IOSR Journal of Engineering (IOSRJEN) **www.iosrjen.org**
ISSN (e): 2250-3021, ISSN (p): 2278-8719
Vol. 05, Issue 03 (March. 2015), ||V1|| PP 16-23

Bioremediation of Irradiated and non-irradiated Sewage Sludge by *Fusarium oxysporium* fungi

Ahmed. A. Moursy., O.A. Abdel Aziz. and A.Z. Mustafa.
Atomic Energy Authority, Nuclear Research Center, Soil & Water Research Department Abou-Zaabl, 13759, Egypt,

Abestract:Heavy metal pollution has become a major environmental concern nowadays and the bioremediation of polluted soil is an increasingly popular strategy due to both its efficiency and safety. Low cost mitigation measures like phytoremediation and mycoremediation are commonly employed. Mycoremediation using macro fungi (*Fusarium oxysporium*) have proven to provide effective tolerance using an efficient accumulation mechanism in removing heavy metals from soil. It was found that the soils treated with *Fusarium oxysporium* showed a much lower concentration of total and exchangeable Cd, Cu and Sr compared with the control. The highest cadmium content was occurred with non irradiated sewage sludge (NISS) while the lowest one was occurred with irradiated sewage sludge (ISS). Higher reduction ratio of copper element along interval times was showed at 45 days incubation time between non-fungal and fungal treatments with more than 60 % compared with other intervals incubation times. Reduction of lead was noted in most treated NISS with non-fungus especially intervals incubation times at 0, 30, 45 and 60 days compared to the same NISS treated with *fusarium oxisporum* fungi.

Key word: *Heavy metal, bioremediation, Fusarium oxysporium, irradiated sewage sludge (ISS)*

I. INTRODUCTION

Heavy metal pollution is nowadays one of the most important environmental concerns. Anthropogenic activities like metalliferous mining and smelting, agriculture, waste disposal or industry discharge a variety of metals such as Ag, As, Au, Cd, Co, Cr, Cu, Hg, Ni, Pb, Pd, Pt, Rd, Sn, Th, U and Zn, which can produce harmful effects on human health when they are taken up in amounts that cannot be processed by the organism. Damage may cause adverse reactions in different organs and biological functions, including reproduction and birth defects[1] (Malik, 2004). Fauna and flora are also affected by these detrimental effects.

The disposal of sewage sludge on land as a fertilizer is an old practice due to the presence of nitrogen, phosphorus and other nutrients in the sludge[2] (Lombardi and Garcia, 1999). However, the presence of heavy metals in sludge restricts its use on land. The application of sewage sludge contaminated with heavy metals poses serious threat to the environment due to the potential risk of metal leaching to ground water and surface water and is also because of their entry in the food chain[3] (Fytianos and Charantoni 1998). Therefore, removal of heavy metals from contaminated sewage sludge assumes great importance to ensure safe disposal of the sludge on land.

Physical and chemical methods have been proposed for the removal of these pollutants. Nevertheless, they have some disadvantages, among them cost-effectiveness limitations, generation of hazardous by-products or inefficiency when concentration of polluted materials is below 100 mg l-1[4,5] (Gavrilescu, 2004; Wang and Chen, 2009). Biological methods solve these drawbacks since they are easy to operate, do not produce secondary pollution and show higher efficiency at low metal concentrations [6](De *et al*., 2008).

Microorganisms and plants are usually used for the removal of heavy metals. Mechanisms by which microorganisms act on heavy metals include biosorption (metal sorption to cell surface by physiochemical mechanisms), bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), biomineralization (heavy metal immobilization through the formation of insoluble sulfides or polymeric complexes) intracellular accumulation, and enzyme-catalyzed transformation (redox reactions)[7] (Lloyd, 2002). On the basis of energetic requirements, biosorption seems to be the most common mechanisms[8] (Haferburg and Knothe, 2007). Furthermore, this is the only option when dead cells are applied as bioremediation agent. Nevertheless, systems with living cells allow more effective bioremediation processes as they can self-replenish and remove metals via different mechanisms[9] (Malik *et al*., 2004). On the other hand, living cells show higher sensitivity to environmental conditions and demand nutritional and energetic sources. In this context, the present study deals with the ability of *Fusarium oxysporium* fungi on reduction of some heavy metals in sewage sludge and contribution in enrichment agriculture system with clean organic source.

II. MATERIALS AND METHODS

An incubation experiment was carried out in microbiological, nutrition and pollution laboratories in Soil and Water Research Department, Nuclear Research Center, Atomic Energy Authority to evaluate the impact of the ability of fungal inoculations on the reduction of heavy metals in sewage sludge samples along many intervals incubation times.

