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(RESEARCH ARTICLE)

The use of different concentrations of Nickel and Lead for the inhibition growth of some types of soil microorganisms

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Abstract

Three types of Mycorrhiza has been chosen *R. rosulus, S. varigatus* and *P. involutus* in removing and lowering the ascending concentration of nickel and lead elements during three incubation and contact periods (5,7 and 10 days), and comparing the isolated growth with control sample. Type of *P. involutus* is the most efficient in the removal by observing the growth of isolates by increasing it's diameter compared to the control samples and the concentration of nickel and lead, and for all the days of incubation, while the type *S. varigatus* was the highest growth at low concentration of nickel and lead only for all periods of incubation, for type *R. rosulus*, the diameter was smaller as compared to control samples and for all elements concentration of nickel and lead, except at concentration (2.5ppm), which showed increase in diameter for nickel, and is generally observed increase diameters of the isolates of the three types by increasing periods of incubation and all elemental concentrations of nickel and lead, and this increase is less than at high concentrations of these elements and of all species studied.

Keywords: Mycorrhizae; Heavy metals; Biological treatment; Soil pollution removal

1. Introduction

The current global trends towards the concept of pollution are only a result of realizing the importance of preserving the environment from pollution in all the interlocutor, and soil is one of the pillars of the environment that is no less important than water and air, as soil pollution is one of the most prominent, and common environmental problem, complex and difficult situation [1] the heavy elements are one of the important examples of soil pollutants, as their danger lies in the concentration doubled through the food chains as a result of bioaccumulation and bio-magnification in the tissues of organisms. Plant and animal life over time, which is characterized by a high stability of non-disintegration and dehydration into simple components and thus reaching the human [2]. On the other hand the low concentration of it play an important role in the metabolic activities of living organisms as it enters into many enzymatic reactions, and used as auxiliary materials for the reaction and the transfer of electrons, and the cumulative characteristic may give solution by exploiting microorganisms that have the adaptive ability to withstand high percentages and concentrate them in their bodies, and thus work to free the environment of them and produce inert compounds that are not harmful to the environment [3] which calls for serious consideration of using these organisms for treatment and the fungi are one of these organisms, as it can store and bear high concentrations of heavy elements, and filamentous fungi (mycobacteria), play a role in reducing and removing the concentration of many heavy elements in the soil, by having several mechanisms for removal of these elements, by the formation of sedimentary complexes, or the mechanism of biological drinking and storage of elements inside their cell [4].

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2. Material and methods

The study included three types of Mycorrhiza fungi, namely *R. rosulus*, *S. varigatus*, and *P. involutus*, which belong [Basidiomycota (and the Cultivar) Agaricomycetes]. Obtained from the environmental research center of the university of Bristol U.K, these isolates were activated and grown on selected culture media approved by (Sigma) company, namely SDA and PDA, with a ratio of three duplicate for each type, different concentrations were added to the selective value elements for the study as follows:

Nickel (Ni): 2.5, 7.5, 10 mg/L

Lead (Pb): 0.5, 1.0, 1.5 mg/L

The selected isolates were grown in culture media treated with this concentration and the samples were incubated for (5,7,10 days), respectively.

The measurements were taken using Vexnia to observe the effect of the concentration on the growth of the studied species.

All tools used were sterilized and dried using an autoclaving devise for 40 minutes at a temperature of 121degree Celsius, and at a pressure of 1 atmosphere, using the oven at a temperature of 90^0C, the culture media was used according to the manufactures protocol (Sigma), then the media was sterilized using autoclave at a temperature of 121 degree Celsius for 15 minutes, and under pressure of 1 atmosphere (APHA, 1998).

The plates were left to harden for 24 hours and to suppress the growth of bacteria 30 mg/L of the antibiotic Streptomycin was added. The plates are inverted for 24 hours at room temperature for the purpose of dehydration of the agar, and to facilitate the cultivation process, use SPSS 2010 and Excel for the purpose of performing statistical analysis, and the F-test and T-test variance loading were adopted. The significant differences were compared with the least significant difference test (LSD) at probability ($P \leq 0.05$).

3. Results and discussion

Table 1 The growth of isolates (colony diameter), at different concentrations of nickel (Ni), and lead (Pb).

