of Kok and *al.,* but results given by actual study shown the capacity of biomass absorption is Pb>Zn>Cu.

All test series experience, used ph: 5, 6 according to Roy and Pan*,* 2005. For an optimum uptake was fast at ph: 6 maximum. Because the tolerance may be also influenced by abiotic factors such as PH, temperature and nature of nutriment and environnement for growing. The absorption of heavy metals depends on cell density present in medium culture following the results found in work of Iqbal; 2005. Who talking about mycelia growth occurs in for pallets under submerged conditions in liquid medium with agitation. Finally *Trichoderma sp.* growth in all heavy metals but to different degrees he is one of the *Trichoderma* species who has demonstrated in study of Tellez Vergaz and *al.,* in 2017 a great amelioration in stress in onion plants due to excess of Cu, the *Trichoderma sp.* supports excess and highest concentration proven in our study , for Cd the last study for Nonghmithem and *al.,* in 2016 exibits the *Trichoderma* isolates to Ni and Cd ; it's through his study that we can support our hypothesis on Cd tolerance to some concentrations, the increase Cd concentrations affecting germination and growth , even at higher concentrations the stress of fungi increase , the regression is unregistered up to 800 mg/l, the zinc also is assimilated and absorbed already he enters in cellular needs and metabolism activities, Ting and Jioe demonstrated in 2016 that isolates of *Trichoderma sp.* in their study of tolerance to metals ; the Zn is tolerated at 100 ppm at this concentration the diameter growth was 80 mm, at 200 mg/l or 200 ppm in present study the diameter was 43 mm , this fungi is generally able to tolerate metals via cellular adaptation, for Ting and Jioe the fungi has an exceptional tolerance to Al up to 500 ppm and Pb which is the subject of our study; they mentioned a tolerance of Pb up to 300 ppm, according to our results le *Trichoderma sp*. assimilate and resist to lead even at 4000 mg/l , it is considered the best metal assimilated and absorbed by *Trichoderma* in many studies .

CONCLUSION

Our findings indicate that fungal populations isolated from heavy metal-contaminated sites and sea water has the ability to resist higher concentrations of metals. The *Trichoderma sp.* have a better tolerance to all metals, a comparative level of metal resistance was also shown in the present study concludes that *Trichoderma sp.* have an ability to resist higher concentrations of Pb, it grows up to 4000 mg/l. The tolerance and the resistance of the isolates depended much more on the fungus tested than on the sites of its isolation, the *Trichoderma sp.* potential metal biosorbents is mentioned through the results obtained shown a great tolerance with success each heavy metals cited at the front, especially lead, and pushes in liquid solution up to 1000 mg/l, the process is based on their capacity uptake with fungal expansion, the fungi develop metal resistance and performance. *Trichoderma sp*. can be exploited as potential metal biosorbents and used as potent bioremediation.

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