Sewage sludge samples: Freshly deposited sewage sludge was sampled from ditch of Al-Gabal Al Asfar farm, Abo-Zabal, Egypt, in Augustus. Sewage sludge samples were collected in polyethylene bags and brought to the laboratory, air dried and grinding with crusher to pass through a 2 mm sieve. Air dried and sieved sewage sludge samples were radiated with 10.0 kGy γ radiation.
Virgin sand soil samples were collected from experimental farm of Soil and Water Research Department, Nuclear Research Center, Atomic Energy Authority.

Microorganism and inoculum preparation: The selected fungal isolate of *Fusarium oxysporum* were kindly provided by[10] (Abdel Aziz, 2005) and allowed to grow on plates containing Dox-agar medium (CDAM) and incubated for 7 days at 30^{o}C. A spore suspension of fungal isolates was prepared by inoculation of broth Dox media with 10 discs/L (1.7 cm) obtained from the previous growth plates. At the end of the incubation period, the flask containing fungal growth was shaked for 30 min to obtain the spore suspension. A spore suspension was added in quantity of 5 X 10^{8}spores/cup containing mix of 50% air dried sewage sludge (50 gm) and 50% of sand soil (50 gm).

Characterization of sewage sludge: The pH of the sludge was determined using pH meter. The total nitrogen content and total phosphorous content was determined according to the standard methods[11] (APHA 1989). For determination of total heavy metals content, the sludge samples were subjected to di-acid digestion (HNO_3 $^{+}$, $HClO_4$) and the heavy metals in the digested liquid were determined using atomic absorption spectrophotometer .The chemical properties of applied sewage sludge were pH (1:5) 6.80, EC 4.11 dS m^{-1}, C/N ratio 8.50, O.M 45.92%, N 3.2%, P 1.25%, K 0.23%, Fe 14650 mg kg^{-1}, Cu 593 mg kg^{-1}, Mn 413.42 mg kg-1, Zn 1459 mg kg^{-1}, Cd 8.68 mg kg^{-1},Sr 130.2 mg kg-1,Pb1260 mg kg^{-1} .

Incubation Experiment: A laboratory incubation experiment with three replicates was conducted with the incubation of *Fusarium oxysporum* and examined irradiated and non-irradiated 50 % of sewage sludge samples, the water holding capacity was adjusted to 60 % until the end of experiment. The cups was incubated for intervals times (0, 15, 30, 45 and 60) days at 30 ^{o}C in the lab of the Soil and Water Microbiology unit, Soils and Water Research Department, Nuclear Research Center, Atomic Energy Authority, Inshas, Egypt.

Total and available metal contents in incubation sewage sludge: Total cadmium, copper, strontium and lead contents were determined after digestion with both *aqua regia* (1.0 g dried sewage sludge/soil mix: 2.5 ml nitric acid, 7.5 ml hydrochloric acid and 0.5 ml 30% hydrogen peroxide)[12] (Kilburn, 2000) and 2 M nitric acid (5 g soil: 100 ml concentrated nitric acid) [13](UN/ECE, 1998). The amounts of elements were determined by atomic absorption spectrometry (AAS).

EDTA extraction method: 10 g of air-dried sewage sludge/soil mix was digested and extracted with 20 ml EDTA extract (0, 01 M ethylene-diaminetetraacetic acid (EDTA) and 1M (NH4)2C03, adjusted to pH 8, 6) and shaken for 30 minutes. Therefore, soil: solution ratio was 1:2[14] (Trierweiler and Lindsay, 1969). Extract was filtered and concentrations of trace metals were determined by atomic absorption spectrometry (AAS).

III. RESULTS AND DISCUSSION

3.1. Cadmium content mg kg^{-1} soil in sewage sludge materials as affected by different treatments and of incubation:

Cadmium content in sewage sludge media as affected by additive and time of incubation was graphically illustrated by Fig (1). It is obvious that the different treatments induced different Cadmium content. As indicated by the overall means, the highest cadmium content was occurred with NISS while the lowest one was induced by ISS. In this regard, the composting treatments could be ranked as following:
NISS > NISS Non Fungus > ISS > ISS Non Fungus

Concerning the incubation intervals, the overall mean reflected that cadmium content was decreased with increasing time intervals where the lowest cadmium content was noticed after 60 days. It means that the swage sludge could be poorly the end incubation at this time where it was poor in swage sludge content comparing to the other time intervals. In conclusion, both the treatments and incubation intervals have significant effects on cadmium content of the sewage sludge materials. In addition, we can select NISS Fungus as the best treatment, which enriched cadmium content the sewage sludge materials.