Species	Days of incubation	(Ni) ppm			(Pb) ppm			control
		2.5	7.5	10	0.5	1.0	1.5	
		Isolates diameter (mm)			Isolates diameter (mm)			Isolates diameter (mm)
P. involutus	5	29	33	38	12	15	18	10
	7	51	41	44	26	24	21	16
	10	79	60	49	45	38	37	34
S. varigatus	5	52	45	11	57	50	42	48
	7	65	58	12	66	53	45	59
	10	72	60	15	70	57	46	62
R. rosulus	5	31	25	19	22	19	16	27
	7	38	33	25	26	25	21	35
	10	54	43	29	38	28	23	41

At the level of significance ($P \leq 0.05$)

Between table (1), and (2), the fungus P. *involutus*, proved it's ability to grow, and it's physiological activities continued through increasing the diameter of the two colonies, compared to the diameter of the control samples, for both

concentrations of nickel and lead on three days (5,7,10), of incubation where some fungi types has been regarded as storage and big accumulation, and have the ability to absorb and concentrate heavy metals inside the fungus through it's adoption of multiple mechanisms to tolerate and eliminate the toxic effect of metals, and the secretion of enzymes and acids outside the cell which liquefies exploitatively, the acidic medium caused by the heavy elements is suitable for fungi in their growth environment [4] and also the adsorption on the surface of the cell, and the building and synthesis of thiol compounds that have the ability to bind with compounds elements, and thus reduce it's toxicity to the cell by metal deposition in the cell walls, or by linking it with other compounds as a mechanism to remove the poisoning [5]. This is in agreement with many studies, which examined the ability of fungi for the removal of heavy metals [6]. When comparing the diameter with the increase in the concentration of the studied elements, we note that the incubation periods (5 days), was the appropriate or most efficient, as the diameters of the isolate were increasing with increasing concentration. It was the diameter of isolate (29 mm) at a concentration of (2.5 ppm), while it became (38mm) at (10 ppm), for the nickel as well as for lead.

The isolate diameter increased from (12mm) at concentration of (0.5 ppm), to (18mm) at concentration of (1.5 ppm), while at incubation (7,10 days), the diameters of the isolates were gradually reduced by increasing the concentration of the two components as shown in table (1), this could be attributed to the effect of this concentration on the gradual resistance of the isolates with increase of the time period [7].

The statistical analysis of this fungus showed table (2,3), that there are significant differences for the three concentrations of both elements nickel and lead compared with the control sample, while there were no significant differences between these concentrations.

Table 2 Statistical analysis of the average diameters of isolates (the three incubation periods), and the different concentrations of nickel (Ni)

Species / **Isolates** / **Diameters average**	***P. involutus***	***S. Varigatus***	***R. Rosulus***	**LSD P≤0.05**
	20.00 Cb	56.33 Aa	34.33 Ba	
Control	±	±	±	10.30
	1.73	4.26	2.33	
	53.00 ABa	63.00 Aa	41.00	
Con. 2.5	±	±	±	20.67
	8.14	5.86	2.52	
	44.67 ABa	54.33 Aa	33.67 Ba	
Con. 7.5	±	±	±	19.81
	8.01	4.70	3.48	
	43.67 Aa	12.67 Cb	24.33 Bb	
Con. 10	±	±	±	8.93
	3.18	1.20	2.91	
LSD P≤0.05	22.63	14.22	9.27	

Table 3 Statistical analysis of the average diameters of isolates (the three incubation periods), and the different concentrations of lead (Pb).

Species / Isolates / Diameters average	***P. involutus***	***S. Varigatus***	***R. Rosulus***	**LSD P≤0.05**
Control	20.00 Cb	56.33 Aab	34.33 Ba	
	±	±	±	10.30
	1.73	4.26	2.33	
Con. 0.5	27.67 Ba	64.33	28.67 Bab	
	±	±	±	8.07
	1.20	2.19	3.18	
Con. 1	25.67 Ba	53.33 Ab	24.00 Bab	
	±	±	±	7.08
	1.20	2.03	2.65	
Con. 1.5	25.33 Ba	44.33 Ac	20.00 Cc	
	±	±	±	5.12
	0.88	1.20	2.08	
LSD P≤0.05	4.21	8.70	8.46	