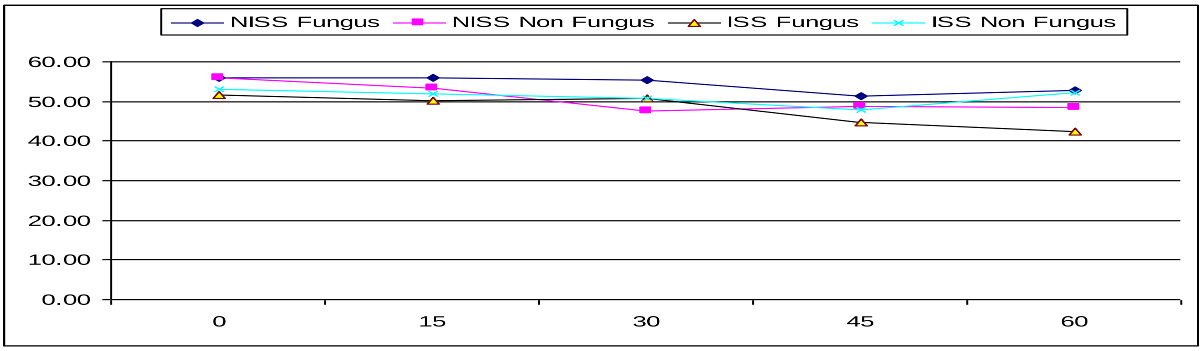

Fig (1) Interaction between swage sludge treatments and time of incubation on cadmium content changes.

Data presented in Fig (2) show the effect of Irradiated and non-irradiated sewage sludge and fungi added to the sandy soil on changes in its content of cadmium available (mg cd kg^{-1} soil) at the different incubation periods up to 45 days. Data revealed that cadmium available of the soil tended to increase by increasing incubation periods up to 60 days was regardless of type of the added Irradiated and non-irradiated Sewage Sludge. However cadmium available of the investigated added Sewage Sludge. was frequently affected type of the added Sewage Sludge.. For example, the highest soil cadmium available was indicated due to application of NISS Non Fungus treatment on the basis of overall mean. On the other hand, the lows cadmium available occurred with ISS treatment while, there was no a pronounced difference between the NISS Non Fungus and ISS[15] María del Carmen et al (2012) found that fungal action on Cd(II), Cr(VI) and Zn(II) ranged between 85% and 60%. Fusarium and Penicillium arewell-recognized fungi in terms of their capability in the bioremediation of heavy metals[15,16,17] (Pan et al., 2009; Sen et al., 2007; Sun and Shao, 2007).

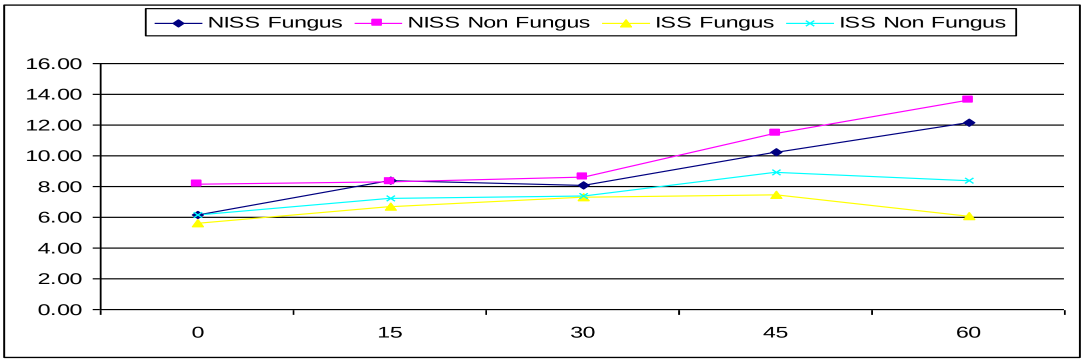

Fig (2) Interaction between sewage sludge treatments and time of incubation on cadmium available mg kg^{-1} soil changes.

These results indicate that there is direct relation between the cadmium original contents of the studied sewage sludge and the corresponding cadmium contents of the treated soil where the sewage sludge was of highest cadmium available content whereas the cadmium available content of sewage sludge treated soil seemed relatively low.

The overall mean of time intervals indicated that the highest amount of cadmium available was induced due to application the inoculation periods of at 60 days while the lowest one was noticed after 1day of incubation.

The Sewage Sludge could be ranked according to their effects on the soil content of cadmium available as follow: NISS Non Fungus > ISS Non Fungus > NISS > ISS .

3.2. Sewage sludge Copper concentrations

Concentrations of total copper were showed in Fig (3). Total copper in non-irradiated sewage samples was increased in most non fungus treatments along intervals incubation times compared to fungal treatments applied. In this respect, higher reduction ratio of copper element along interval times was showed at 45 days incubation time between non-fungal and fungal treatments with more than 60 % compared with other intervals incubation times. Data of the estimated copper in non-irradiated sewage samples showed that the less value of the element was recorded at 30 days interval incubation time in fungal and non-fungal treatments (159.08 and 186.08 mg/kg) respectively. Generally, data of copper in non-fungal and fungal treatments along interval times was indicated to decrease of copper element at 0 and 15 days interval times compared to concentrations of copper at 45 and 60 days was increased. On the other hand, in irradiated sewage samples copper was regularly increased along to intervals incubation days times under fungal treatment (0<15<30<45). In contrast, under non-fungal interval incubation times, non fixed trend was showed between recorded values of copper element along incubation interval times (0> 15 < 30 >45 < 60).

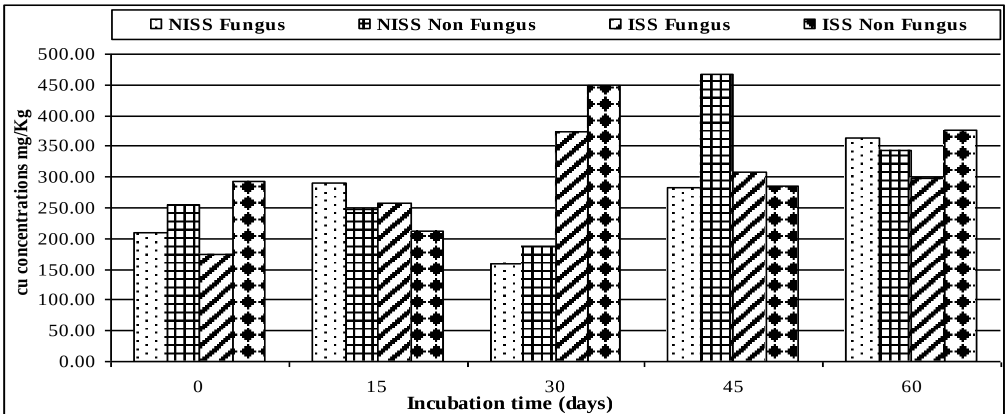

Fig (3) Interaction between treated sewage sludge with fungal inoculations and incubation times on copper content changes

Available copper content was recorded and showed in fig (4). In common, reduction of copper element was showed in irradiated and non-irradiated sewage samples under fungal treatments compared to non-fungal treatments at most interval incubation times except recorded values at interval incubation times 15 and 60 days irradiated treated sewage samples. Also, data of available copper element was indicated to ascending increase in recorded values along to interval incubation times under irradiated swage that treated with fungal inoculation. On the other hand, simplified range of available copper content was recorded at 0 and 15 incubation days and increased at 30 incubation day and stable decreased at 45 and 60 incubation days under non-irradiated sewage samples untreated with fungal inoculations. The Presented data was indicated to the higher reduction of available copper is recorded in irradiated sewage sample and non-fungus treated at incubation time 15 days (25.11 mg/kg) comparable other showed data along incubations time in irradiated and non-irradiated sewage samples.

Also, irregular decreasing and increasing of available copper content was noted and recorded in irradiated sewage sludge under fungal and non-fungal treated samples along incubation intervals times. In this respect, Filamentous fungi are used widely in industrial fermentation and bioremediation[18,19] (Bosshard *et al*., 1996; Plaza *et al*., 1996). They are preferred over other organisms for bioremediation because they are easier to remove from liquid substrates. Data are presented by [20]Price et al. (2001) support an active process as being responsible, at least in part, for the fungus' ability to remove such large amounts of copper and zinc from solution.[18] Bosshard *et al.* (1996) reported the use of *A. niger* to remove copper, zinc and other metals from incinerated municipal fly ash by organic acid leaching. The fungus is known to produce large quantities of citrate and gluconate, both of which are capable of leaching or precipitating metals out of materials.[21] Akthar and Mohan (1995) used killed mycelium of *A. niger* to remove copper and zinc from contaminated lake waters. The killed biosorbent was able to remove 17.02 µg Cu and 912.5 µg Zn/g fungal dry weight from the contaminated lake water. [18]Bosshard *et al.* (1996) reported the use of *A. niger* to remove copper, zinc and other metals from incinerated municipal (fly) ash by organic acid leaching. The fungus is known to produce large quantities of citrate and gluconate, both of which are capable of leaching or precipitating metals out of materials. Data obtained by[22] Price *et al*., (2001) support an active process as being responsible, at least in part, for the fungus' ability to remove such large amounts of copper and zinc from solution. A live fungal biomass is able to remove more than twice the amount of copper from solution than a dead biomass. They found that *A. niger* removes metals, especially copper, from its environment mainly by absorption. Initial studies in untreated wastewater showed all of the removed metal associated with the fungus, and no metal associated with the HCl wash. *A. niger* was able to remove 51.1 µg Cu and 963 µg Zn/g fungal dry weight from treated swine effluent, which is superior to the killed fungus biosorption method used by[21] Akthar and Mohan (1995). The absorptive properties of *A. niger* have not been previously reported for use in bioremediation.