As for the type *S. varigatus*, the diameters of the isolates of this type compared with the control samples is higher at lower concentration only and for all three incubation periods (5,7,10 days), when a concentration of (2.5 ppm), of nickel became (52-65-72mm), respectively, and at a concentration of (0.5 ppm) for lead, it became (57-66-70mm) respectively, compared to the control samples, which were (48-59-62mm) respectively, but at high concentration (10 ppm) and (1.5 ppm) for the two components, we note that isolates of this type are affected by the toxicity of these elements at their higher concentration, so that it's diameters were smaller compared to the control samples. (Table 1), and figure (1&2), as for the absorption of the elements by the isolates depends on the ability of membrane and include rapid attachment to the cell wall then inside the cell (Fernander et al, 2012), (Bayramoglu et al, 2003), and when comparing the diameters of the isolates with an increase in the concentration of the studied elements, we not that they begin to gradually decrease with each increase in concentration from the statistical analysis of this type, it was found that there are significant differences between the concentration (10 ppm), compared to the control sample, while the concentration (2.5 ppm) and (7.5 ppm), did not show significant differences with the control sample and that the concentration (10 ppm), also showed significant differences between each of both concentrations (2.5 ppm), and (7.5 ppm), which were not with nickel element as for the element lead, the significant differences appeared between the concentration (1.5 ppm), only compared to the control sample, and the three concentrations showed significant differences between them and at the level of probability (P ≤ 0.05). Tables (1) and (2). As for the species (*R. rosulus*), the diameters of isolates were generally lower compared to the control samples for all elements concentrations of nickel, lead and for all three incubation periods (5,7,10 days), except for nickel (2.5 ppm) concentration which increased and became (31-38-54mm), respectively compared to the control samples which were (27-41-35 ppm) respectively as shown in the (Table 1), and figure (1&2). And this is likely due to the possibility of the elements linking on the cell walls of the isolates without absorbing to the inside the cell, where the cell walls of most types of fungi contain a high amount chitin, which has been proven to bind minerals ions and act as a barrier to permeability which can limit the entry of elements into the cell (Dursun et al, 2003), which was in agreement what has been found by (Ahmed et al, 2007). Also noticed the statistical analysis for this species for nickel element there is significant differences between the concentration (10 ppm), compared to the control sample, while the concentration (2.5ppm), and (7.5 ppm) did not show any significant differences, and that the concentration (10 ppm), also showed significant differences between each of both concentrations (2.5 ppm), and (7.5 ppm), which have no significant differences between them. As for lead significant differences appeared between concentrations (1 ppm), and (1.5 ppm), compared to the control sample as

well as significant differences between concentration (0.5 ppm), and (1.5 ppm), other than the concentration (1 ppm), which there were no significant differences between them and at the probability level ($P \leq 0.05$). (Table 2 and 3). In general , we note that the diameter of the three studied species increase with increasing incubation days, and for all concentrations of nickel and lead elements, because the increase in the incubation period gives an opportunity to increase the contact period between the fungi and the contaminated metal, and thus the greater time to remove it, feed on it and get rid of it as living organism have the ability to transform these elements into a form of little toxicity by their formation for many of the compounds that are related together. Also the incubation period (10 days), had the largest diameters of all studied fungal species, compared with the rest of the incubation days for each concentration of the different elements added to the isolates (table 1), so that we note that the fungi adopt themselves a mechanisms defense and resistance to the nutrients and getting rid of that element at this particular concentration, which lead to growth and increase in the diameter of the isolates. This is in agreement with the research of (Mohammed et al, 2014), for the biodegradation of naphthalene compound by filamentous fungi.