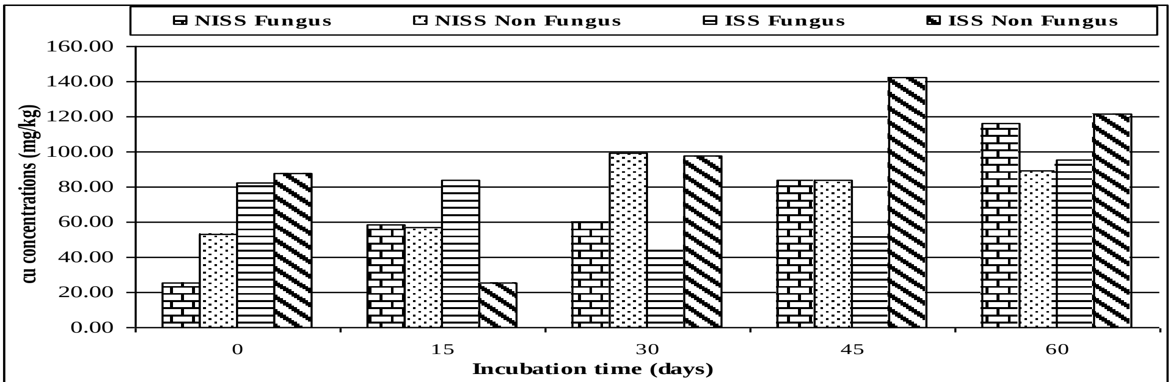

Fig (4) Interaction between treated sewage sludge with fungal inoculations and incubation times on available copper content changes

3.3. Sewage sludge Strontium concentrations

Sr concentrations in irradiated (ISS) and non irradiated sewage sludge (NISS) varied during incubation (Fig 5, 6). The total Sr content in NIS – IS sludge displayed the highest value on day 0 (Fig 5), and then remarkably decreased with incubation. After 60 days of incubation, the concentration of Sr in NISS fungal and ISS fungal even dropped to 83.25 and 86 mg kg^{-1}, respectively, while that in NISS non fungal and ISS non fungal remained 107 and 96 mg kg^{-1}, respectively. It was generally shown that in NISS fungus and ISS fungal a slight decrease on the content of Sr when compared with non incubated. The available Sr in NIS – IS sludge increased obviously after 15-day incubation, and tend to increase with the time to reach the maximum values at the end of the experiment whereas still higher in non irradiated sewage sludge in both incubated and non-incubated treatment (Fig 6). Compared with the control NISS, the highest concentration of available Sr were found in non irradiated sewage sludge while, the lowest concentration of soluble exchangeable Sr were found in irradiated sewage sludge after incubation (Fig 6), which offered the evidence for the lowest mobility and bioavailability of Sr in non-irradiated sewage sludge. In this respect, the living cells of *Penicillium, Aspergillus, Rizopus, Mucor, Saccharomyces* and *Fusarium* have been shown to biosorb metal ions[23] Merrin *et al*., (1998). *Penicillium* biomass has been found to biosorb heavy metals (Cr, Ni, Zn, Pb & As) Tan and Cheng (2003). The living cells of *Penicillium, Rizopus* and *Saccharomyces* can biosorb radionuclides (U, Th & Sr) [24]Tsezos *et al*., (1997).

Metal ion uptake by living cells is a function of cell age, composition of growth media, contact time, pH of metal solution and temperature. The biosorption of heavy metal ions on the cell surface occurs by ion exchange and complexation reaction with functional groups like carboxyl, amine, amides, hydroxyl, phosphate and sulphydryl groups (Remacle 1990). Metal uptake can also take place by an active mode, which is dependent on the cell metabolic cycle, and metal ions are transported into the cell material across the cell wall. Metal uptake by active mode has been observed for Cu, Cd, Ni, Zn, Co, Mn, Sr, Mg and Ca[25] Volesky (1994). Gadd (1990) suggested that at high metal concentrations, active mode might not contribute significantly to metal uptake, especially for filamentous fungi.