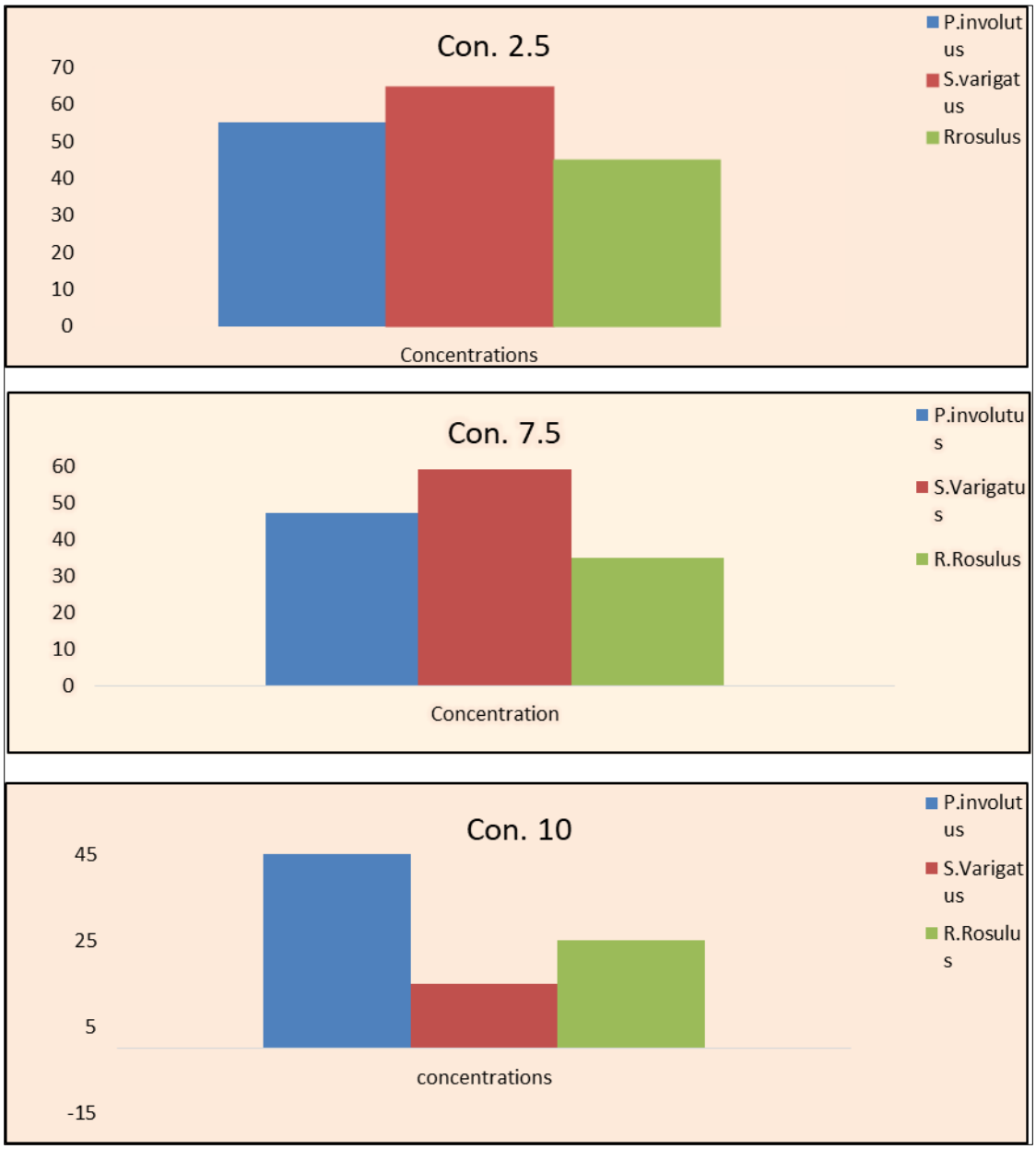

Figure 1 The average diameter of isolates of nickel with different concentrations

The exploitation of the elements depend on the difference in concentration, incubation period and the type of fungi as fungi are among the most affected organisms in the circles in which lived and affected by any small change in the nutritional environment, so that this leads to undesirable results it increases or decreases it's growth drops significantly

below the required limit (Mohammed et al, 2005). Through statistical analysis (Table 2 and 3), we note the effect of concentrations on the studied species for the nickel element significant differences were found between the species (*R. rosulus*), and species (*S. varigatus*). At a concentration of (2.5 ppm) and (7.5 ppm), while there were no significant differences between the type (*P. involutus*), and the type (*S. varigatus*) at these concentrations and significant differences were also found between the three studied species at a concentration of (10 ppm), as for the lead element. Significant differences were found between (*P. involutus*) and (*R. rosulus*), with species (*S. varigatus*), at a concentration of (0.5 ppm) and (1 ppm), while no significant differences were found between species (*P. involutus*), and (*R. rosulus*) at this concentration, and found significant differences as well among the three species at a concentration of (1.5 ppm), and at a level of probability ($P \leq 0.05$).

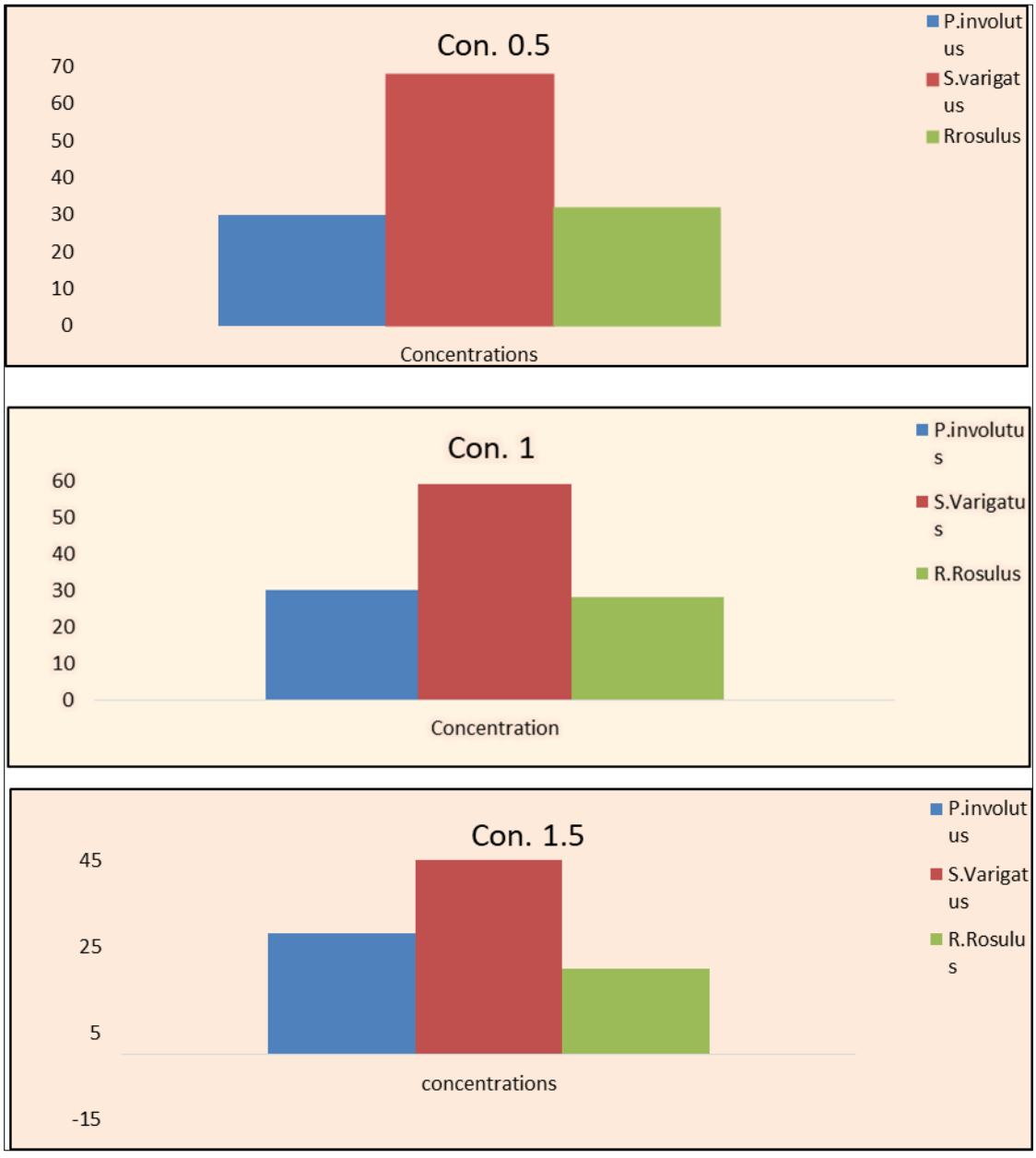

Figure 2 The average diameter of isolates of lead with different concentrations

So can summarized our conclusion from the data shows above that the species (*P. involutus*), is the most efficient in restricting and treating the elements of nckel and lead for all concentrations and incubation days, through increasing it's diameters compared to the control samples, and thus possibility of using it in biological treatment. Also the highest concentration of nickel (2.5 ppm), was the most appropriate for all types of fungi studied, as their diameters increased at these concentrations compared to the control samples and for all incubation days.

4. Conclusion

This study shows that P.involutus is the most efficient in the removal of Nickel and Lead at all concentrations during incubation and contact periods, there for it can be used for biological treatment, and it's generally observed that the low concentration of Nickel (2.5ppm) is the most suitable for all species in this study.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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