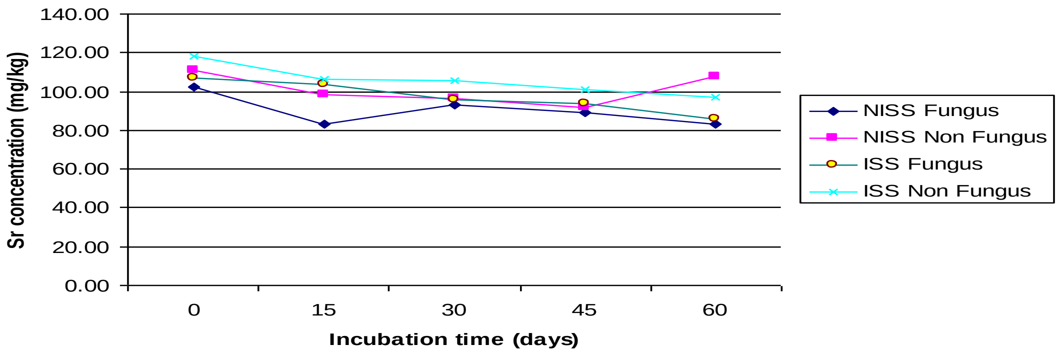

Fig (5) Interaction between sewage sludge treatments and time of incubation on Strontium content changes

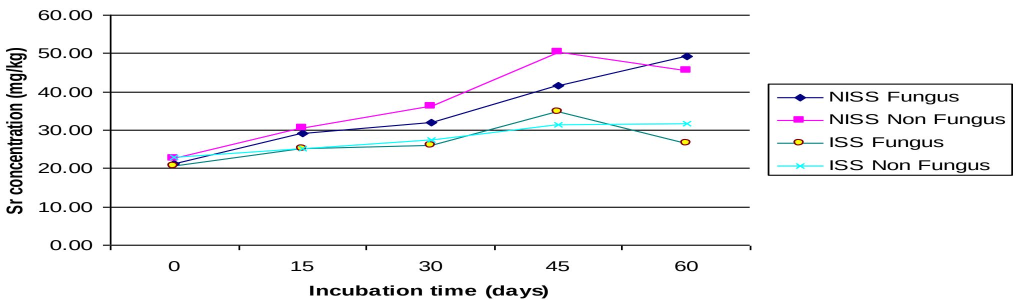

Fig (6) Interaction between sewage sludge treatments and time of incubation on Strontium content changes.

Lead (Pb^{2+})

Lead concentrations in irradiated (ISS) and non irradiated sewage sludge (NISS) are varied during fungal treatments with *Fusarium oxysporium* for different intervals incubation times were showed in (fig 7, 8). Total lead content in NISS fungal and non-fungal treatments was increased at 15 days incubation times compared to 0 day incubation time. Reduction of lead was noted in most treated NISS with non-fungus especially intervals incubation times at 0, 30, 45 and 60 days compared to the same NISS treated with *fusarium oxisporum* fungi. In this respect, recorded data of lead might be attributed to antagonistic negatively effect of fungal inoculations and microflora in reduction of element in NISS treated with fungi compared to NISS-non fungus treatments. On the other hand, reduction of lead element was recorded in most treated ISS with fungal inoculations at different interval times 0, 30, 45 and 60 days comparable to the same ISS non-fungus treatments. This recorded data were clarified the solely positive effect of applied fungal inoculation on lead reduction in tested ISS samples. Also, the highest ratio of element reduction was noted and can detected in fungal ISS treatment 60% comparable to the same treatment ISS non-fungus at 45 days incubation time followed by fungal ISS treatment that compared to the non-fungal ISS with ratio 50.5%. In this respect, Microorganisms (especially fungi), are known have the ability to scavenge to increase the bioavailability of heavy metals during adsorption [26](Agunwamba *et al.*, 2013). Generally, on the reason why the organisms were all able to adsorb the heavy metals, the issue of complexation and ion exchange comes to play. Organisms like fungi and bacteria are known to have negatively charged walls and some functional groups like lipids, hydroxyl, phosphonate, carboxylic, chitin (fungi) and amino which are responsible for ion exchange with positively charged metals like $pb^{2}+$, $pb^{4}+$, $zn^{2}+$, $cd^{2}+$ thus reducing and adsorbing them in exchange of ions. Again, ionic compounds can be exchanged for another [27] (Agunwamba *et al*., 2013). For instance,[28] Mashitah *et al*., (1999) also holds that some ions (calcium ions) present in the cell wall of organisms are released in exchange for lead. The highest adsorption efficiency was recorded in Sample C for each heavy metal. The combination of fungi and bacteria is seen to have an efficiency of 94%, 82%, and 98% for cadmium, lead and zinc respectively.

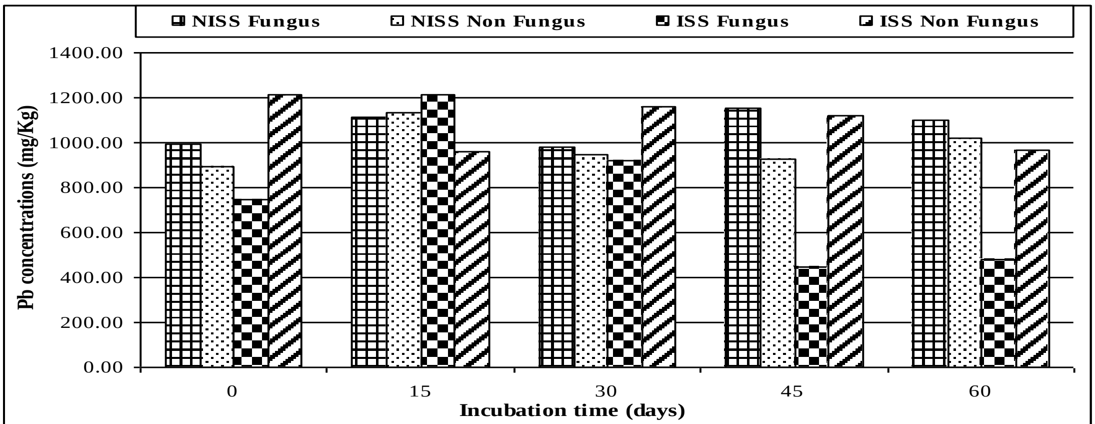

Fig (7) Interaction between treated sewage sludge with fungal inoculation and icubation times on lead content change

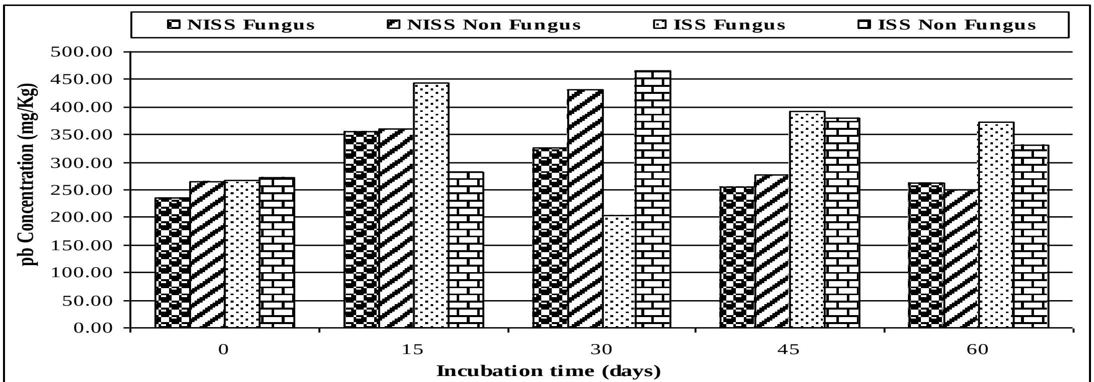

Fig (8) Interaction between treated sewage sludge with fungal inoculations and icubation times on available lead content changes

Data of available lead in fungal and non-fungal NISS treatments are slightly increased at incubation times 15 and 45 days comparable with others interval incubation times. Commonly, available lead was increased at 15 and 30 days and finally decreased at 45 and 60 day incubation time in fungal and non-fungal NISS treatments. On the other hand, available lead in ISS is regular increased especially with non-fungal treatments at 0, 15 and 30 days incubation time and sable trend was recorded at 45 and 60 days incubation time. Available lead reduction was induced by fungal treatments and recorded the highest ratio in ISS at 30 days incubation time 56.1 % comparable with other treatments. In this finding results obtained by[27] (Agunwamba *et al*., 2013) showed that the combination of fungi and bacteria performed best for the sewage water with efficiencies of 94%, 82%, and 98% for cadmium, lead and zinc respectively. For the sewage sludge, the combinations of fungi and plant, and bacteria with 99% each performed best in the adsorption cadmium whereas for zinc, fungi with 98% performed the least. All other samples had efficiencies of 99% each. For lead, fungi and bacteria with 96% and 97% performed the least.

REFERENCES

[1]. Abdel-Aziz,O.A. (2005).Bioremediation of soil contaminated with some heavy metals using nuclear techniques. M.Sc. thesis in Al-Azhar Univ., Faculty of Science, Botany and Microbiology Department, Cairo, Egypt.

[2]. Agunwamba J.C., Onyekweredike K. O. and Mmonwuba N. (2013). Comparative analysis of bioremediation of heavy metals using plants and microorganisms. INT J CURR SCI 2013, 6: 153-160

[3]. Akthar, M.N., Mohan, P.M., 1995. Bioremediation of toxic metal ions from polluted lake waters and industrial effluents by fungal biosorbent. Current Science 69, 1028-1030.

[4]. APHA 1989. Standard Methods for the Examination of Water and Wastewater. 17th edition, American Public Health Association, Washington D.C., 1,268 pp.

[5]. Bosshard, P., Bachofen, R., Brandl, H., 1996. Metal leaching of fly ash from municipal waste incineration by Aspergillus niger. Environmental Science and Technology 30, 3066-3070.

[6]. De, J, Ramaiah N, Vardanyan L 2008. Detoxification of toxic heavy metals by marine bacteria highly resistant to mercury. Marine Biotechnology 10, 471-477.

[7]. Fytianos, K.; Charantoni, E. (1998). Leaching of heavy metals from municipal sewage sludge. Environmental. International., 24 (4), 467-475.

[8]. Gadd G M, (1990). Fungi and yeast for metal accumulation, in Microbial Mineral Recovery, edited by H L Ehrlich & C L Brierley (McGraw-Hill Publishing Company, New York), 249-276.
[9]. Gavrilescu M 2004. Removal of heavy metals from the environment by biosorption. Engineering in Life Sciences 4, 219-232.
[10]. Haferburg G, Kothe E 2007. Microbes and metals: Interactions in the environment. Journal of Basic Microbiology 47, 453-467.
[11]. Kilburn M. 2000. Aqua Regia Digestion for Soils/Sludges. Analytical methods manual. Method NO: H115.
[12]. Lloyd J R 2002. Bioremediation of metals: The application of microorganisms that make and break minerals. Microbiology Today 29, 67-69.
[13]. Lombardi, A. T.; Garcia, Jr. O. (1999). An evaluation into the potential of biological processing for the removal of metals from sewage sludges. Critical Reviews in Microbiology., 25 (4), 275-288.
[14]. Malik A 2004. Metal bioremediation through growing cells. Environment International 30, 261-278.
[15]. Malik, S.I., H.N. Malik, N.M. Minhas and M. Munir, 2004. General and specific combining ability studies in maize diallel crosses. Int. J. Agric. Biol., 6: 856–859.
[16]. Mashitash MD, Zulfadhly Z, Bhatia S (1999). Binding Mechanism of heavy metal biosorption by *pycnoporus sanguineus.* Artif Cells Blood Substit Immobil Biotechnology. 27(5-6): 441-5.
[17]. Merrin J S, Sheela R, Saswathi N, Prakasham R S & Ramakrishna S V, (1998). Biosorption of chromium (VI) using *Rhizopus arrhizus,* Ind J Exp Biol, 36: 1052-1055.
[18]. Plaza, G., Lukasik, W., Ulfig, K., 1996. Sorption of cadmium by filamentous soil fungi. Acta Microbiologica Polonica 45, 193-201.
[19]. Price M. S., Classen J. J., Payne G. A., 2001. Aspergillus niger absorbs copper and zinc from swine wastewater. Bioresource Technology 77 : 41-49
[20]. Remacle J, (1990). The cell wall and heavy metals, in Biosorption of Heavy Metals, edited by B Volesky (CRC Press, Boca Raton, Florida), 83-92.
[21]. Tan T and Cheng P, (2003). Biosorption of metal ions with Penicillium chrysogenum, Appl Biochem Biotechnol, 104: 119-128.
[22]. Trierweiler J E and Lindsay W L 1969. EDTA-ammonium carbonate soil test for zinc. Soil Sci. Soc. Am. Proc. 39, 49–54.
[23]. Tsezos M, Georgousis Z & Remoudaki E, (1997).Mechanism of aluminium interference on uranium biosorption by Rhizopus arrhizus, Biotechnol Bioengg, 55: 16-27.
[24]. UN/ECE ICP on Integrated Monitoring. Manual. August 1998.
[25]. Volesky B, (1994). Advances in biosorption of metals: selection of biomass types, FEMS Microbiol Rev, 14: 291-302.
[26]. Wang J, Chen C 2009. Biosorbents for heavy metals removal and their future. Biotechnology Advances 27, 195-226.